Risk assessment and prevention of spontaneous preterm birth

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Publication date
2020
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Final published version
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of SPONTANEOUS PRETERM BIRTH

Bouchra Koullali

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COLOPHON

Cover design:	James Jardine | www.jamesjardine.nl
Layout:	James Jardine | www.jamesjardine.nl
Print:	Ridderprint | www.ridderprint.nl
ISBN:	978-94-6416-298-1

Financial support for the publication of this thesis was kindly provided by Chipsoft and Bridea Medical.

Risk assessment and prevention of spontaneous preterm birth

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Universiteit van Amsterdam
op gezag van de Rector Magnificus
prof. dr. ir. K.I.J. Maex
ten overstaan van een door het College voor Promoties ingestelde commissie,
in het openbaar te verdedigen in de Agnietenkapel
op woensdag 9 december 2020, te 13.00 uur

door

Bouchra Koullali

geboren te Utrecht

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CHAPTER 1.1

Aims and outline of this thesis

Preterm birth, defined as birth before 37 weeks of gestation, is the most common cause of neonatal morbidity and mortality worldwide.[1] Children that survive preterm birth are at increased risk of respiratory immaturity, intracranial hemorrhages, and infections. These conditions can result in long-term neurodevelopmental sequelae such as intellectual impairment, cerebral palsy, chronic lung disease, deafness, and blindness. Morbidity and mortality rates increase with decreasing gestational age.[2]

Preterm birth can be classified as either spontaneous or iatrogenic. The overall worldwide incidence of preterm birth is 10.6%, which results in nearly 15 million children born preterm each year.[3] In The Netherlands, the overall incidence of preterm birth in singleton pregnancies is 5.3%, of which 2.8% spontaneous preterm births. In multiple pregnancies, the preterm birth percentage is 52.5% of which 14.3% spontaneous births.[4]

Spontaneous preterm birth is a complex issue with a multifactorial etiology and the pathogenesis remains not well understood. Multiple risk factors have been identified that can be categorized in (1) maternal characteristics, (2) obstetric and/or gynaecological history and (3) current pregnancy characteristics.[5] Despite the identification of risk factors, which will be discussed in more detail in **Chapter 1.2**, their exact role in the pathway leading to spontaneous preterm birth and their prognostic interaction is not well understood which makes it difficult to select women at high risk for preterm birth. Additionally, preventive management of spontaneous preterm birth remains a challenging task for clinicians.

The two main treatment strategies to prevent preterm birth that are used in clinical practice worldwide include progesterone and cervical cerclage. However, these treatment strategies are not effective in all patient populations at risk for preterm birth.[5] In addition, the role of a cervical pessary in the prevention of spontaneous preterm birth is currently being studied extensively in randomized controlled trials. Randomized controlled trials about the effectiveness of a cervical pessary that have been published so far show conflicting results and the exact potential of a cervical pessary is still being debated and further studied in trials.[6-9] There remains a strong need for alternative, effective therapies for preventing spontaneous preterm birth in women at risk.

The aim of the research described in this thesis is (1) to elucidate the role of various risk factors for spontaneous preterm birth and (2) to study preventive management strategies in women at risk for spontaneous preterm birth.

Part I of this thesis focuses on the risk assessment of spontaneous preterm birth. **Chapter 2** describes the association between interpregnancy interval and the risk of recurrent spontaneous preterm birth using data from the population based Netherlands Perinatal Registry (PERINED). **Chapter 3** describes the role of parity in the risk of spontaneous preterm birth, assessing first, second, third, fourth and fifth pregnancies, also based on PERINED data. In **Chapter 4**, we assess the utility of mid-trimester uterine artery Doppler in the prediction of spontaneous preterm delivery in a single center prospective cohort study. In **Chapter 5**, we explore whether verification of short cervical length with a second measurement improves the identification of patients with short cervical length who are at increased risk of preterm delivery.

Part II focuses on the prevention of spontaneous preterm birth in women with cervical insufficiency. In **Chapter 6**, we conduct a systematic review and meta-analysis to assess the effect of an emergency cerclage in singleton pregnancies with cervical dilation before 24 weeks of gestation. **Chapter 7** is a protocol of a randomized controlled trial that compares a cervical pessary with a cervical cerclage in the prevention of preterm delivery in women with short cervical length and a history of preterm birth (PC-Study).

Part III focuses on novel interventions in the prevention of spontaneous preterm birth. In **Chapter 8**, we discuss two novel interventions to treat cervical insufficiency, including (1) an injectable, silk protein-based biomaterial for cervical tissue augmentation (injectable cerclage) and (2) a patient-specific pessary. In **Chapter 9,** we describe the biocompatibility of an injectable silk-based hydrogel for augmenting cervical tissue *in vivo* in a pregnant rat model. The rationale for the development of an injectable gel is to provide support to the cervical stroma to prevent cervical shortening and thereby reduce the risk for preterm birth. We hypothesize that further development of this hydrogel can lead to therapeutic use as an alternative to cerclage in preterm birth due to cervical insufficiency. In **Chapter 10**, we discuss tissue engineering scaffolds (1) to study remodelling of the cervical stroma and (2) to repair cervical tissue in pregnancies at risk for preterm birth because of cervical insufficiency.

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CHAPTER 1.2

Risk assessment and management to prevent preterm birth

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Seminars in Fetal & Neonatal Medicine. 2016 Apr;21(2):80-8

ABSTRACT

Preterm birth is the most important cause of neonatal mortality and morbidity worldwide. In this review, we review potential risk factors associated with preterm birth and the subsequent management to prevent preterm birth in low and high risk women with a singleton or multiple pregnancy. A history of preterm birth is considered the most important risk factor for preterm birth in subsequent pregnancy. General risk factors with a much lower impact include ethnicity, low socio-economic status, maternal weight, smoking, and periodontal status. Pregnancy-related characteristics, including bacterial vaginosis and asymptomatic bacteriuria, appear to be of limited value in the prediction of preterm birth. By contrast, a mid-pregnancy cervical length measurement is independently associated with preterm birth and could be used to identify women at risk of a premature delivery. A fetal fibronectin test may be of additional value in the prediction of preterm birth. The most effective methods to prevent preterm birth depend on the obstetric history, which makes the identification of women at risk of preterm birth an important task for clinical care providers.

1. INTRODUCTION

Preterm birth, defined as delivery before 37 weeks of gestation, is an important complication of both singleton and multifetal pregnancies worldwide. Children born preterm are at increased risk of mortality and are more likely to have long-term neurological and developmental disorders than those born at term. The incidence of preterm birth varies between countries with a range of 5-13%, resulting in 15 million preterm deliveries worldwide each year. More than 60% of all preterm births occur in Sub-Saharan Africa and South(-eastern) Asia. The highest rates are found in South-eastern and South Asia where 13.4% of the children are born preterm. The preterm birth rate in Europe ranges from 5% to 10%, where relatively low rates are observed in Scandinavian countries and relatively high rates occur in Cyprus and Hungary. Of the 1.2 million preterm births that occur in high income countries, more than 0.5 million (42%) occur in the USA where the estimated preterm birth rate is 11-12%.[1]

Mortality and morbidity rates of babies born preterm increase with decreasing gestational age. The worldwide incidence of preterm birth at <32 weeks is 16% of all preterm births. Although survival rates have greatly improved in recent years for children born very (<32 weeks) and extremely (<28 weeks) preterm, mortality and morbidity are highest among these children, especially in low income countries. Mortality and morbidity rates in late preterm births (32-37 weeks) are less pronounced, though they remain substantial compared to rates in children born at term.

The identification of women at risk is important, as several treatment strategies have been effective in the reduction of spontaneous preterm birth. For an accurate risk assessment, several factors may be taken into account including general risk factors, obstetric history and specific pregnancy-related risk factors (**Table 1**). This article aims to review potential risk factors associated with preterm birth and the subsequent management to prevent preterm birth in both low and high risk singleton and multiple pregnancies.

2. RISK FACTORS

2.1. General

2.1.1. Maternal characteristics

Ethnicity, socio-economic status, and body mass index (BMI; kg/m^2) all seem to be associated with poor pregnancy outcome including preterm birth. Several studies report a positive association between certain ethnic groups and preterm birth. Women classified

as African and Afro-Caribbean are considered to be at high risk for preterm birth (odds ratio (OR): 2.0; 95% confidence interval (CI): 1.8-2.2) when compared to Caucasian women as well as women of low socio-economic and low educational status.[2,3] It should not be excluded that the physiological duration of pregnancy in women of different ethnicities is different, and that African and Afro-American women have a shorter duration pregnancy. Indeed, preterm children from Afro-Caribbean women do better when born preterm as compared to women from other ethnicities.[4]

TABLE 1. Risk factors for preterm birth and possible interventions.

	Risk Factor	Intervention
Maternal characteristics	Low socio-economic status	Information
	Ethnicity	Information
	Smoking	Stop smoking
	Low body mass index	Lifestyle, nutrition information
	Periodontitis	Referral to dentist
Medical history	Cervical surgery (LEEP conization)	Information
	Uterus anomaly	Information
Obstetrical history	Preterm birth	Progesterone
	Pregnancy loss >16 weeks GA	Progesterone
	Cervical insufficiency	History indicated cerclage (singletons)
Current pregnancy	Mode of conception (in-vitro fertilization)	Information
	Multiple pregnancy	Information
	Short cervix in women without a history of PTB (singleton and twin pregnancies)	Progesterone or pessary
	Short cervix in women with a history of PTB (singleton pregnancies only)	Ultrasound-indicated cerclage (or pessary)

Abbreviations: *LEEP, loop electrosurgical excision procedure; GA, gestational age; PTB, preterm birth.*

Furthermore, as compared to normal-weight women, higher preterm birth rates are observed in women with both low BMI (OR: 1.35; 95% CI: 1.14-1.60) and in overweight and obese women (OR: 1.26; 95% CI: 1.15-1.37 for BMI 25-30). The higher the BMI, the higher the risk, especially for extreme preterm birth (OR: 1.58; 95% CI: 1.39-1.79 for BMI 30-35; OR: 2.01; 95% CI: 1.66-2.45 for BMI 35-40; and OR: 2.99; 95% CI: 2.28-3.92 for BMI ≥40).[5] The mechanism by which these maternal demographics are related to preterm birth remain unclear.

In addition to these general maternal characteristics, it is known that singleton pregnancies after in-vitro fertilization (IVF) are at increased risk of preterm birth (risk ratio (RR): 2.13;

95% CI: 1.26-3.61).[6] Additionally, previous studies indicate that either a short or a long interval between pregnancies is associated with adverse perinatal outcomes, including preterm birth; however, whether this association is confounded remains unclear.[7,8]

2.1.2. Medical history

Maternal periodontal disease is associated with preterm birth (RR: 1.6; 95% CI: 1.1-2.3), and the risk seems to increase when periodontal disease progresses during pregnancy, potentially due to haematogenous transmission of oral microbial pathogens and release of inflammatory mediators and prostaglandins into the maternal circulation.[9]

Cervical surgery after cervical intraepithelial neoplasia (CIN) is also associated with preterm birth. Various studies have shown that the increased risk is due to the cervical surgery, especially when performed during pregnancy, and does not seem to be related to the neoplasia itself.[10,11] Castanon et al. observed that large excisional treatment (>15 mm) of cervical transformation zone is associated with a doubling of the risk of preterm birth (RR: 2.04; 95% CI: 1.41-2.96). This risk does not decrease with increasing time to conception. This implies that all women who have had cervical surgery with large excisions of the cervical transformation zone should be closely monitored during pregnancy.[12]

2.1.3. Smoking

Smoking is strongly related to preterm birth (OR: 3.21; 95% CI: 1.42-7.23) and this risk is directly correlated to the number of cigarettes smoked per day.[13] It has been hypothesized that smoking is associated with a systemic inflammatory response, leading to preterm birth. The association between smoking and preterm birth appears to be stronger for very preterm birth (<32 weeks) than for moderate preterm birth (≥32 weeks).[14]

Previous studies report that 20-40% of smokers quit smoking during pregnancy; of those, the majority quits early in pregnancy. Women with low education, women who started smoking at a young age, heavy smokers, women exposed to passive smoking, and multiparous women are more at risk for continued smoking during pregnancy.[14]

The assessment of risk factors varies between different pregnancy populations. In this review we discuss the following sub-groups: low risk pregnancies, i.e. women with a singleton pregnancy without a history of preterm birth; and high risk pregnancies, i.e. women with a multiple pregnancy and women with a history of preterm birth.

2.2. Low risk pregnancies

2.2.1. Women with singleton pregnancy without a history of preterm birth

2.2.1.1. Bacterial vaginosis. Bacterial vaginosis is an abnormal vaginal condition that results from an overgrowth of atypical micro-organisms in the vagina, including Gardnerella vaginalis, Prevotella spp., Bacteroides spp., Mobiluncus spp., Gram-positive cocci, and genital mycoplasma.[15] The presence of at least three of the following four criteria is considered to be consistent with the presence of bacterial vaginosis: vaginal pH >4.5, clue cells on saline wet mount, release of a fish amine odour on addition of 10% KOH to a drop of vaginal discharge, and abnormal vaginal discharge.[16] A scoring system of vaginal smears to diagnose bacterial vaginosis was described by Nugent et al., in 1991. The Nugent score is based on a weighted combination of the different micro-organisms on wet mount, ranging from 0 to 10.[17] A meta-analysis from 2003, which included 18 studies and 20,232 low risk singleton pregnancies showed that bacterial vaginosis during pregnancy is associated with an increased risk of miscarriage (RR: 9.91; 95% CI: 1.99-49.34) and preterm birth (RR: 2.19; 95% CI: 1.54-3.12).[18]

2.2.1.2. Asymptomatic bacteriuria. Asymptomatic bacteriuria is defined as the presence of significant bacteriuria without symptoms of a urinary tract infection, occurring in 5-10% of pregnancies.[19] Bacteriuria is considered to be associated with obstetric complications such as preterm birth and low birth weight in low risk pregnant women in various studies.[20,21] However, a more recent prospective cohort study with an embedded randomized controlled trial (RCT) by Kazemier et al. did not confirm the association between asymptomatic bacteriuria and preterm birth in uncomplicated singleton pregnancies (OR: 1.5; 95% CI: 0.6-3.5).[22]

2.2.1.3. Cervical length. The risk of spontaneous preterm birth is increased in women with a mid-pregnancy short cervix.[23-25] In low risk singleton pregnancies with a mid-pregnancy cervical length of ≤35 mm and without any known risk factors, the risk of spontaneous preterm birth before 37 weeks of gestation is 13% (RR: 2.35; 95% CI: 1.42-3.89). This risk is inversely proportional to the size of the cervix, with a shorter cervix predicting a higher risk. Once the cervix is <26 mm the risk of preterm birth will be more than double (RR: 6.19; 95% CI: 3.84-9.97).[24] Although a short cervical length is associated with a higher risk for preterm birth, change in transvaginal sonographic cervical length over time does not appear to be a clinically useful test to predict preterm birth.[26]

Cervical length measurements can be performed by using transabdominal or transvaginal ultrasound. In contrast to trans-abdominal ultrasound evaluation of the cervix, transvaginal cervical ultrasonography has been shown to be a reliable and

reproducible method to assess the cervical length and is the gold standard for cervical length measurement.[27] In addition, trans-vaginal evaluation of the cervix is safe and well accepted by women.[28]

The role of mid-pregnancy screening for short cervical length in a low risk population is currently being debated while not routinely recommended.[29] Limiting cervical length screening for short cervical length to women with one or more identified risk factors decreases the number of transvaginal ultrasound examinations and increases the specificity from 62.8% to 96.5%. However, this results in nearly 40% of women with short cervix not being detected. Before the introduction of a universal screening program, it is important to be aware of potential limiting factors, such as a high number needed to screen to prevent one preterm birth[30], and the poor image qualities of many cervical length measurements. This could lead to overdiagnosis of cervical shortening and possible unnecessary interventions such as bed rest and hospitalization.[31] Developing an optimal screening and treatment program is a challenging yet important task for clinical investigators.

2.2.1.4. Fetal fibronectin. Fetal fibronectin is a glycoprotein found in amniotic fluid, membranes, and in placental tissue which is normally present in low concentrations in cervical and vaginal secretions between 18 and 34 weeks of gestation. Although its exact function is unclear, it appears to act as an adhesive glue between fetal membranes and the decidua. It is hypothesized that fetal fibronectin is released through mechanical and infection-mediated damage to the membranes or placenta prior to birth. Elevated concentrations of fetal fibronectin indicate an increased likelihood of (preterm) delivery[32], making it one of the most effective predictors of preterm birth in all pregnant populations, including low and high risk singleton and twin pregnancies, and especially in women with symptoms of preterm labour.[33]

A prospective study with 2929 low risk singleton pregnancies evaluated the correlation between positive fetal fibronectin and the prediction of spontaneous preterm birth in low risk singleton pregnancies, finding an association between a positive test and preterm birth (sensitivity 63%, specificity 98%, resulting in a positive predictive value of 13%).[34] An additional study confirmed this association, particularly in women with a short cervix.[35] Abbott et al. performed a prospective observational cohort study in which they evaluated quantitative fetal fibronectin concentration in asymptomatic women at high risk of spontaneous preterm birth. Quantitative measurement of fetal fibronectin improved the accuracy for defining risk of spontaneous preterm birth in high risk asymptomatic women.[36]

2.3. High risk pregnancies

2.3.1. Women with a multiple pregnancy

As more than 50% of all women with twin pregnancies deliver at <37 weeks of gestation, women with multiple gestation contribute to 20% of all preterm births and to an even larger proportion of preterm children.[37,38]

2.3.1.1. Bacterial vaginosis. In contrast to low risk singleton pregnancies, the presence of bacterial vaginosis in twin pregnancies appears not to be associated with an additional increased risk of spontaneous preterm birth. A meta-analysis performed by Conde-Agudelo et al. reported that the presence of bacterial vaginosis has very low predictive values for spontaneous preterm birth at <32, <35, and <37 weeks of gestation with sensitivities and specificities, between 0-23% and 78-92%, with corresponding likelihood ratios of positive and negative tests ranging between 0.6-1.8 and 0.9-1.2, respectively.[39]

2.3.1.2. Cervical length. There are conflicting results regarding cervical length measurements and the prediction of preterm birth in twin gestations. Conde-Agudelo et al. reported in a meta-analysis that a mid-pregnancy cervical length measurement is considered as a good predictor of spontaneous preterm birth (pooled sensitivities and specificities of 39% and 96%, and likelihood ratios of positive and negative tests of 10.1 and 0.64, respectively, for preterm birth <32 weeks).[38] In addition, various studies report that a cervical length of >35 mm in women with a twin pregnancy is associated with a low risk of 4% for preterm delivery.[40,41] In contrast, Pagani et al. showed that, despite an independent association between cervical length and preterm birth (OR: 0.94; 95% CI: 0.90-0.99), a mid-pregnancy cervical length measurement is a poor predictor of preterm birth <32 weeks in asymptomatic twin gestations.[42]

A meta-analysis by Kindinger et al. showed that prediction of preterm birth in twin gestations depends on both cervical lengths and the gestational age at screening. The authors conclude that the best prediction of preterm birth ≤28 weeks is provided by screening at ≤18 weeks, and prediction of birth between 28 and 36 weeks by screening at ≥24 weeks. It is therefore recommended to screen twins ≤18 weeks for cervical length shortening.[43]

2.3.1.3. Fetal fibronectin. A meta-analysis by Conde-Agudelo et al. on the accuracy of fetal fibronectin test in predicting preterm birth in 1009 asymptomatic women with twin pregnancies included a total of 11 studies and found only limited accuracy in predicting preterm birth before 32, 34, and 37 weeks of gestation (pooled sensitivities and specificities between 33-39% and 80-94%, and likelihood ratios of positive and negative

tests ranged from 2.0-5.1 and 0.7-0.8, respectively).[44] In addition, two retrospective cohort studies found similar disappointing results for the prediction of preterm birth before 32 weeks of gestation in asymptomatic women.[45,46]

2.3.2. Women with a previous preterm birth

The most important risk factor for preterm birth is a previous preterm birth. Women with a history of spontaneous preterm birth are considered as high risk and they have an average risk of 20% (range: 15.8-30.2%) of recurrence of spontaneous preterm birth before 37 weeks.[47] The risk increases with a lower gestational age at index pregnancy and the number of spontaneous preterm births.[48]

2.3.2.1. Cervical length. Many studies evaluating screening for short cervical length in women with a prior preterm birth have been performed. In this high risk group, a cervical length <25 mm is associated with an increased risk of preterm birth in a subsequent pregnancy (RR: 4.5; 95% CI: 2.7-7.6).[49] Women with a previous preterm birth should be screened with serial cervical length measurements before 24 weeks of gestation, as some may benefit from interventions to prevent preterm birth when a short cervix is found.[49]

2.3.2.2. Fetal fibronectin. In a prospective study by Iams et al. on predictors of spontaneous preterm birth in singleton gestations, the relationship between fetal fibronectin and recurrence rate of spontaneous preterm birth was assessed. The study compared 378 women with a prior spontaneous preterm birth before 37 weeks of gestation to 904 women without a history of spontaneous preterm birth. This study concluded that fetal fibronectin was the best single predictor in women with a history of preterm birth, with a short cervical length also contributing independently to the recurrence risk. The recurrence risk was 64% in women with a positive fetal fibronectin test and a cervical length of ≤25 mm, compared to 25% when the fetal fibronectin test was negative.[25] Romero et al. found dissimilar results in a retrospective cohort of 176 patients with a prior spontaneous preterm birth. These authors did not find a similar association between fetal fibronectin and recurrent preterm birth in patients with a history of spontaneous preterm birth (OR: 0.647; 95% CI: 0.043-9.759).[50] There is no hard evidence endorsing the clinical value of fetal fibronectin tests in asymptomatic singleton pregnancies so far.[51]

3. RISK REDUCTION

Interventions aiming at risk reduction of spontaneous preterm birth vary between different populations, including low and high risk singleton and twin pregnancies. This section reviews preventive interventions to reduce the risk of spontaneous preterm birth.

3.1. General

3.1.1. Maternal characteristics

Clearly, ethnicity and socio-economic status are fixed characteristics, making these factors unsuitable for preventive interventions; however, this information may be of great value in providing perinatal care adjusted to an individual woman's risk profile.

For maternal overweight and obesity, there is no evidence that exercise during pregnancy reduces the risk of preterm birth.[52] Available data even suggest that insufficient gestational weight gain and gestational weight loss may increase the risk of preterm delivery (OR: 1.38; 95% CI: 1.12-1.71). Because of this association with preterm birth, for women with a low BMI and for overweight women it is recommended not to lose weight during pregnancy.[53]

The relationship between IVF and preterm birth has been demonstrated in various studies; we therefore advise performing IVF only in those women with a sound medical indication. In addition, it is recommended to perform a single embryo transfer which gives a lower rate of preterm birth compared to a double or multiple embryo transfer.[6]

Various studies propose that there is a relationship between interpregnancy interval and preterm birth, suggesting that there is an optimal interval between pregnancies and that spacing pregnancies appropriately might help to prevent these adverse perinatal outcomes. The World Health Organization recommends a minimum interpregnancy interval of two years based on the available information and evidence. However, it has been hypothesized that this association is confounded by unknown maternal factors, which would counter the suggestion of an optimal interval.

3.1.2. Medical history

Whether treatment of periodontal disease decreases the risk of preterm birth remains uncertain since several studies report conflicting and inconclusive findings. An RCT from 2009 included 1087 women with periodontal disease who were randomly assigned to dental treatment or no additional care (control group) during pregnancy. This study did not find a reduction in the preterm birth rate in the treatment group (OR: 1.05; 95% CI: 0.7-1.58).[54] In 2010, a meta-analysis found similar results and showed no difference in preterm birth when periodontal disease was treated (OR: 1.15; 95% CI: 0.95-1.40).[55] In contrast, a meta-analysis from 2011 showed that periodontal treatment significantly decreased preterm birth (OR: 0.65; 95% CI: 0.45-0.95).[56] A meta-analysis from 2012 did not find this association, but a subgroup analysis of women at high risk for preterm birth showed a decrease in the preterm birth rate (RR: 0.66; 95% CI: 0.54-0.80).[57] Treatment of

periodontal disease solely for the purpose of reducing the risk of preterm birth should therefore not be recommended, as results are conflicting. However, consideration of treatment after pregnancy is advisable for dental reasons.

The risk of progression of CIN to invasive cervical cancer during pregnancy is minimal and a significant number regresses spontaneously postpartum. Treatment of CIN with cervical surgery during pregnancy is associated with preterm birth and with a high rate of recurrence or persistence. Therefore, these data suggest that cervical surgery in cases of CIN should be postponed until after delivery and that the only indication for therapy during pregnancy is invasive cancer.[10,58] Furthermore, large excisional treatment should be avoided when CIN is detected during the reproductive age of a woman. It is recommended to excise the entire lesion while preserving as much healthy cervical tissue as possible.[12]

3.1.3. Smoking

Since smoking is associated with an increased risk for preterm birth, all women should be advised to quit smoking before pregnancy or early in pregnancy. A prospective cohort study from 2009 examined pregnancy outcomes of 1992 non-smokers, 261 women who had stopped smoking before 15 weeks of gestation, and 251 smokers. There were no differences in preterm birth between non-smokers and women who had stopped smoking (OR:1.03;95% CI:0.49-2.18). Continuing smokers had significantly higher rates of spontaneous preterm birth (OR: 3.21; 95% CI: 1.42-7.23). This study indicates that stopping smoking early in pregnancy reduces the risk of preterm birth to the level of non-smokers.[13]

Potentially all the above-mentioned general risk factors are interrelated. Women of lower socio-economic status tend to have a higher BMI, appear to smoke more frequently, and will probably have worse body and dental hygiene. Thus, reduction in preterm birth may potentially be achieved by tailor-made education programmes creating awareness not just in the general population, but more especially in the lower educated.

3.2. Low risk pregnancies

3.2.1. Women with singleton pregnancy without a history of preterm birth

3.2.1.1. Bacterial vaginosis. The association of bacterial vaginosis and preterm birth resulted in the hypothesis that screening for and treatment of bacterial vaginosis might reduce the preterm birth rate. In a meta-analysis from 2011, treatment with clindamycin was associated with a significantly reduced risk of preterm birth before 37 weeks (pooled RR: 0.60; 95% CI: 0.42-0.86).[59] On the contrary, a Cochrane review from 2013 including 21

trials reported a reduced risk of late miscarriage (RR: 0.20; 95% CI: 0.05-0.76); however, no effect on the preterm birth rate before 37 weeks of gestation (RR: 0.88; 95% CI: 0.71-1.09) was seen when asymptomatic bacterial vaginosis was treated.[60]

3.2.1.2. Treatment of asymptomatic bacteriuria. In a recent study from 2015, 248 out of 4283 low risk women were screened positive for asymptomatic bacteriuria, of whom 40 were randomly assigned to treatment with nitrofurantoin and 45 to placebo. No difference in preterm birth was observed when asymptomatic bacteriuria was treated (risk difference: -0.4; 95% CI: -3.6 to 9.4).[22]

3.2.1.3. Treatment of short cervix. Many strategies and interventions to prevent preterm birth in low risk women with a short mid-pregnancy cervix have been investigated. We discuss the cervical cerclage, pessary, and progesterone.

Cerclage. A cervical cerclage is a surgical procedure that involves occlusion of the cervix by means of a cervical suture or stitch, which is performed under general or spinal anaesthesia as proposed by Shirodkar in 1955[61] and by McDonald in 1957.[62] Cervical cerclage aims to give mechanical support to the cervix and to keep the cervix closed during pregnancy. In asymptomatic singleton pregnancies without a prior preterm birth with a short cervix of <25 mm, cerclage has not been shown to be of benefit in the reduction of preterm birth (RR: 0.76; 95% CI: 0.52-1.15).[63,64] This was confirmed by a meta-analysis from 2010 showing no reduction in preterm birth in 344 women with an asymptomatic short cervix <25 mm.[65]

Pessary. The cervical pessary is a soft and flexible silicone device, used since 1959 in women with recurrent miscarriage.[66] Although the exact mechanism of the cervical pessary remains unknown, it has been hypothesized that the pessary relieves direct pressure on the internal cervical os by changing the position of the cervical canal and distributing the weight of the pregnant uterus.[67] Hence, it may prevent premature dilatation of the cervix and premature rupture of the membranes. Another possible mechanism is that the pessary might support the immunological barrier between chorioamnion-extraovular space and the vaginal microbiological flora.[68]

The largest RCT evaluating the effect of a cervical pessary in women with a short cervical length was the Spanish PECEP trial from 2012. In this study, 385 women with a singleton pregnancy and a cervical length of ≤25 mm at ~20 weeks of gestation were randomized either to a cervical pessary or to expectant management. This trial showed that a cervical pessary reduces the risk of spontaneous preterm birth before 37 weeks of gestation (OR: 0.19; 95% CI: 0.12-0.30), spontaneous preterm birth before 34 weeks (OR: 0.18; 95% CI:

0.08-0.37) and improves neonatal outcome (RR: 0.14; 95% CI: 0.04-0.39).[68] A Chinese study from 2013 with 108 randomized singleton pregnancies did not reproduce these results, and did not find a positive effect of the pessary (RR: 0.96; 95% CI: 0.81-1.14).[69]

Progesterone. It has been suggested that progesterone plays an important role in maintaining pregnancy. Progesterone has suppressive actions on the immune system and lymphocyte proliferation and activity. In addition, progesterone suppresses the activity of uterine smooth muscle to ensure maintenance of pregnancy.[70,71] Progesterone concentration in peripheral blood decreases before the onset of labour in most mammalian species, but this mechanism is not described in humans. The hypothesis of the working mechanism of progesterone is based on the cervical ripening action of progesterone antagonists, which leads to cervical shortening.[72]

A Cochrane meta-analysis from 2013 including 36 studies with a total of 8523 women shows that the use of vaginal progesterone reduces the risk of preterm birth before 34 weeks (RR: 0.64; 95% CI: 0.45-0.90) and before 28 weeks of gestation (RR: 0.59; 95% CI: 0.37-0.93) in women with a singleton pregnancy and a short cervix (<25 mm).[73] In addition, another meta-analysis from 2012 shows a reduction in composite adverse neonatal outcome when vaginal progesterone is used in singleton pregnancies with a cervical length of ≤25 mm.[74] The use of vaginal progesterone appears to be cost-effective when screening for short cervical length in a low risk population.[75]

3.3. High risk pregnancies

3.3.1. Women with a multiple pregnancy

3.3.1.1. Cerclage. A Cochrane review from 2014 concludes that there is currently no evidence available that a cerclage is an effective intervention for preventing preterm births and improving perinatal and neonatal outcomes.[76] A meta-analysis from 2015 assessed the effect of ultrasound-indicated cerclage and found no effect on the preterm birth rate (before 37 weeks OR: 1.13; 95% CI: 0.17-8.66; before 28 weeks 1.66; 0.62-4.01).[77] Both the Cochrane review and the meta-analysis indicate an increased rate of very low birth weight and respiratory distress syndrome in twin gestations with a short cervical length and a cerclage.[76,77] However, these results are based on limited data and large trials concerning this issue remain necessary.

3.3.1.2. Pessary. Liem et al. performed a large RCT including 808 twin gestations to assess the effect of a pessary in twin gestations. Overall the pessary did not improve neonatal outcome; however, in a subgroup of women with a cervix <38 mm (p25), neonatal outcome was improved (RR: 0.40; 95% CI: 0.19-0.83), and preterm birth rates

<28 and <34 weeks were decreased in the pessary group.[78] An RCT recently performed by Goya et al. evaluated the effect of a pessary in twin pregnancies and a cervical length of ≤25 mm. A reduction in spontaneous preterm birth before 34 weeks of gestation was observed (16.2% versus 25.7%; P < 0.0001).[79] Nicolaides et al. performed a trial to evaluate the effect of a pessary on twin pregnancies. No benefit was present in the reduction of preterm birth <34 weeks (RR: 1.054; 95% CI: 0.787-1.413) or neonatal outcome (RR: 1.094; 95% CI: 0.851-1.407). A subgroup analysis of women with a cervical length of ≤25 mm also showed no benefit from the cervical pessary on the preterm birth rate or neonatal outcome.[80]

These conflicting results may be due to the difference in gestational age at which the pessary was inserted between the studies. In studies where the pessary was inserted at an earlier gestational age, the effect seems to be present. Future research is needed to give more information about the optimal time and cervical length of intervention.

3.3.1.3. Progesterone. Dodd et al. concluded in a meta-analysis that there is no effect of both 17a-hydroxyprogesterone caproate and vaginal progesterone in multiple pregnancies on pregnancy outcome[73] Another meta-analysis from 2014, including 13 trials with 3768 twin gestations, found no effect of progesterone in unselected women with an uncomplicated twin gestation. However, vaginal progesterone reduced adverse perinatal outcomes in women with a cervical length of <25 mm (RR: 0.56; 95% CI: 0.42-0.75).[81] An RCT also published in 2015 included 288 twin pregnancies of which 194 women were allocated to weekly 17α-hydroxyprogesterone caproate. There was no reduction in preterm birth, whereas there was a significant reduction in composite neonatal outcome (OR: 0.53; 95% CI: 0.31-0.90).[82]

The conflicting findings of various studies assessing the effect of progesterone in twin and multiple pregnancies may be due to the range of cervical lengths in women, since there is evidence that progesterone reduces preterm birth in twin pregnancies with a short cervical length.[81] This implies that future studies should focus on women who may benefit from the interventions to prevent preterm birth.[83]

3.3.2. Women with a previous preterm birth

3.3.2.1. Bacterial vaginosis: antibiotics. A Cochrane meta-analysis by Brocklehurst et al. showed no effect of the use of antibiotics in women with a history of preterm birth and bacterial vaginosis (RR: 0.57; 95% CI: 0.22-1.50).[60] However, Thinkhamrop et al. performed a meta-analysis to assess the effect of antibiotic prophylaxis during the second and third trimester on adverse pregnancy outcome and morbidity. A reduction in preterm delivery in the subgroup of pregnant women with a prior preterm birth and bacterial vaginosis

during the current pregnancy was observed (RR: 0.64; 95% CI: 0.47-0.88).[84] There is still no clear evidence whether the use of antibiotics is effective in the prevention of preterm birth in this subgroup.

3.3.2.2. Progesterone. The preventive effect of progesterone in the reduction of spontaneous preterm birth in women with a history of spontaneous preterm birth has been thoroughly investigated. Dodd et al. performed a meta-analysis including 11 studies encompassing 1899 singletons with a prior spontaneous preterm birth to assess the benefits of progesterone administration for the prevention of preterm birth. There was a significant reduction in spontaneous preterm birth before 34 weeks (RR: 0.31; 95% CI: 0.14-0.69) and of perinatal mortality (RR: 0.50; 95% CI: 0.33-0.75) in the progesterone group. There is no strong evidence for a difference in effectiveness between the different routes of administration of progesterone; therefore, it is recommended to offer women with a prior spontaneous preterm birth either vaginal progesterone (gel capsules 200 mg daily of vaginal gel 90 mg daily) or 17α-hydroxyprogesterone caproate intramuscular (250 mg weekly) starting between 16 and 24 weeks of gestation, until 36 (intramuscular) or 37 (vaginal) weeks of gestation.[51,73]

3.3.2.3. Cerclage: history indicated. Primary cerclage, also elective cerclage, is considered to be effective in the prevention of preterm birth in women with a cervical insufficiency. Cervical insufficiency is characterized by progressive shortening and dilatation of the cervix before 24 weeks of gestation without signs of preterm labour, and is associated with mid-trimester pregnancy loss. However, due to the lack of objective findings and clear criteria, the clinical diagnosis of cervical insufficiency remains challenging.

Primary cerclages have been studied in several RCTs and meta-analyses. The first RCT from 1984 included 194 women with a singleton pregnancy and high risk of preterm birth, and showed no benefit of cervical cerclage compared to conservative treatment in the reduction of preterm birth, neonatal morbidity, and neonatal mortality.[85] Similar results were found in another RCT including 506 women; however, this study included women at moderate risk for preterm birth and excluded women at high risk.[86] The largest trial was performed with 1292 women with singleton pregnancies published in 2003, which showed a significant reduction in preterm birth before 33 weeks of gestation (13% versus 17%; P=0.03). An increased incidence of postpartum fever in the cerclage cohort was found in this study.[87] In addition, a meta-analysis from 2003 demonstrated that an elective cervical cerclage had a significant effect in preventing spontaneous preterm birth before 34 weeks of gestation, yet the authors recommended further research with a focus on the identification of women who would benefit most from cerclage.[88] Based on current, yet limited, clinical information, an elective history-indicated cerclage should be

limited to patients with a history of one or more unexplained second-trimester deliveries in the absence of painful cervical dilation or labour.[64] However, the indication for a history-indicated cerclage may vary between, and even within, countries worldwide.

3.3.2.4. Short cervix

Cerclage: ultrasound-indicated. The effectiveness of cervical cerclage in women with a high risk of spontaneous preterm birth based on their history of previous spontaneous preterm birth and mid-pregnancy short cervix, an ultrasound-indicated cerclage, has been studied in a number of trials. A meta-analysis from 2011 included 504 women with a prior preterm birth and short cervix (<25 mm) receiving a cerclage. The authors observed a reduction in both preterm birth (before 37 weeks of gestation RR: 0.70, 95% CI 0.58-0.83; 35 weeks: 0.70, 055-0.89; 32 weeks: 0.66, 0.48-0.91; 28 weeks: 0.64, 0.43-0.96) and in composite perinatal mortality and morbidity (0.64, 0.45-0.91).[89] A Cochrane review of 2012 also concluded that cerclage is associated with a reduction in preterm birth before 37 weeks of gestation (RR: 0.80; 95% CI: 0.69-0.95), before 34 weeks (0.79; 0.68-0.93) and before 28 weeks (0.80; 0.64-1.00). Yet, no significant effect on perinatal death nor on composite outcome of perinatal mortality and morbidity was reported in this review.[90] Szychowski et al. assessed the optimal cervical length for placing an ultrasound-indicated cerclage and concluded that cerclage is beneficial in women with shortened cervical length <25 mm when placed between 16 and 24 weeks of gestation.[91]

Pessary. When the pessary was first described in 1959, it was used in women with habitual abortions and possible cervical incompetence. In addition, the PECEP study from 2012 included 11% of women with at least one prior preterm birth. This study compared expectant management with pessary treatment in women with a short cervix, showing a significant decrease in preterm birth in the intervention (pessary) group; however, no subgroup analysis was performed for women with a previous preterm birth.[68] There are currently no recent large studies available with information on the effectiveness of a pessary in women with a previous preterm birth. There are ongoing RCTs evaluating the effect of a cervical pessary in women at risk of preterm birth based on their obstetric history.

Practice points

- Identification of risk factors early in pregnancy is an essential component of clinical obstetric care, since early interventions may be effective to reduce the risk of preterm birth. Preconceptional counselling regarding these factors may further reduce the risk of preterm birth.

- Differentiation between low risk and high risk pregnancies is important to assess the best strategy of preventing preterm birth (**Table 1**).
- In low risk singleton women without a history of preterm birth, cervical length measurements may be of value to identify women at risk for preterm birth; however, the number needed to screen is relatively high. When a mid-trimester measurement of the cervix of ≤25 mm is detected, women can be offered treatment with either vaginal progesterone 200 mg or a cervical pessary (see also **Figure 1**).
- In multiple pregnancies, cervical length measurement may be of value to identify women at higher risk for preterm birth. Both vaginal progesterone and a cervical pessary may be beneficial to reduce the risk of preterm birth in twin pregnancies with a mid-trimester short cervical length; however, optimal timing of intervention should be investigated (see also **Figure 1**).
- Women at high risk for a preterm birth, i.e. women with one or more preterm births in their history, should be offered routine progesterone starting at 16 weeks of gestation until 36 weeks. In addition, serial cervical length screening is indicated between 16 and 24 weeks of gestation. In case of a cervix <25 mm, ultrasound-indicated cerclage is recommended. The pessary is being evaluated in this subgroup of women. In women with cervical insufficiency, i.e. women with one or more mid-pregnancy deliveries in the absence of signs of labor, a history-indicated cerclage might be considered (see also **Figure 1**).

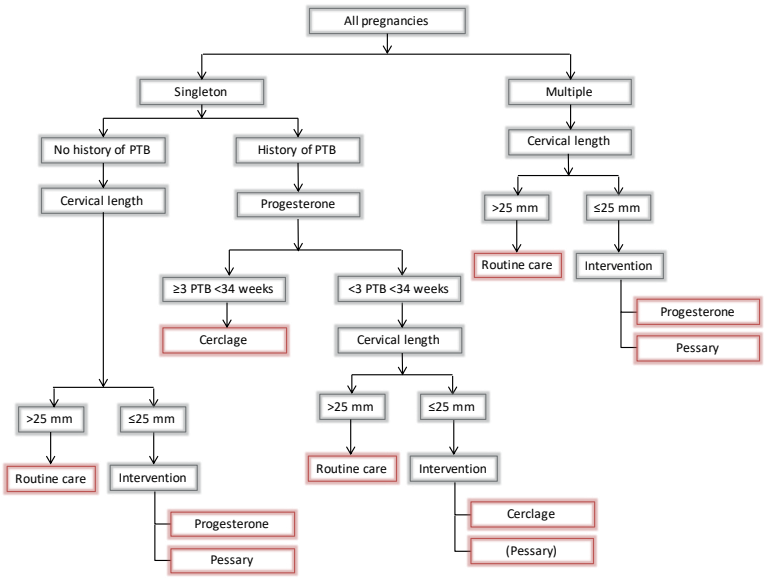

FIGURE 1. Algorithm for all pregnancies as a tool to identify possible interventions to prevent preterm birth (PTB).

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PART I

RISK ASSESSMENT OF SPONTANEOUS PRETERM BIRTH

CHAPTER 2

The effect of interpregnancy interval on the recurrence rate of spontaneous preterm birth: a retrospective cohort study

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American Journal of Perinatology. 2017 Jan;34(2):174-182

ABSTRACT

Objective: We assessed, in women with a previous spontaneous preterm birth, the effect of interpregnancy interval on the subsequent preterm birth rate.

Design: Retrospective cohort study.

Setting: A nationwide longitudinal dataset of the Netherlands Perinatal Registry.

Population: Women with three sequential singleton pregnancies between 1999 and 2009 and a spontaneous preterm birth <37 weeks in the first pregnancy.

Methods: We evaluated the impact of interpregnancy interval on the course of the next pregnancies. Antenatal death and/or congenital abnormalities were excluded. Conventional and conditional logistic regression analysis were applied. We adjusted for maternal age, ethnicity, socioeconomic status, artificial reproductive techniques, and year of birth. Main Outcome Measures Outcomes studied were preterm birth <37 weeks, <32 weeks, low birth weight <2500 g, and small for gestational age <10th percentile.

Results: Among 2,361 women with preterm birth in the first pregnancy, logistic regression analysis indicated a significant effect of a short interpregnancy interval (0–5 mo) on recurrent preterm birth <37 weeks (odds ratio [OR], 2.22; 95% confidence interval [CI], 1.62–3.05), <32 weeks (OR, 2.90; 95% CI, 1.43–5.87), and low birth weight (OR, 2.69; 95% CI, 1.79–4.03). In addition, a long interval (≥60 mo) had a significant effect on preterm birth <37 weeks (OR, 2.19; 95% CI, 1.29–3.74). Conditional logistic regression analysis confirmed the effect of a short interval on the recurrence of preterm birth rate <37 weeks and low birth weight.

Conclusion: In women with a previous spontaneous preterm birth, a short interpregnancy interval has a strong impact on the risk of preterm birth before 37 weeks and low birth weight in the next pregnancy, irrespective of the type of analysis performed.

INTRODUCTION

Preterm birth, defined as birth before 37 weeks of gestation, is the most common cause of perinatal mortality and neonatal morbidity in developed countries,[1] mostly due to respiratory immaturity, intracranial hemorrhages, and infections. These conditions can result in long-term neurodevelopmental sequelae such as intellectual impairment, cerebral palsy, chronic lung disease, deafness, and blindness.[2] The incidence of preterm birth in the Netherlands is 7.7% of all pregnancies and 1.3% occur before 32 weeks. The rate of spontaneous preterm birth in singleton pregnancies is 5.4%.[3]

Risk factors for spontaneous preterm birth are various and include black ethnicity, low maternal body mass index, and low socioeconomic status. It is also known that preterm birth is associated with an increased risk of preterm birth in a subsequent pregnancy.[4,5] In addition, previous studies indicate that either a short or a long interval between pregnancies is associated with adverse perinatal outcomes, including preterm birth, low birth weight, and small for gestational age (SGA). This suggests that there is an optimal interval between pregnancies and that spacing pregnancies appropriately could help to prevent these adverse perinatal outcomes.[6–8] The World Health Organization recommends a minimum interpregnancy interval of 2 years based on the available information and evidence.[9]

However, whether this association is confounded by other risk factors, including various aspects of socioeconomic status, ethnicity, demographics, and lifestyle, is unclear.[7] Several authors propose that the relation between interpregnancy interval and perinatal outcome is entirely due to these confounders, that is, other (maternal) factors which are correlated with interpregnancy interval and the perinatal outcome in subsequent pregnancy.[10,11] Recently, Ball et al re-evaluated the link between interpregnancy interval and adverse birth outcome. They stated that previous analyses based on between mother-comparison may have inadequately adjusted for such unknown confounders. Ball et al subsequently analyzed the interpregnancy interval using a matched model, in which each mother was used as her own control for risk factors, to adjust completely for persistent maternal factors. While conventional logistic regression analysis in their data showed strong relations between a short interpregnancy interval and adverse outcome in a subsequent pregnancy, only small effects of a short interpregnancy interval on the total preterm birth rate <37 weeks, SGA, and low birth weight remained when they used conditional logistic regression. Based on these results, the authors concluded that the impact of short interpregnancy interval and adverse outcome of the subsequent pregnancy is minimal.[11]

It is of high importance to determine whether there is an independent association between extreme interpregnancy intervals and adverse neonatal outcome. This information could reasonably be provided to counsel women about birth spacing, particularly in those women with a previous preterm birth, which makes this issue relevant to public health and clinical practice.

The aim of the present study was to evaluate the effect of the time to conception of the next pregnancy on the preterm birth rate in the subsequent pregnancy in women who suffered spontaneous preterm birth. We explored this issue by using both conventional and conditional logistic regression analyses, as suggested recently by Ball et al, in a cohort of women who have had three births.

METHODS

Dataset

This study was based on data from the Netherlands Perinatal Registry (PRN). The PRN consists of population-based data that contain information on all deliveries ≥22 weeks of gestation and readmissions until 28 days after birth in the Netherlands. The PRN database is obtained by a validated linkage of three different registries: the midwifery registry, the obstetrics registry, and the neonatology registry of hospital admissions of newborn infants.[12,13] The coverage of the PRN registry is ~96% of all deliveries in the Netherlands.

Longitudinal linkage

A probabilistic linkage procedure was performed in which records of children born between January 1, 1999 and December 31, 2009 of the same mother were linked. All children born from their mothers' second with the first (391,026) and the third with the second pregnancies (61,664) in the PRN registry were linked. The linkage was based on the following variables: day, month, and year of the mother's birthday; day, month, and year of the (previous) child's birthday; and the postal code of the mother (four digits). The record linkage was performed with a "two-stage" procedure. In the first stage, the dataset with first-born children was linked to the dataset containing the second-born children. The method to link these two records is described in more detail by Schaaf et al.[14,15] A temporary dataset was made from all record pairs with a posterior probability >0.80 of belonging to the same mother. In the second stage of the procedure, the temporary dataset was linked to the dataset containing the third-born children. To determine whether a mother with two children also gave birth to a third child, we compared the

linkage variables of her second child to the linkage variables of the children in the dataset containing the third-born children. We performed the same linkage procedure as in the first stage. All combinations of mothers with two children and a third-born child with a probability >0.80 were used to create the dataset which was used for the analysis. The final linked longitudinal cohort with complete data on first, second, and third deliveries of the same mother consisted of 61,664 women and 184,992 (3 x 61,664) deliveries.

Ethical approval

The data in the perinatal registry are anonymous, and therefore, ethical approval was not needed. The Netherlands Perinatal Registry gave their approval for the use of their data for this study (approval no. 14.11).

Inclusion and exclusion criteria

From this longitudinal database, we selected women with three sequential singleton pregnancies. Women whose first, second and/or third pregnancy was complicated by congenital abnormalities, antepartum fetal mortality, birth after 44 weeks, or a primary caesarean section were excluded. Next, we selected only women with a spontaneous preterm birth before 37 weeks in the first pregnancy. Whether a delivery commences as spontaneous (i.e., with spontaneous rupture of the membranes or contractions), or as iatrogenic (i.e., primary caesarean section or induction), is a required field within the PRN dataset, which makes it possible to distinguish between spontaneous and nonspontaneous births. The final database thus contained data of women who had three pregnancies, a first, a second, and a third, amongst whom the first pregnancy had ended spontaneously before 37 weeks. These inclusion and exclusion criteria are collected in the PRN and are thereby identifiable from the database.

Outcome measures

Our primary outcome measure was preterm birth before 37 weeks in the second and third pregnancy. Additional outcomes studied were preterm birth before 32 weeks, birth weight less than 2,500 g, and SGA less than the 10th percentile. Interpregnancy interval was documented as the time interval between delivery of the first pregnancy and the conception of the subsequent pregnancy (delivery date minus gestational age at birth). Interpregnancy interval was divided in categorical variables, classed as 0 to 5 months, 6 to 11 months, 12 to 17 months, 18 to 23 months (the reference group), 24 to 59 months, and 60 months or longer.

Statistics

Women with a spontaneous preterm birth before 37 weeks in the first pregnancy were selected. In this group, we compared the recurrence rate of preterm birth before 37 and 32 weeks, the incidence of low birth weight (less than 2,500 g) and SGA (less than the 10th percentile) in the second and third pregnancy between women with different interpregnancy intervals. These intervals were subdivided into six categories based on widely used intervals in literature. To estimate the effect of the different interpregnancy intervals on the outcomes in the second and third pregnancy, univariate logistic regression modelling was used, and the results were expressed as odds ratios (OR) with 95% confidence interval (CI). We used multivariate logistic regression analysis to adjust for low maternal age (<25 y) and high maternal age (>35 y), non-White ethnicity, low socioeconomic status, artificial reproductive techniques, and year of birth. The information about these factors is combined by using the variables of the first pregnancy for the interval between first and second pregnancy, and the variables of the second pregnancy for the interval between second and third pregnancy.

In addition, to correct for all possible maternal confounders, we used univariate and multivariate conditional logistic regression analysis to measure the effect of interpregnancy interval in the same group of women.[11] Using this analysis we were able to perform an individual analysis for each mother. We adjusted for the same risk factors in the same way as described previously. The probabilistic longitudinal linkage procedure was performed with the R statistical software environment (version 2.13.1; R Foundation for Statistical Computing, Vienna, Austria), and the data were analyzed with the SAS statistical software package (version 9.3; SAS Institute Inc, Cary, NC).

RESULTS

There were 61,664 women identified as having three sequential deliveries between 1999 and 2009. Women, who in their first, second, or third pregnancy had a twin pregnancy (1,907=3.1%), gestational age of 44 weeks or more (25=0.04%), antepartum fetal mortality (240=0.4%), or congenital abnormalities (1,284=2.1%), were excluded. After selecting women with a spontaneous preterm birth before 37 weeks in the first pregnancy and excluding women with a negative pregnancy interval (37=0.06%), 2,361 women with complete follow-up data remained (**Figure 1**). Baseline characteristics of our cohort are presented in **Table 1** (second births) and **Table 2** (third births). The prevalence of adverse outcomes in the women with spontaneous preterm birth in first pregnancy were higher in the second than in the third pregnancy.

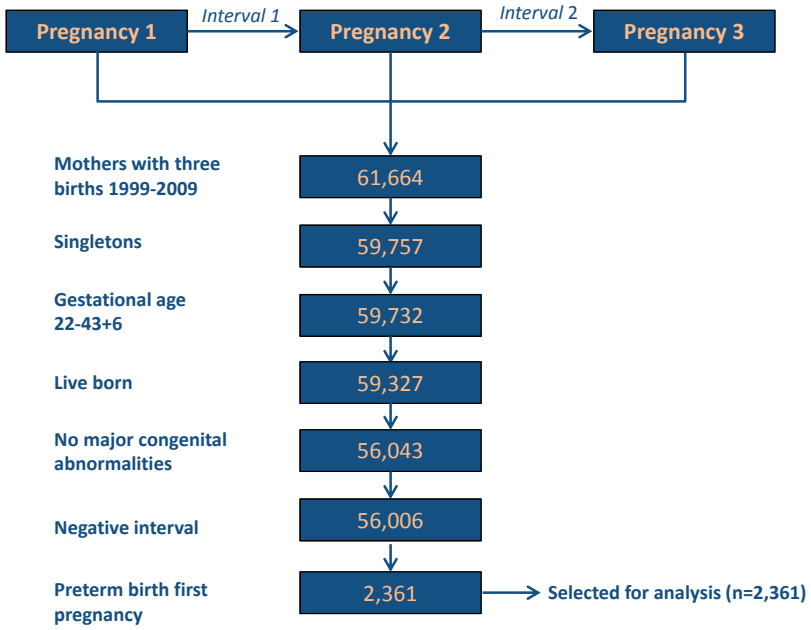

FIGURE 1. The selection of records used in this study.

Conventional logistic regression analysis

Using the interval of 18 to 23 months as the reference, logistic regression analysis showed a strong effect of a short interpregnancy interval (0–5 mo) on the rate of preterm birth before 37 weeks (OR, 2.22; 95% CI, 1.62–3.05), rate of very preterm birth before 32 weeks (OR, 2.90; 95% CI, 1.43–5.87), and low birth weight (OR, 2.69; 95% CI, 1.79–4.03) after adjusting for confounders. There was an insignificant effect on SGA (OR, 1.39; 95% CI, 0.92–2.12). In addition, a long interval (≥60 mo) showed a significant effect on preterm birth before 37 weeks (OR, 2.19; 95% CI, 1.29–3.74), with no significant effect on the incidence of preterm birth before 32 weeks (OR, 0.60; 95% CI, 0.08–4.72), low birth weight (OR, 1.83; 95% CI, 0.87–3.84), and SGA (OR, 1.45; 95% CI, 0.72–2.93) (**Table 3**).

Conditional logistic regression analysis

When conditional logistic regression analysis was used, the significant effect of a short interpregnancy interval (0–5mo) on the preterm birth rate before 37 weeks (OR, 1.90; 95% CI, 1.03–3.50) and low birth weight (OR, 2.58; 95% CI, 1.24–5.36) persisted after adjusting for confounders. However, no significant effect on preterm birth before 32 weeks (OR, 2.75; 95% CI, 0.64–11.77) and SGA (OR, 0.96; 95% CI, 0.47–1.95) was seen. Large interpregnancy intervals showed no significant effect on any of the outcomes (**Table 3**).

TABLE 1. Characteristics of second births

Second births					
Characteristics	PTB < 37 weeks (%)	PTB < 32 weeks (%)	LBW (%)	SGA (%)	Total
Total	419 (17.8)	62 (2.6)	237 (10.0)	215 (9.1)	2361
Interpregnancy interval (months)					
0–5	79 (26.4)	16 (5.4)	53 (17.7)	33 (11.0)	299
6–12	105 (17.1)	14 (2.3)	59 (9.6)	54 (8.8)	614
12–17	74 (13.2)	8 (1.4)	32 (5.7)	49 (8.7)	562
18–23 (ref)	54 (15.3)	8 (2.3)	28 (7.9)	27 (7.6)	354
24–59	98 (19.4)	16 (3.2)	61 (12.1)	46 (9.1)	505
2–60	9 (33.3)	0 (0.0)	4 (14.8)	6 (22.2)	27
Maternal age (years)					
< 25	115 (22.1)	18 (3.5)	76 (14.6)	63 (12.1)	521
20–35 (ref)	298 (16.6)	42 (2.3)	154 (8.6)	147 (8.2)	1800
> 35	6 (15.0)	2 (5.0)	7 (17.5)	5 (12.5)	40
Ethnic origin					
White (ref)	359 (17.3)	53 (2.6)	200 (9.6)	173 (8.3)	2077
Non-White	60 (21.1)	9 (3.2)	37 (13.0)	42 (14.8)	284
Socio-economic status					
High/middle (ref)	311 (17.3)	41 (2.3)	169 (9.4)	147 (8.2)	1802
Low	108 (19.3)	21 (3.8)	68 (2.9)	68 (12.2)	559
Year of birth					
1999	67 (16.4)	9 (2.2)	43 (10.5)	33 (8.1)	408
2000	73 (16.5)	10 (2.3)	49 (11.1)	41 (9.3)	443
2001	77 (19.2)	14 (3.5)	35 (8.7)	29 (7.2)	401
2002	61 (17.4)	7 (2.0)	29 (8.3)	33 (9.4)	350
2003 (ref)	59 (19.1)	6 (1.9)	26 (8.4)	36 (11.7)	309
2004	38 (15.8)	8 (3.3)	28 (11.7)	22 (9.2)	240
2005	31 (19.6)	5 (3.2)	18 (11.4)	18 (11.4)	158
2006	10 (22.7)	3 (6.8)	6 (13.6)	2 (4.6)	44
2007	3 (37.5)	0 (0.0)	3 (37.5)	1 (12.5)	8
ART					
No (ref)	320 (17.6)	47 (2.6)	183 (10.0)	164 (9.0)	1822
Yes	99 (18.4)	15 (2.8)	54 (10.0)	51 (9.5)	539

Abbreviations: *ART, artificial reproductive techniques; LBW, low birth weight; PTB, preterm birth; ref, reference; SGA, small for gestational age*

TABLE 2. Characteristics of third births

Third births					
Characteristics	PTB < 37 weeks (%)	PTB < 32 weeks (%)	LBW (%)	SGA (%)	Total
Total	274 (11.6%)	34 (1.4)	160 (6.8)	194 (8.2)	2361
Interpregnancy interval (months)					
0–5	26 (19.6)	6 (4.5)	15 (11.3)	12 (9.0)	133
6–11	52 (13.0)	7 (1.8)	26 (6.5)	41 (10.2)	401
12–17	37 (8.2)	2 (0.4)	25 (5.5)	31 (6.9)	452
18–23 (ref)	36 (8.7)	5 (1.2)	16 (3.9)	27 (6.5)	415
24–59	109 (12.2)	13 (1.5)	72 (8.1)	78 (8.7)	894
2–60	14 (21.2)	1 (1.5)	6 (9.1)	5 (7.6)	66
Maternal age (years)					
< 25	46 (17.6)	6 (2.3)	35 (13.4)	33 (12.6)	262
20–35 (ref)	214 (10.9)	24 (1.2)	110 (5.6)	150 (7.6)	1968
> 35	14 (10.7)	4 (3.1)	15 (11.5)	11 (8.4)	131
Ethnic origin					
White (ref)	216 (10.5)	27 (1.3)	122 (5.9)	158 (7.7)	2056
Non-White	58 (19.0)	7 (2.3)	38 (12.5)	36 (11.8)	305
Socio-economic status					
High/middle (ref)	198 (10.9)	24 (1.3)	116 (6.4)	142 (7.8)	1823
Low	76 (14.1)	10 (1.9)	44 (8.2)	52 (9.7)	538
Year of birth					
1999	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2
2000	13 (12.3)	4 (3.8)	8 (7.6)	9 (8.5)	106
2001	29 (11.2)	1 (0.4)	10 (3.9)	20 (7.8)	258
2002	33 (9.7)	2 (0.6)	18 (5.3)	30 (8.8)	342
2003 (ref)	42 (9.7)	6 (1.4)	25 (5.8)	29 (6.7)	431
2004	42 (11.8)	8 (2.3)	23 (6.5)	28 (7.9)	356
2005	41 (12.5)	6 (1.8)	32 (9.7)	32 (9.7)	329
2006	41 (12.9)	3 (0.9)	26 (8.2)	30 (9.4)	318
2007	25 (13.9)	4 (2.2)	15 (8.3)	13 (7.2)	180
2008	8 (20.5)	0 (0.0)	3 (7.7)	3 (7.7)	39
ART					
No (ref)	203 (11.3)	22 (1.2)	118 (6.6)	147 (8.2)	1799
Yes	71 (12.6)	12 (2.1)	42 (7.5)	47 (8.4)	562

Abbreviations: *ART, artificial reproductive techniques; LBW, low birth weight; PTB, preterm birth; ref, reference; SGA, small for gestational age*

TABLE 3. Relation between interpregnancy interval (in months) and adverse neonatal outcomes

Interpregnancy interval (months)	Logistic regression analysis		Conditional regression analysis	
	Unadjusted	Adjusted	Unadjusted	Adjusted
PTB < 37 weeks				
0–5	2.42 (1.78–3.31)	2.22 (1.62–3.05)	1.86 (1.05–3.28)	1.90 (1.03–3.50)
6–11	1.38 (1.05–1.82)	1.35 (1.02–1.78)	1.03 (0.65–1.64)	1.14 (0.69–1.88)
12–17	0.93 (0.69–1.25)	0.93 (0.69–1.25)	0.90 (0.55–1.45)	0.89 (0.53–1.49)
18–23 (ref)	1	1	1	1
24–59	1.31 (1.01–1.71)	1.29 (0.98–1.68)	0.71 (0.46–1.10)	0.74 (0.45–1.20)
≥60	2.48 (1.47–4.17)	2.19 (1.29–3.74)	1.50 (0.61–3.72)	1.17 (0.41–3.31)
PTB < 32 weeks				
0–5	3.12 (1.56–6.26)	2.90 (1.43–5.87)	1.00 (0.33–3.01)	2.75 (0.64–11.77)
6–11	1.23 (0.61–2.47)	1.22 (0.61–2.46)	0.69 (0.23–2.09)	1.55 (0.39–6.24)
12–17	0.58 (0.25–1.33)	0.57 (0.25–1.31)	0.62 (0.19–2.11)	0.74 (0.17–3.19)
18–23 (ref)	1	1	1	1
24–59	1.23 (0.64–2.38)	1.21 (0.62–2.36)	0.88 (0.30–2.52)	1.23 (0.34–4.48)
≥60	0.63 (0.08–4.89)	0.60 (0.08–4.72)	0.39 (0.03–5.29)	0.67 (0.03–17.67)
LBW < 2,500 grams				
0–5	3.08 (2.06–4.59)	2.69 (1.79–4.03)	1.97 (1.02–3.84)	2.58 (1.24–5.36)
6–11	1.51 (1.03–2.20)	1.44 (0.98–2.10)	1.28 (0.70–2.33)	1.38 (0.72–2.68)
12–17	0.98 (0.66–1.47)	0.96 (0.64–1.45)	1.32 (0.67–2.56)	1.60 (0.78–3.30)
18–23 (ref)	1	1	1	1
24–59	1.73 (1.22–2.25)	1.72 (1.20–2.46)	1.23 (0.69–2.20)	1.48 (0.78–2.81)
≥60	1.99 (0.96–4.09)	1.83 (0.87–3.84)	0.75 (0.27–2.09)	0.79 (0.24–2.64)
SGA < p10				
0–5	1.54 (1.02–2.33)	1.39 (0.92–2.12)	0.91 (0.47–1.78)	0.96 (0.47–1.95)
6–11	1.37 (0.97–1.94)	1.33 (0.94–1.89)	1.47 (0.86–2.52)	1.53 (0.86–2.72)
12–17	1.13 (0.79–1.62)	1.14 (0.80–1.63)	0.97 (0.54–1.72)	0.98 (0.53–1.79)
18–23 (ref)	1	1	1	1
24–59	1.29 (0.92–1.80)	1.20 (0.86–1.68)	1.05 (0.61–1.79)	0.95 (0.54–1.69)
≥60	1.78 (0.89–3.53)	1.45 (0.72–2.93)	0.59 (0.19–1.85)	0.41 (0.12–1.43)

Abbreviations: *LBW, low birth weight; PTB, preterm birth; ref, reference.* **Note:** *SGA < p10 = small for gestational age, less than the 10th percentile. Values are odds ratios (OR; 95% confidence intervals)*

DISCUSSION

Main findings

We assessed the effect of interpregnancy interval on adverse neonatal outcomes in the subsequent second and third pregnancy of a subgroup of women with three sequential deliveries, after spontaneous preterm birth before 37 weeks in the first pregnancy. After controlling for confounders, we found that a short interpregnancy interval is associated with a higher risk of preterm birth before 37 weeks, very preterm birth before 32 weeks, and low birth weight. However, after controlling for possible confounders using conditional logistic regression analysis, no effect on preterm birth before 32 weeks was seen, whereas the effect of a short interpregnancy interval on both preterm birth before 37 weeks and low birth weight persisted. This effect was, however, less prominent compared with the results of the conventional method of analysis. For the large intervals, conventional logistic regression analysis revealed an association with preterm birth before 37 weeks, however, this did not persist after applying the conditional method of analysis.

Strengths and limitations

There are several potential strengths and limitations in our study. To perform a conditional analysis, we based our outcomes of second and third births on mothers delivering their first three singleton births as live infants at the start of labor. We could not include women with more or less than two consecutive pregnancies after the first (85% of the population in the longitudinal dataset had only two consecutive pregnancies), who might differ in sociodemographic and medical characteristics from women with three births. In addition, interpregnancy intervals were calculated only between pregnancies that ended at a gestational age of 22 or more weeks. Pregnancies ended before 22 weeks, including miscarriages before 16 weeks and spontaneous immature births between 16 and 22 weeks, were not included in our database. This may distort the interpregnancy interval values in some cases.

To perform our analysis, a probabilistic linkage method to follow up mothers with three sequential births was used. A risk of using data from a record linkage procedure is the presence of nonlinkage, which could be due to missing values for the linkage variables, or the presence of false matches, caused by the partially identifying nature of the linkage variables. Missing values mainly result from the fact that the first child was born before the start of the PRN registry in 1999, or after the period we included in our linkage procedure, that is, 2009. The postal code of the mother was one of the linkage variables, thus, changes of home address over time will lead to nonlinkage. However, we found that the longitudinally-linked dataset was comparable with the national pregnancy

characteristics for our main outcomes, so we do not think that nonlinked pregnancies have influenced our results to a large degree. Furthermore, false matches in linked data could distort the dataset. However, we used a high probability to define a linking match and therefore we expect that the false matching rate in this cohort is rather low.

A major strength of our study is that we were able to use a conditional logistic regression approach to analyze the data, which allowed adjustment for possible unknown risk factors for adverse outcomes. In addition, we performed classic logistic regression analysis on the same population, thus mimicking the many previous studies of interpregnancy intervals and neonatal outcomes.

Interpretation

To our knowledge, this is the first study that has analyzed the effect of interpregnancy interval in this subgroup of woman with a previous spontaneous preterm birth on the recurrence rate of preterm birth and other adverse neonatal outcomes in a conditional model. Most previous studies assessed the effect of interpregnancy interval on a general population, while we selected women who were at high risk of preterm birth since they already delivered a spontaneous preterm infant. DeFranco et al assessed the effect of a short interpregnancy interval on the recurrence rate of preterm birth and suggested that a short interpregnancy interval is a risk for the recurrence of preterm birth,[16] which is confirmed in our study.

The conditional logistic regression analysis allowed us to analyze several interpregnancy intervals for the same mother, rather than comparing intervals between mothers, therefore better controlling for possible unmeasured and unknown mother specific covariates. Ball and colleagues were the first to apply a within-mother method of analysis, in which mothers essentially act as their own controls, to examine the relationship between interpregnancy interval and adverse neonatal outcome including total preterm birth.[11] In their study, they show that short intervals (less than 18 mo) are not associated with adverse neonatal outcomes. However, an association between a long interpregnancy interval (more than 59 mo) and a higher incidence of SGA and low birth weight was evident, which supports earlier findings linking large interpregnancy intervals with poor neonatal outcomes.[17] Our results do not support this association when applying the conditional method of analysis. Although we could not confirm the relationship between a large interpregnancy interval and SGA, a large interval may have an adverse effect on maternal health,[18] and risk of antenatal deaths or early neonatal death.[19]

Furthermore, an association between short interpregnancy interval and an increased incidence of poor neonatal outcome has been supported by strong and consistent

findings over many years and in a wide range of countries.[6,7,16,17] Although our study confirms the relationship between a short interpregnancy interval and increased risk of preterm birth before 37 weeks and low birth weight, an effect on preterm birth before 32 weeks and SGA was not supported by our conditional logistic regression analysis model. This might be due to the small number of women having a preterm birth before 32 weeks in their second and/or third pregnancy in our dataset. We performed additional analyses in which we divided the interpregnancy interval in quartiles (25, 50, and 75%) to create larger groups in each interval. Using the interval of the second quartile (25–50%) as the reference, these results showed a significant effect for preterm birth before 32 weeks after a short interval (<25%) when conditional logistic regression analysis was applied (OR, 3.8; 95% CI, 1.2–12.3). This supports the theory of a small number of women in the shortest interpregnancy interval (0–5mo) used in our main analysis. Another explanation might be that preterm birth before 32 weeks depends on different risk factors compared with preterm birth before 37 weeks.

In comparison to our study, earlier studies may have inadequately adjusted for possible confounders as different interpregnancy intervals were compared between mothers, as proposed by Ball et al. However, in contrast to the latter study, we have shown persistent effects of a short interpregnancy interval on preterm birth before 37 weeks and low birth weight. Nevertheless, these effects were found in a subgroup of women with a previous spontaneous preterm birth before 37 weeks where Ball et al studied all births in Australia during their study period.

The biological mechanisms linking interval between births and incidence of preterm labor are unknown and are challenging to investigate. Additionally, the causes and mechanisms leading to preterm labor remain elusive.[20] Since both sterile and infectious stimuli can activate the inflammation that leads to preterm labor,[20] and the quality of placental development and function may also contribute,[21,22] the maternal immune response and physical structure and function of the uterus may be two contributing factors. It seems reasonable to assume that the biological functions of the uterus take some time to return to homeostasis after birth, and that conception before complete recovery from recent birth may interfere with optimal future placental development and capacity to sustain a following pregnancy. Additionally, it might be speculated that since the maternal immune response is affected by previous pregnancies,[23] particularly the adaptive immune compartment that generates T regulatory cells, immune tolerance may be compromised when conception occurs in the phase following recent parturition during which maternal immunity re-equilibrates.[24] Since an active maternal immune response is essential to protect from infection and gestational disorders stemming from placental incompetence[25] including preterm labor,[26] appropriate recovery of endometrial

immune activity following previous pregnancy may be necessary. In rodent model systems, the strength of suppressive capability in the regulatory T cell pool is regulated by exposures to paternal seminal fluid before and between pregnancies, as well as gestational antigens,[25] and varies according to memory of any previous pregnancy,[23] so coital activity between pregnancies could also be a factor. Preterm birth may alter the kinetics by which uterine structure and immune function return to normal status in the postpartum phase, but whether and how this occurs requires further investigation.

CONCLUSION

Both conventional and conditional analyses demonstrated an increased risk of preterm birth before 37 weeks and low birth weight after a short interpregnancy interval in a subgroup of women with a previous spontaneous preterm birth before 37 weeks. This increased risk was found after controlling for known and possible unknown confounders. Despite the fact that the impact of interpregnancy interval in an unselected population has been called into question by Ball et al, we found, in a subgroup of women with a history of a spontaneous preterm birth before 37 weeks, a significant effect on pregnancy and neonatal outcomes when applying the controlled method suggested by Ball et al.

Our results may assist clinicians in the counseling of women with a history of spontaneous preterm delivery. We recommend these women to consider an interval of at least 12 months before conception of the next pregnancy. To investigate the impact of interpregnancy interval in a variety of populations, we encourage the use of conditional methods of analysis.

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CHAPTER 3

The association between parity and spontaneous preterm birth: a population based study

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BMC Pregnancy and Childbirth. 2020 Apr 21;20(1):233

ABSTRACT

Background: Preterm birth is the leading cause of perinatal mortality and neonatal morbidity worldwide. Many factors have been associated with preterm birth, including parity. The aim of the present study was to investigate associations between parity and risk of spontaneous preterm birth.

Methods: We conducted a retrospective study including live singleton births (≥22 weeks) of women with a first, second, third, fourth or fifth pregnancy in The Netherlands from 2010 through 2014. Our primary outcome was risk of spontaneous preterm birth < 37 weeks. Secondary outcomes were spontaneous preterm birth < 32 and < 28 weeks.

Results: We studied 802,119 pregnancies, including 30,237 pregnancies that ended spontaneously < 37 weeks. We identified an increased risk for spontaneous preterm birth < 37 weeks in nulliparous women (OR 1.95, 95% CI 1.89–2.00) and women in their fifth pregnancy (OR 1.26, 95% CI 1.13–1.41) compared to women in their second pregnancy. Similar results were seen for spontaneous preterm birth < 32 and < 28 weeks.

Conclusion: Our data show an independent association between nulliparity and spontaneous preterm birth < 37, < 32 and < 28 weeks. Furthermore, we observed an increased risk for spontaneous preterm birth in women in their fifth pregnancy, with highest risk for preterm birth at early gestational age.

INTRODUCTION

Preterm birth, defined as birth before 37 weeks of gestation, is the leading cause of perinatal mortality and neonatal morbidity worldwide, mostly due to respiratory immaturity, intracranial hemorrhages and infections.[1,2] Morbidity and mortality rates increase with decreasing gestational age.[3] Fifteen million children are born preterm worldwide each year, of which almost two and a half million children are born before 32 weeks of gestation.[4]

Preterm birth is considered a syndrome that can be initiated by multiple mechanisms such as intrauterine infection and inflammation, uteroplacental ischemia and hemorrhage, uterine overdistension, cervical insufficiency, hormonal disorders, and other immunologically mediated processes.[5] Defining maternal risk factors for preterm birth in epidemiological studies can provide important insights into mechanisms that lead to preterm birth and help to identify women at risk. This can lead to the introduction of risk-specific treatment and counseling.[6]

There are many maternal characteristics that have been associated with preterm birth, including demographic characteristics (i.e. low socioeconomic status), low or high body-mass index (BMI), smoking and a previous preterm birth.[6,7] Parity is another factor associated with preterm birth, with the highest rates reported in nulliparous women and the lowest rates reported in second births.[8] Studies on the association between high parity and adverse pregnancy outcomes show conflicting results. A number of studies did report an association between high parity and adverse pregnancy outcomes.[9,10] In contrast, other studies state that, under satisfactory socioeconomic and health care conditions, high parity should not be considered as a risk factor for adverse pregnancy outcomes.[11] A systematic review from 2010 shows that grand multiparity and great grand multiparity were not associated with increased risk of preterm birth.[12]

The principal aim of the present study was to investigate associations between parity and risk of spontaneous preterm birth, assessing first, second, third, fourth and fifth pregnancies, using a large population-based study.

METHODS

Dataset

This study was based on data from the Netherlands Perinatal Registry (PERINED). This database is a population based registry that covers approximately 97% of all deliveries

in The Netherlands and contains information on deliveries at ≥22 weeks of gestation and birth weight of ≥500 g. Furthermore, all admissions to the neonatology care unit are registered until 28 days after birth. The perinatal database is obtained by a validated linkage of 3 different registries: the midwifery registry (LVR1), the obstetrics registry (LVR2), and the neonatology registry (LNR) of hospital admissions of newborn infants.[13,14] It is used primarily for an annual assessment of the quality indicators of obstetric care.

Ethical approval

The data in the perinatal registry are anonymous; therefore, ethical approval was not mandatory under Dutch law. The Netherlands Perinatal Registry gave their approval for the use of their data for this study (approval no. 17.34).

Inclusion and exclusion criteria

We studied singleton first, second, third, fourth and fifth pregnancies (P0 through P4) resulting in delivery between 22 and 43 weeks of gestation in the 5-year period from 2010 through 2014. We excluded multiple pregnancies and pregnancies that were complicated by congenital abnormalities or stillbirth.

Outcome measures

Our primary outcome was risk of spontaneous preterm birth < 37 weeks of gestation per parity. Other outcome variables were spontaneous preterm birth < 32 and < 28 weeks. We performed additional analyses for the outcome late spontaneous preterm birth between 34 and 37 weeks to assess pregnancies in women that were not offered additional screening or treatment to prevent recurrent preterm birth. The PERINED registry contains data on whether a delivery started spontaneous (i.e., with spontaneous rupture of the membranes or contractions) or iatrogenic (i.e., planned Caesarean section or induction of labor).

Statistical analysis

To estimate the effect of parity on spontaneous preterm birth < 37, < 32 and < 28 weeks (and between 34 and 37 weeks), we used a univariate logistic regression model and expressed the effect estimates as odds ratios (OR) and corresponding 95% confidence intervals (CI). We used multivariate logistic regression analysis to adjust for the most common known risk factors for preterm birth that were available in the national perinatal registry that we used for our study. The chosen variables were based on previous studies about risk factors for (spontaneous) preterm birth.[6,15,16] First, we adjusted for possible maternal confounders (**correction model A**) including maternal age (< 20 years, ≥40 years and continuous), non-White ethnicity, low socioeconomic status (SES), and, in

multiparous women, a prior preterm birth. Additional analyses were performed to adjust for potentially mediating factors occurring in the pathway between the independent (parity) and dependent (spontaneous preterm birth) variables (**correction model B**). Correction model B included the maternal confounders as in model A and in addition artificial reproductive techniques (ART), male fetal gender, hypertension, preeclampsia and small for gestational age (SGA) < p10. All variables were extracted from PERINED, including SES which was based on the 4-digit postal code of the woman's home address. SES was divided into low (< 25%), middle (25–75%) and high (> 75%) status. In multiparous women (P1 through P4), we used the Cochran-Armitage Trend Test to test for a trend in parity on the incidence of spontaneous preterm birth < 37, < 32 and < 28 weeks (and between 34 and 37 weeks). The data were analyzed with the SAS statistical software package (version 9.3; SAS Institute Inc., Cary, NC).

RESULTS

We identified 837,226 singleton pregnancies of women who delivered ≥22 weeks of gestation from 2010 through 2014. We excluded pregnancies complicated by stillbirth (3118, [0.37%]) or congenital abnormalities (25,444, [3.04%]). The total of first, second, third, fourth and fifth pregnancies (P0 through P4) with complete follow-up data was 802,119, of which 30,237 (3.8%) were spontaneous preterm births < 37 weeks of gestation. The proportion of pregnancies per parity was 45.8% (n = 367,676) in P0, 36.1% (n = 289,391) in P1, 13.1% (n = 105,014) in P2, 3.8% (n = 30,585) in P3 and 1.2% (n = 9453) in P4 (**Table 1**).

Maternal and pregnancy characteristics per parity

The proportion of pregnancies per parity plus the maternal and pregnancy characteristics of the parity groups are presented in **Table 1**. The mean maternal age increased with higher parity from 29.25 years in P0 to 35.03 years in P4 (p < .0001). The percentage of non-White ethnicity increased with higher parity, 17.3% non-White in P0 compared to 39.8% in P4 (p < .0001). Also, the percentage of women with a low SES increased with higher parity, 24.9% low-SES in P0 increasing to 34.0% in P4 (p < .0001). Hypertension and preeclampsia occurred more often in nulliparous women while these rates remained relatively stable in multiparous women (**Table 1**).

Preterm birth incidence by parity

The overall incidence of preterm birth < 37 weeks of gestation among singletons without congenital anomalies was 5.4% in The Netherlands during the 5-year study period. Rates of spontaneous and iatrogenic preterm birth < 37 weeks of gestation were 3.8 and

TABLE 1. Comparison of maternal and pregnancy characteristics and outcomes between the different parity groups.

	Total	P0	P1	P2	P3	P4	P-value*
Number of subjects (%)	802,119 (100)	367,676 (45.8)	289,391 (36.1)	105,014 (13.1)	30,585 (3.8)	9,453 (1.2)	NA
Mean GA in weeks (SD)	39.1 (1.8)	39.08 (2.0)	39.18 (1.6)	39.15 (1.7)	39.03 (1.8)	38.94 (2.0)	NA
Mean maternal age (SD)	30.8 (4.8)	29.25 (4.9)	31.53 (4.4)	32.98 (4.3)	34.03 (4.4)	35.03 (4.5)	NA
Maternal age <20 yrs (%)	10,201 (1.3)	9,230 (2.5)	902 (0.3)	61 (0.1)	7 (0.02)	1 (0.01)	<.0001
Maternal age ≥40 yrs (%)	24,797 (3.1)	6,891 (1.9)	8,505 (2.9)	5,310 (5.1)	2,785 (9.1)	1,306 (13.8)	<.0001
Non-White (%)	152,899 (19.1)	63,754 (17.3)	49,303 (17.0)	25,427 (24.2)	10,650 (34.8)	3,765 (39.8)	<.0001
Low SES (%)	197,136 (24.6)	91,433 (24.9)	65,885 (22.8)	27,006 (25.7)	9,602 (31.4)	3,210 (34.0)	<.0001
Prior PTB (%)	6,858 (0.9)	0	4,208 (1.5)	1,825 (1.7)	623 (2.0)	202 (2.1)	<.0001
ART (%)	29,763 (3.7)	19,838 (5.4)	8,148 (2.8)	1,436 (1.4)	276 (0.9)	65 (0.7)	<.0001
Male fetal gender (%)	409,097 (51.0)	187,681 (51.1)	147,586 (51.0)	53,361 (50.8)	15,588 (51.0)	4,881 (51.63)	0.6184
Hypertension and preeclampsia (%)	70,745 (8.8)	43,656 (11.9)	18,610 (6.4)	5,972 (5.7)	1,869 (6.1)	638 (6.8)	<.0001
SGA <p10 (%)	67,744 (8.5)	30,252 (8.2)	25,431 (8.8)	8,493 (8.1)	2,724 (8.9)	844 (8.9)	0.0002
Total PTB	43,653 (5.4)	25.483 (6.9)	11,769 (4.1)	4,353 (4.2)	1,491 (4.9)	557 (5.9)	
Spontaneous PTB	30,237 (3.8)	18,171 (4.9)	8,065 (2.8)	2,761 (2.6)	893 (2.9)	347 (3.7)	
Iatrogenic PTB	13,416 (1.7)	7,312 (2.0)	3,704 (1.3)	1,592 (1.5)	598 (2.0)	210 (2.2)	
Previous PTB	1,446 (8.0)	NA	942 (8.0)	343 (7.9)	121 (8.1)	40 (7.2)	

Abbreviations: *GA, gestational age; SD, standard deviation; yrs, years; SES, socio-economic status; PTB, preterm birth; ART, artificial reproductive techniques; SGA, small for gestational age.*

1.7%, respectively (**Table 1**). The incidence of total, spontaneous and iatrogenic preterm birth stratified for parity are presented in **Figure 1** and **Table 1**. The highest incidence of spontaneous preterm birth was observed among nulliparous women (P0, 4.9%) and women in their fifth pregnancy (P4, 3.7%) (**Figure 1** and **Table 1**). In addition, among the 18,170 women in their second, third, fourth or fifth pregnancy who had a preterm birth, 8% (n = 1446 out of 18,170 women) had a prior preterm birth and 92% were new preterm births (**Table 1**). These percentages per parity were 8.0% for P1, 7.9% for P2, 8.1% for P3 and 7.2% for P4 (**Table 1**).

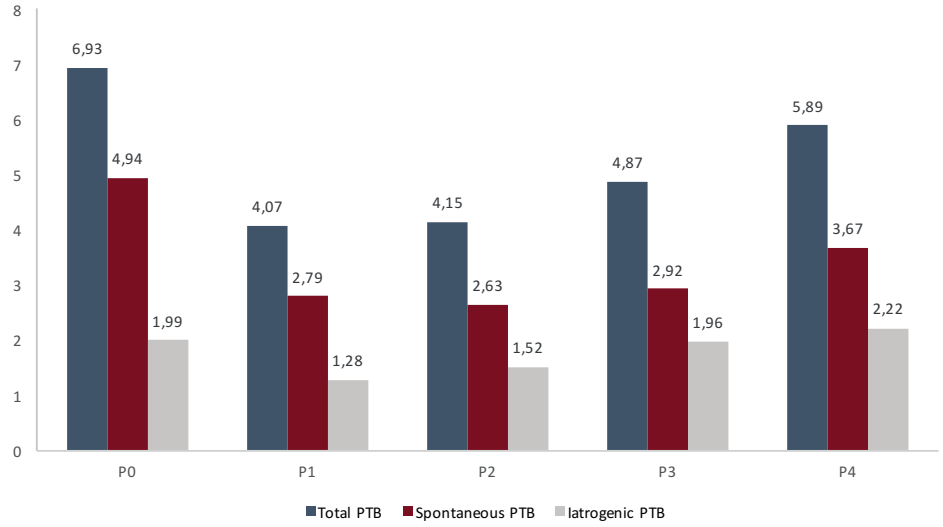

FIGURE 1. Incidence rates of overall preterm birth and stratified for spontaneous and iatrogenic preterm birth for women in their first (P0), second (P1), third (P2), fourth (P3) and fifth (P4) pregnancy from 2010 through 2014 in The Netherlands. **Abbreviations:** PTB, preterm birth.

Parity and risk of spontaneous preterm birth by gestational age

Spontaneous preterm birth risks by gestational age were examined for parity. We used women in their second pregnancy (P1) as reference; the results are demonstrated in **Table 2** and **Figure 2**.

Both nulliparous women and women in their fifth pregnancy had the highest risk for all preterm birth outcomes. Preterm birth risk in nulliparous women slightly increased after adjusting for confounders compared to the unadjusted risk, whereas in women in their fifth pregnancy the risk slightly decreased after correcting for the same confounders (**Table 2** and **Figure 2**).

TABLE 2. Relation between parity and spontaneous preterm birth <37, <32, <28 and between 34-37 weeks of gestation.

	Unadjusted OR (95% CI)	A*: Adjusted OR (95% CI)	B**: Adjusted OR (95% CI)
PTB <37 weeks			
P0	1.83 (1.78-1.88)	1.95 (1.89-2.00)	1.93 (1.88-1.98)
P1 (ref)	1.0	1.0	1.0
P2	0.94 (0.90-0.99)	0.92 (0.88-0.97)	0.93 (0.89-0.97)
P3	1.06 (0.99-1.13)	1.00 (0.93-1.08)	1.01 (0.94-1.08)
P4	1.34 (1.20-1.50)	1.26 (1.13-1.41)	1.27 (1.14-1.42)
PTB <32 weeks			
P0	2.04 (1.89-2.21)	2.15 (1.98-2.33)	2.19 (2.02-2.38)
P1 (ref)	1.0	1.0	1.0
P2	1.10 (0.97-1.25)	1.05 (0.92-1.19)	1.06 (0.93-1.20)
P3	1.32 (1.09-1.59)	1.15 (0.95-1.40)	1.17 (0.97-1.42)
P4	2.05 (1.57-2.67)	1.72 (1.31-2.25)	1.76 (1.34-2.31)
PTB <28 weeks			
P0	1.95 (1.72-2.20)	2.02 (1.78-2.29)	2..11 (1.86-2.39)
P1 (ref)	1.0	1.0	1.0
P2	1.07 (0.89-1.30)	1.00 (0.82-1.21)	1.03 (0.85-1.25)
P3	1.65 (1.27-2.16)	1.38 (1.05-1.81)	1.44 (1.10-1.88)
P4	3.10 (2.21-4.35)	2.44 (1.73-3.45)	2.59 (1.84-3.66)
PTB 34-37 weeks			
P0	1.75 (1.70-1.80)	1.85 (1.79-1.91)	1.83 (1.77-1.89)
P1 (ref)	1.0	1.0	1.0
P2	0.91 (0.87-0.96)	0.90 (0.85-0.94)	0.90 (0.85-0.95)
P3	1.00 (0.93-1.09)	0.97 (0.89-1.05)	0.97 (0.90-1.06)
P4	1.22 (1.08-1.39)	1.17 (1.03-1.33)	1.18 (1.03-1.34)

Abbreviations: *OR, odds ratio; CI, confidence interval; PTB, preterm birth.*

Correction* **model A*: Adjusted for maternal age (<20 years, ≥40 years and continuous), non-White ethnicity, low socioeconomic status, and a prior preterm birth.*

Correction* **model B: Adjusted for A and artificial reproductive techniques, male fetal gender, hypertension, preeclampsia, and small for gestational age <p10.*

For spontaneous preterm birth < 37 weeks we observed the highest risk in nulliparous women (OR 1.95, 95% CI 1.89–2.00) and women in their fifth pregnancy (OR 1.26, 95 CI 1.13–1.41) (**Figure 2a**). For spontaneous preterm birth < 32 weeks, nulliparous women had the highest risk (OR 2.15, 95% CI 1.98–2.3) followed by women in their fifth pregnancy (OR 1.72, 95% CI 1.31–2.25) (**Figure 2b**). Although we observed an increased risk for spontaneous preterm birth < 32 weeks in women in their fourth pregnancy, no effect was seen after adjusting for confounders (OR 1.15, 95% CI 0.95–1.40). The risk for spontaneous preterm birth < 28 weeks was highest in women in their fifth pregnancy (OR 2.44, 95% CI 1.73–3.45), followed by nulliparous women (OR 2.02, 95% CI 1.78–2.29)

and women in their fourth pregnancy (OR 1.38, 95% CI 1.05–1.81) (**Figure 2c**). Women in their first pregnancy had the highest risk for a spontaneous preterm birth between 34 and 37 weeks of gestation (OR 1.85, 95% CI 1.79–2.29) followed by women in their fifth pregnancy (OR 1.17, 95% CI 1.03–1.33) (**Figure 2d**). We did not observe significant differences between results obtained from **model A** and **model B** in all parity groups and outcomes (**Table 2**).

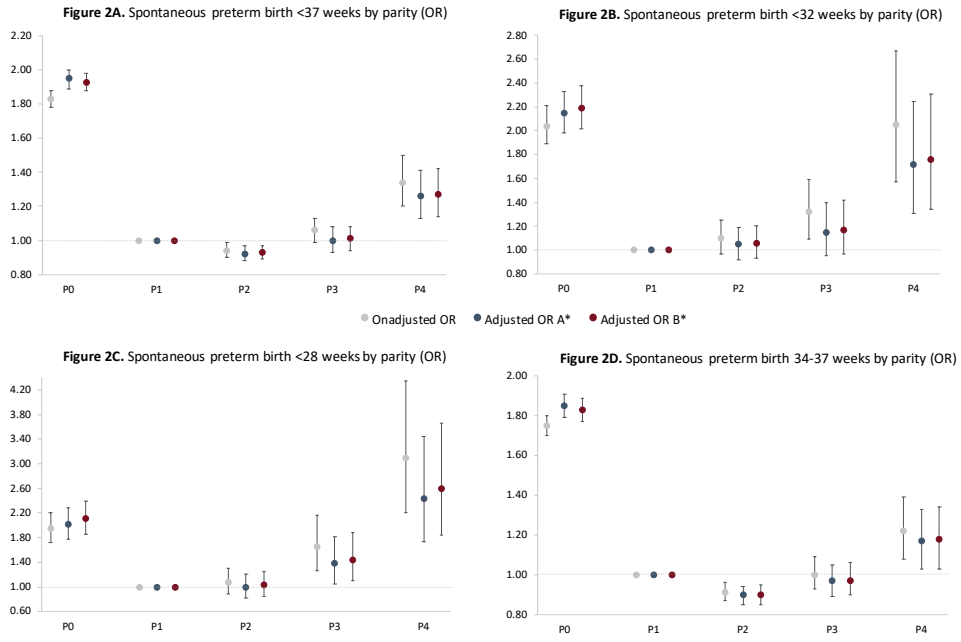

FIGURE 2. Odds ratio's for spontaneous preterm birth (**A**) < 37 weeks, (**B**) < 32 weeks, (**C**) < 28 weeks and between (**D**) 34–37 weeks of gestation per parity. **Abbreviations:** OR, odds ratio. *Correction **model A**: adjusted for maternal age (< 20 years, ≥40 years and continuous), non-White ethnicity, low socioeconomic status, and a prior preterm birth. Correction **model B**: adjusted for A and artificial reproductive techniques, male fetal gender, hypertension, and small for gestational age < p10

Trend in incidence of spontaneous preterm birth in multiparous women

After exclusion of nulliparous women, we observed an increase in incidence of spontaneous preterm birth < 37 weeks (p = 0.0178), < 32 weeks (p < .0001) and < 28 weeks (p < .0001), with increasing parity in multiparous women (**Table 3**). This trend was in line with our observation of increasing odds ratio's in multiparous women for all three outcomes. No trend was seen in spontaneous preterm birth between 34 and 37 weeks (**Table 3**).

TABLE 3. Trend test in spontaneous preterm birth incidence rates in multiparous women

	P1	P2	P3	P4	P-value*
Spontaneous PTB					
<37 weeks	8,065 (2.8%)	2,761 (2.6%)	893 (2.9%)	347 (3.7%)	0.0178
<32 weeks	873 (0.3%)	349 (0.3%)	121 (0.4%)	58 (0.6%)	<.0001
<28 weeks	367 (0.1%)	142 (0.1%)	64 (0.2%)	37 (0.4%)	<.0001
34-37 weeks	6,406 (2.2%)	2,118 2.0%)	675 (2.2%)	251 (2.7%)	NS

Abbreviations: *PTB, preterm birth. * Two-sided Cochran-Armitage Trend Test*

DISCUSSION

In this nationwide retrospective study, we found that nulliparity (P0) was independently associated with an overall increased risk for spontaneous preterm birth compared to women in their second pregnancy (P1). We also observed an increase in incidence of spontaneous preterm birth < 37, < 32 and < 28 weeks with higher parity in multiparous women, with highest risk for spontaneous preterm birth < 28 weeks in women in their fifth pregnancy.

The association between nulliparity and spontaneous preterm birth is supported by other studies.[17,18] Our study also finds an association between high parity and spontaneous preterm birth. Previous studies mostly assessed the effect of (high) parity in the context of advanced maternal age[19] or state that the effect of parity is influenced by socioeconomic and health care conditions.[11] More studies have been conducted to assess the association between parity and adverse pregnancy outcomes, however, these studies do not assess preterm birth as a primary outcome.[9,10]

The conflicting results of the different studies point to the complexity of the association between possible risk factors, including parity, and spontaneous preterm birth. It also highlights the possible influence of factors that contribute to a higher risk of spontaneous preterm birth, such as ethnicity and socio-economic status. However, in the current study we found an association between high parity and spontaneous preterm birth while adjusting for established risk factors such as ethnicity and socio-economic status. This possibly points to other factors that may contribute to a higher risk of spontaneous preterm birth. One of the factors that may play a role could be a damaged cervix. The cervix plays an important role in maintaining pregnancy. It is well known that damage to the cervix, for instance by dilatation and curettage or loop excisions of the cervix for premalignant lesions, contributes to a higher risk of spontaneous preterm birth.[20] The risk

of such procedures being performed is higher in women at higher age or parity, which may be an explanation for the association of parity and spontaneous preterm birth we found.

The overall risk for spontaneous preterm birth was significantly increased in nulliparous women compared to women in their second pregnancy, including the risk of birth between 34 and 37 weeks. According to the national prevention of preterm birth protocol in The Netherlands, women with a prior spontaneous preterm birth between 34 and 37 weeks are not offered additional screening or treatment (such as administration of progesterone, pessary or cerclage, and cervical length screening or bacterial vaginosis screening) and receive similar obstetric care as women without a prior preterm birth.[21] Also, it is unlikely that these treatment effects can explain these differences.

Although our results show that nulliparity and high parity is associated with an increased risk for spontaneous preterm birth, we observed remarkable differences between the association with nulliparity compared to high parity. While the risk of spontaneous preterm birth < 37, < 32 and < 28 weeks in nulliparous women is relatively similar, women in their fourth and women in their fifth pregnancy have a particularly high risk of spontaneous preterm birth occurring at early gestational age (**Table 2**).

We observed that odds ratios in nulliparous women increased after adjusting for confounders whereas odds ratio's in multiparous women decreased after adjusting. These data point to differences in the effect of established confounders on spontaneous preterm birth between different parity groups. This is in line with the significant differences we observed in the confounders low and high maternal age, non-White ethnicity and low socio-economic status between nulliparous and multiparous women.

Strengths and limitations of this study

The strengths of this study include the high quality of data in the PERINED registry which covers approximately 97% of all deliveries in the Netherlands. We were able to study a large recent set of pregnancies (n = 802, 119), including first, second, third, fourth and fifth pregnancies, and 30,237 spontaneous preterm births < 37 weeks of gestation.

Multiple epidemiologic studies have reported associations of nulliparous women with increased risk of preterm birth.[9,18,22–24] Yet, in many of these studies, parity has been categorized as nulliparous and multiparous, with women with their second pregnancy often grouped in with those of higher-order parity. In our study, we evaluated the effect per parity separately which allowed us to identify the increased risk in both nulliparous women and women with higher parity.

Unfortunately, due to low reporting within the perinatal database, we were not able to correct for smoking during pregnancy and maternal body mass index (BMI) in our analyses. The general incidence of smoking in The Netherlands is 22.4% in the population > 18 years old, 19.2% of all women are smokers.[25] The incidence of smoking in pregnant women in The Netherlands is 7.4%.[26] The general incidence of obesity in The Netherlands is 50.2% in the population > 18 years, of all women 47.2% has obesity (30.4% has moderate obesity and 16.9% has severe obesity).[27] Smoking and very low or very high maternal BMI are known risk factors for spontaneous preterm birth.[28,29] This may have influenced our results. Because we corrected for low socio-economic status in our analyses, and it is known that low socio-economic status is strongly correlated to both smoking and maternal obesity, we do not think that this issue of missing adjustment factors has influenced our results to a large degree. In addition to smoking and BMI, we were not able to correct for other potential risk factors that contribute to the risk of preterm birth, such as polyhydramnios, intra-uterine infection, single marital status, short interpregnancy interval (< 6 months) and specific maternal diseases (uterus anomaly, cervical excision procedures, maternal surgery during pregnancy, depression).[6]

Pregnancies ending < 22 weeks were not included in our national database. Although we corrected for a prior preterm birth, which was available in our dataset, we did not have information on multiple occurrence nor severity of the prior preterm birth. Because no longitudinal linked obstetric database was available, pregnancies could not be related to the level of the individual woman in this study. We therefore could not identify women that were included multiple times due to multiple pregnancies between 2010 and 2014 which may have influenced our results.

Conclusion

Our findings indicate that high parity, as well as nulliparity, is involved as a risk factor in the complex pathways that lead to spontaneous preterm birth. These results highlight the importance of the effect of parity on spontaneous preterm birth and may assist in preterm birth risk stratification and counseling.

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CHAPTER 4

The predictive capacity of Uterine artery Doppler for preterm birth - a cohort study

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Acta Obstetricia et Gynecologica Scandinavica. 2020 Apr;99(4):494-502

ABSTRACT

Introduction: Mid-trimester uterine artery resistance measured with Doppler sonography is predictive for iatrogenic preterm birth. In view of the emerging association between hypertensive disease in pregnancy and spontaneous preterm birth, we hypothesized that uterine artery resistance could also predict spontaneous preterm birth.

Material and methods: We performed a cohort study of women with singleton pregnancies. Uterine artery resistance was routinely measured at the 18-22 weeks anomaly scan. Pregnancies complicated by congenital anomalies or intrauterine fetal death were excluded. We analyzed if the waveform of the uterine artery (no notch, unilateral notch or bilateral notch) was predictive for spontaneous and iatrogenic preterm birth, defined as delivery before 37 weeks of gestation. Furthermore, we assessed whether the uterine artery pulsatility index was associated with the risk of preterm birth.

Results: Between January 2009 and December 2016 we collected uterine Doppler indices and relevant outcome data in 4521 women. Mean gestational age at measurement was 19^{+6} weeks. There were 137 (3.0%) women with a bilateral and 213 (4.7%) with a unilateral notch. Mean gestational age at birth was 38^{+6} weeks. Spontaneous and iatrogenic preterm birth rates were 5.7% and 4.9%, respectively. Mean uterine artery resistance was 1.12 in the spontaneous preterm birth group compared with 1.04 in the term group (P = 0.004). The risk of preterm birth was increased with high uterine artery resistance (OR 2.9 per unit; 95% CI 2.4-3.9). Prevalence of spontaneous preterm birth increased from 5.5% in women without a notch in the uterine arteries to 8.0% in women with a unilateral notch and 8.0% in women with a bilateral notch. For iatrogenic preterm birth, these rates were 3.9%, 13.6% and 23.4%, respectively. Likelihood ratios for the prediction of spontaneous preterm birth were 1.6 (95% CI 1.0-2.6) and 1.9 (95% CI 1.0-3.5) for unilateral and bilateral notches, respectively, and for iatrogenic preterm birth they were 3.6 (95% CI 2.5-5.2) and 6.8 (95% CI 4.7-9.9) for unilateral and bilateral notches, respectively. Of all women with bilateral notching, 31.4% delivered preterm.

Conclusions: Mid-trimester uterine artery resistance measured at 18-22 weeks of gestation is a weak predictor of spontaneous preterm birth.

INTRODUCTION

Preterm birth (PTB), defined as delivery before 37 weeks of gestation, remains a major burden in obstetric care, affecting over 15 million babies worldwide each year.[1,2] Despite various preventive measures, 1 million neonatal deaths are attributable to complications of PTB, which makes it the leading cause of death in children under the age of 5 years.[3] To reduce these numbers, it is crucial to understand the mechanisms underlying prematurity and develop targeted interventions for prevention.

During the normal development of the placental structure, trophoblast cells invade the myometrium and cause remodeling of maternal spiral arteries. These spiral arteries convert to a low-resistance, high-flow state.[4,5] The complete transformation of the decidual and myometrial segments of the spiral arteries is also known as deep placentation.[6] Defective deep placentation was first found in women with preeclampsia and intrauterine growth restriction,[7] but in recent years it was also found to be associated with spontaneous preterm labor.[8,9] The disease of the placenta vascular bed that underpins these complications is commonly known as the "great obstetrical syndrome".[6]

It has been suggested that women with manifestations of placental dysfunction have a higher impedance of uterine artery (UtA) blood flow and failure of physiological transformation of the spiral arteries.[10-13] This abnormal mid-trimester UtA resistance measured with Doppler sonography is known to be predictive for preeclampsia and, hence, for iatrogenic PTB.[12,14]

So far, studies have found conflicting results in the association between spontaneous PTB and UtA resistance.[15,16] In addition, it was found that women carrying a male fetus have higher second-trimester UtA resistance and a higher frequency of notching of the UtA.[17]

In view of the emerging association between hypertensive disease in pregnancy and spontaneous PTB,[18] we hypothesized that UtA resistance could also predict spontaneous PTB.

Our objective was to investigate the utility of mid-trimester UtA Doppler in the prediction of spontaneous preterm delivery in a large cohort.

MATERIAL AND METHODS

Study design

We performed a single-center cohort study among consecutive women with a singleton pregnancy who visited the fetal ultrasound department of the Academic Medical Center, Amsterdam for their routine fetal anomaly scan between 1 January 2009 and 31 December 2016. Data were collected using an ASTRAIA database. ASTRAIA is a local registry that is used for collection of all sonographic data and pregnancy outcomes.

Inclusion and exclusion criteria

We studied all women with a singleton gestation who had an anomaly scan between 18 and 22 weeks of gestation. As the Academic Medical Center is a tertiary referral center, women attending antenatal care had pre-existing medical conditions or an increased risk for pregnancy complications. Moreover, the obstetric ultrasound department has a regional function for the surrounding midwifery practices, which leads to a large number of low-risk women being included in our cohort.

Women with a pregnancy complicated by congenital anomalies or antepartum fetal mortality were excluded. Furthermore, women were excluded if measures of interest were not available for both the left and right UtA Doppler, if outcome of pregnancy was unknown, or if the way in which labor started (spontaneous contractions, cesarean section, induction) was not specified.

Data collection

All women received an intake by the sonographer in which obstetrical history, smoking during pregnancy, maternal height and weight, and method of conception (spontaneous, in vitro fertilization, ovulation induction or intracytoplasmic sperm injection) were recorded.

UtA Doppler measurement was performed by a certified sonographer. The UtAs were identified at the crossover with the external iliac artery by an abdominal approach (**Figure 1**). Both arteries were sampled and three consecutive waveforms were evaluated. All measurements were performed using a Voluson™ ultrasonograph (Voluson E8 or E10; GE Healthcare). Both quantitative assessments of the wave by pulsatility index (PI) as well as qualitative analysis of the flow velocity waveform (notching) (**Figure 2**) were performed and reported in the local ultrasound database (ASTRAIA). Notching was defined as a persistent decrease in blood-flow velocity in the early diastole, below the diastolic peak velocity.[5] This can be objectified at none, one or both sides of the UtAs.

If bilateral notching was observed during the fetal anomaly scan, increased surveillance was performed. Women with a bilateral notch were invited for a follow-up ultrasound at 24 weeks of gestation (fetal biometry and UtA Doppler). When there was a persistent bilateral notch in the UtAs, monitoring of fetal growth by ultrasound was suggested until fetal biometry was within the normal range at 30 weeks of gestation.

All included women were followed until delivery. Follow up of the pregnancies was retrieved by questionnaires that were distributed routinely after the 20-week fetal anomaly scan. In addition, we checked the hospital charts of the Academic Medical Center for any missing data.

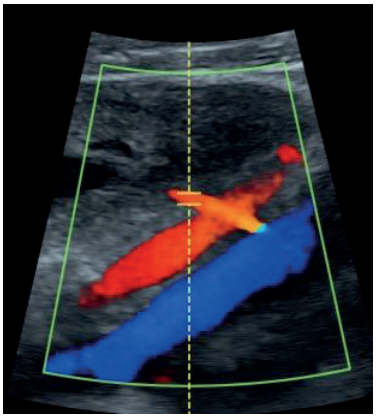


FIGURE 1. Doppler image of uterine artery at crossover iliac artery

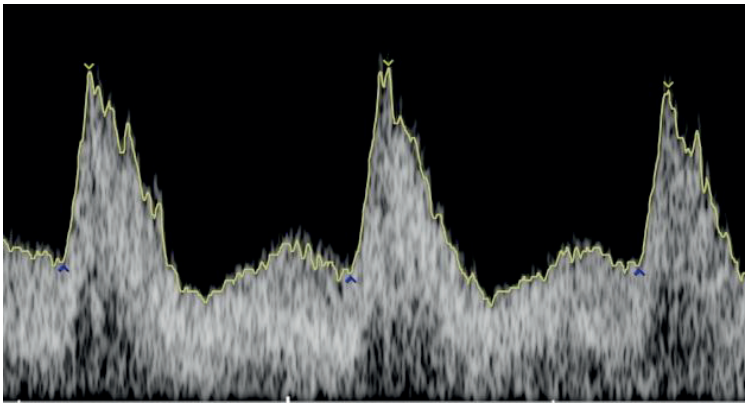


FIGURE 2. Notch waveform in uterine artery

Outcome measures

Preterm birth was defined as a delivery before 37 completed weeks of gestation and categorized as spontaneous or iatrogenic. Gestational age (GA) was based on first trimester crown-rump length. If the first trimester scan was not performed, estimated due date was calculated using the last menstrual period or a second- or third-trimester ultrasound, in concordance with the national guideline.[19]

Our primary outcome was spontaneous PTB before 37 weeks of gestation. Secondary outcomes were overall PTB before 37 weeks, iatrogenic PTB before 37 weeks, spontaneous and iatrogenic PTB between 34 and 37 weeks, between 32 and 34 weeks and between 22 and 32 weeks of gestation, and GA at birth. We used mean UtA PI (left + right PI divided by 2) as a measure of UtA resistance. As UtA PI varies with GA, we constructed GA-adjusted centiles based on the women with a term delivery on our own data set. We defined increased PI as >90th centile (P90) and >95th centile (P95). The presence of notches could be either unilateral or bilateral.

Statistical analyses

In case of missing data, multiple imputation was used to create several "complete" sets of data.[20] Both patient characteristics and outcomes were taken into account to impute missing data. We used an iterative Markov chain Monte Carlo method for the generation of missing values and created 10 imputed data sets to use the pooled estimates.

We assessed demographic and obstetric baseline characteristics. We performed univariate analysis for the comparison of baseline characteristics for the 3 groups (no notch, unilateral notch and bilateral notch) using the chi-squared test for categorical variables or 1-way analysis of variance with the post-hoc Bonferroni correction for comparison of means. Mean GA at birth was assessed for all groups. Incidence of spontaneous and iatrogenic PTB was estimated for the overall study population and for each group.

Overall prevalence of PTB was estimated for the total study population. UtA waveform was categorized into (a) no notch, (b) unilateral notch and (c) bilateral notch.

The association between the waveform in the UtA (no notch, unilateral notch or bilateral notch) and spontaneous or iatrogenic PTB was assessed using likelihood ratios. Time to delivery was expressed for all waveforms. A Kaplan-Meier survival curve was plotted to show any difference in GA at birth between different mid-trimester notching. In pregnancies in which labor was induced, time to delivery was censored.

To estimate the effect of the UtA PI on PTB, logistic modeling was used, expressed as an odds ratio (OR) with a 95% CI. A multivariable logistic regression was performed to adjust for possible confounders that were unequally distributed in the baseline demographics. We tested both the effect on PTB overall and also within the groups with spontaneous or iatrogenic start of labor separately.

After excluding women with an iatrogenic PTB, mean UtA PI, UtA PI > P90 and UtA PI > P95 were compared between women with and without spontaneous PTB < 37 weeks.

To investigate which measure of UtA resistance predicted best for spontaneous PTB < 37 weeks, we constructed receiver operating characteristic (ROC) curves with mean UtA, UtA PI > P90 and UtA PI > P95 and calculated an area under the curve (AUC) for each.

Sensitivity, specificity, and positive and negative predictive values of the mean UtA PI were calculated for different cut-offs of GA (spontaneous PTB between 34 and 37, 32 and 34, 28 and 32 weeks, and at <28 weeks of gestation).

In The Netherlands, the official protocol for standardized ultrasound for dating pregnancies was introduced in September 2011. Previous research reported that the method used for GA estimates (last menstrual period or ultrasound) influences the PTB rates.[21] In the earlier years of our study cohort (2009-2011), standardized ultrasound for dating of the pregnancy was not common practice, so we performed a post-hoc sensitivity analysis over the last 4 years of our cohort to reduce dating errors as an explanation for our findings.

Data were analyzed using SPSS Statistics 24 (IBM SPSS). A $P < 0.05$ was supposed to indicate statistical significance.

Ethical approval

In the Amsterdam University Medical Center all women participating in prenatal screening give written informed consent to use the data of the pregnancy and outcome for research. All measurements and pregnancy and delivery characteristics are stored in an ultrasound registry. The data extracted for our study were anonymous, so no further ethical approval was necessary. This study complies with the Declaration of Helsinki.

RESULTS

We identified 6996 women with a singleton gestation during our study period. Women with a pregnancy complicated by congenital anomalies (n = 46), termination of the pregnancy for any reason (n = 11) and/or an intrauterine death (n = 48) were excluded. After excluding women whose pregnancy outcome was not available (n = 1716), or in whom the method of onset of labor was unknown (n = 654), data of 4521 women were available for analysis.

Baseline characters

Maternal characteristics are shown in **Table 1**. There were 137 (3.0%) women with a bilateral notch and 212 (4.7%) with a unilateral notch. Mean GA at measurement was 19^{+6} weeks. History of PTB was more prevalent within the group with a bilateral notch compared with women without a notch (13.1% vs 7.0%). Women with a bilateral notch were significantly younger than women with a unilateral notch or with no notch (30.6 vs 30.8 and 31.8 years for unilateral and no notch, respectively, P < 0.001).

TABLE 1. Baseline characteristics of the whole cohort in relation to the presence or absence of notching (N=4521)

Baseline characteristics	No notch	Unilateral notch	Bilateral notch	
	N=4171	N=213	N=137	P-value
GA at measurement	19^{+6}	19^{+6}	19^{+6}	0.98
Maternal age (years, mean)	31.6	30.8	30.6	0.01
Maternal BMI (kg/m2, SD)	25.7 (5.3)	25.6 (5.6)	26.9 (6.9)	0.04
Smoking (n, %)	251 (6.7%)	18 (9.3%)	11 (9.1%)	0.24
Nulliparous (n, %)	1793 (44.6%)	98 (48.3%)	61 (45.2%)	0.58
ART (n, %)	61 (8.3%)	12 (7.3%)	8 (8.7%)	0.89
Previous preterm birth (n, %)	290 (7.0%)	22 (10.3%)	18 (13.1%)	0.005

Abbreviations: *ART, assisted reproductive technology; BMI, body mass index; GA, gestational age; SD, standard deviation*

Primary and secondary outcomes

An overview of birth outcomes is presented in **Table 2**. Mean GA at birth was 38^{+6} weeks. **Figure 3** shows the time to delivery for women with different notch findings. The overall incidence of PTB before 37 weeks of gestation was 10.6% (n = 481), spontaneous PTB occurred in 6.3% (n = 259) whereas iatrogenic PTB occurred in 4.9% (n = 222) of the women. The incidence of spontaneous PTB at <37 weeks increased from 5.5% in women without a notch in the UtAs to 8.0% in women with a unilateral notch as well as in women with a bilateral notch (P = 0.02) (**Table 2**). For iatrogenic PTB, these rates were

3.9%, 13.6% and 23.4%, respectively (P < 0.001). Within the group with a bilateral notch, 5.8% of the women had a spontaneous PTB between 34 and 37 weeks compared with 3.3% of the women without a notch (P < 0.001). For the prediction of spontaneous PTB between 32 and 34 weeks and between 22 and 32 weeks, no significant differences were observed.

TABLE 2. Outcomes

	No notch	Unilateral notch	Bilateral notch	
Outcomes	**N=4171**	**N=213**	**N= 137**	**P-value**
GA at delivery	39^{+0}	38^{+1}	37^{+1}	<0.001
Total preterm births, n (%)				
<37 wk	392 (9.4%)	46 (21.6%)	43 (31.4%)	<0.001
34-37 wk	248 (6.0%)	27 (12.7%)	19 (13.9%)	<0.001
32-34 wk	53 (1.3%)	6 (2.8%)	7 (1.7%)	<0.001
22-32 wk	82 (2%)	13 (6.1%)	17 (12.4%)	<0.001
Spontaneous preterm births, n (%)				
<37 wk	231 (5.5%)	17 (8.0%)	11 (8.0%)	0.02
34-37 wk	138 (3.3%)	15 (7.0%)	8 (5.8%)	0.001
32-34 wk	37 (0.9%)	1 (16.7%)	1 (0.7%)	0.87
22-32 wk	47 (1.1%)	1 (0.5%)	2 (1.5%)	0.45
Iatrogenic preterm births, n (%)				
<37 wk	161 (3.9%)	29 (13.6%)	32 (23.4%)	<0.001
34-37 wk	110 (2.6%)	12 (5.6%)	11 (8.0%)	<0.001
32-34 wk	16 (0.4%)	5 (2.3%)	6 (4.4%)	<0.001
22-32 wk	35 (0.84%)	12 (5.6%)	15 (10.9%)	<0.001

Abbreviation: *GA, gestational age; wk, weeks*

Likelihood ratios for the prediction of spontaneous PTB were 1.6 (95% CI 1.1-2.6) and 1.9 (95% CI 1.0-3.5) for unilateral and bilateral notches, respectively. For iatrogenic PTB, presence of notching was significantly related to the risk of PTB (<37, 32-34, 32-22 weeks; P < 0.001). Corresponding positive likelihood ratios were 3.5 (95% CI 2.5-5.2) for unilateral notching and 6.8 (95% CI 4.7-9.9) for bilateral notching (**Table 3**). Of all women with bilateral notching, 31.4% delivered preterm.

Mean UtA resistance was 1.12 in the spontaneous PTB group compared with 1.04 in the term group (P < 0.001). The risk of overall PTB was increased with a higher UtA (OR 3.1; 95% CI 2.5-3.8). Adjustment for maternal age and previous PTB did not change this result (OR 2.9; 95% CI 2.4-3.7). **Table 4** shows the relation between mean UtA resistance and

spontaneous PTB for different GAs. Mean PI > P90 was the best predictor for spontaneous PTB between 32 and 37 weeks. Mean PI > P95 was associated with a higher risk for spontaneous PTB before 32 weeks.

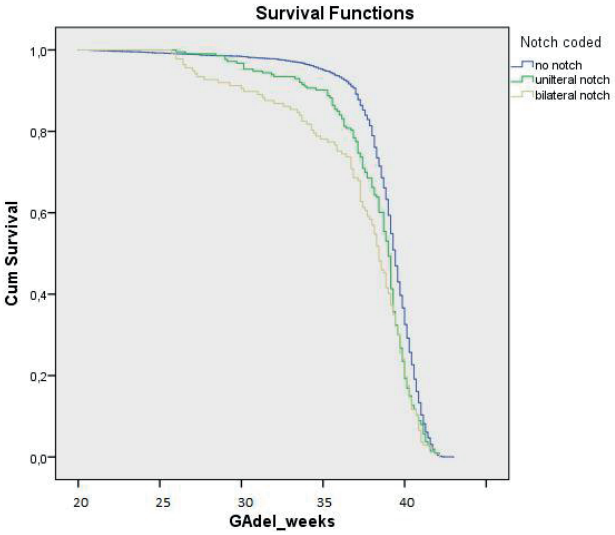


FIGURE 3. Time to delivery expressed for uterine artery waveform (no notch, unilateral notch, bilateral notch). **Abbreviations:** GAdel, gestational age at delivery

Figure 4 shows the different ROC curves for the prediction of spontaneous PTB at <37 weeks. Mean UtA PI had the largest AUC (0.56; 95% CI 0.52-0.60).

Sensitivity analysis

We performed a post-hoc sensitivity analysis for the period between years 2012 and 2016 because during the earlier years there was no routine first-trimester ultrasound scan for estimation of GA. Analysis of 3809 pregnancies in these last 4 years did not change our results (see **Supplementary material**, **Table S1**).

TABLE 3. Rates and likelihood ratios (LR) for spontaneous preterm births, iatrogenic preterm births and term birth

	Term births		
Notch	**N**	**%**	**LR (95% CI)**
No notch	3779	90.6	1.15 (1.1-1.2)
Unilateral	167	78.4	0.40 (0.30-0.55)
Bilateral	94	68.6	0.25 (0.17-0.35)
Total	4040	89.4	

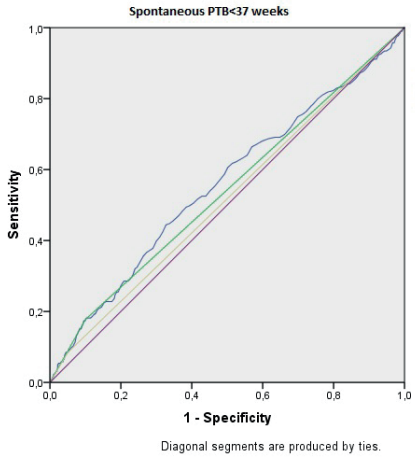
	Spontaneous			Iatrogenic			Total		
Notch	**N**	**%**	**LR (95% CI)**	**N**	**%**	**LR (95% CI)**	**N**	**%**	**LR (95% CI)**
Preterm birth <37 wk									
No notch	231	5.5	0.95 (0.91-1.0)	161	3.9	1.5 (1.4-1.7)	392	14.9	0.9 (0.83-0.91)
Unilateral	17	8	1.6 (1.0-2.6)	29	13.6	3.6 (2.5-5.2)	46	21.6	2.5 (1.8-3.4)
Bilateral	11	8	1.9 (1.0-3.5)	32	23.4	6.8 (4.7-9.9)	43	40	4.1 (2.9-5.8)
Total	259	5.7	-	222	4.9	-	481	10.6	-
Preterm birth <34 wk									
No notch	93	2.2	1.0 (0.97-1.1)	51	1.2	0.62 (0.52-0.74)	144	3.4	0.83 (0.77-0.90)
Unilateral	2	0.9	0.46 (0.12-1.8)	17	8	5.5 (3.5-8.4)	19	8.9	2.5 (1.6-4.0)
Bilateral	3	2.2	1.1 (0.37-3.5)	21	15.3	10.7 (7.1-16.0)	24	17.5	5.2 (3.5-7.9)
Total	98	2.2	-	89	2	-	187	4.2	-
Preterm birth <32 wk									
No notch	1	0.5	1.02 (0.96-1.09)	35	0.8	0.61 (0.49-0.76)	36	1.1	0.81 (0.73-0.90)
Unilateral	2	1.5	0.38 (0.05-2.6)	12	5.6	5.5 (3.3-9.1)	14	7.1	2.7 (1.6-4.5)
Bilateral	56	1.3	1.2 (0.31-4.8)	15	10.9	10.5 (6.6-16.6)	71	12.2	5.5 (3.4-8.8)
Total	59	1.3	-	62	1.4	-	121	2.7	-

Abbreviations: *CI, confidence interval; wk, weeks*

TABLE 4. Association between mean uterine artery pulsatility index and spontaneous preterm birth (PTB) at different gestational age cutoffs

	Sensitivity	Specificity	NPV	PPV	Odds ratio
Mean uterine artery >P90					
Spontaneous PTB <37 wk	0.18 (0.14-0.23)	0.90 (0.90-0.90)	0.95 (0.94-0.95)	0.10 (0.08-0.13)	1.94 (1.39-2.71)
Spontaneous PTB 34-37 wk	0.20 (0-14-0.27)	0.90 (0.90-0.90)	0.97 (0.96-0.97)	0.07 (0.06-0.10)	2.20 (1.48-3.29)
Spontaneous PTB 32-34 wk	0.15 (0.06-0.31)	0.90 (0.90-0.90)	0.99 (0.99-0.99)	0.01 (0.01-0.03)	1.56 (0.65-3.74)
Spontaneous PTB 28-32 wk	0.27 (0.09-0.55)	0.90 (0.90-0.90)	0.10 (0.10-0.10)	0.01 (0.00-0.00)	3.12 (0.99-9.84)
Spontaneous PTB <28 wk	0.09 (0.03-0.22)	0.90 (0.89-0.90)	0.99 (0.99-0.99)	0.01 (0.00-0.00)	0.85 (0.30-2.39)
Mean uterine artery >P95					
Spontaneous PTB <37 wk	0.09 (0.06-0.14)	0.95 (0.95-0.95)	0.94 (0.94-0.94)	0.10 (0.06-0.14)	1.73 (1.09-2.73)
Spontaneous PTB 34-37 wk	0.09 (0.06-0.15)	0.95 (0.95-0.95)	0.96 (0.96-0.97)	0.07 (0.04-0.11)	1.89 (1.09-3.28)
Spontaneous PTB 32-34 wk	0.05 (0.01-0.18)	0.95 (0.95-0.95)	0.99 (0.99-0.99)	0.01 (0.00-0.03)	0.96 (0.23-4.0)
Spontaneous PTB 28-32 wk	0.20 (0.05-0.48)	0.95 (0.95-0.95)	1.00 (1.00-1.00)	0.01 (0.00-0.03)	4.5 (1.26-16.0)
Spontaneous PTB <28 wk	0.05 (0.01-0.17)	0.95 (0.95-0.95)	0.99 (0.99-0.99)	0.01 (0.00-0.03)	0.85 (0.20-3.53)

Abbreviations: *NPV, negative predictive value; PPV, positive predictive value; PTB, preterm birth; wk, weeks.*



Area Under the Curve

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Confidence Interval Lower Bound	Confidence Interval Upper Bound
mean UtA PI	0,56	0,019	0,00	0,52	0,59
a uterina >P90	0,54	0,019	0,04	0,50	0,58
a uterina >P95	0,52	0,019	0,36	0,48	0,55

a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

FIGURE 4. Receiver operating characteristics curve for spontaneous preterm birth <37 wk for different measures of uterine artery resistance. **Abbreviations:** UtA PI, uterine artery pulsatility index.

DISCUSSION

In this study we assessed the utility of mid-trimester UtA Doppler measurement for the prediction of PTB. The main finding of this study is that women with a higher UtA resistance, either manifested in notching or higher PI, are at increased risk for spontaneous PTB before 37 weeks of gestation. The risk is particularly present between 34 and 37 weeks of gestation. No statistically significant effect on spontaneous PTB before 34 weeks was observed.

Our study has both strengths and limitations. A major strength of this study is that we included a cohort with a large sample size. We recruited women with both a low-risk (midwifery practices) and high risk (tertiary center) for spontaneous PTB. Data were derived from a local ultrasound registry (ASTRAIA), in which both data on delivery and other pregnancy outcomes are registered. All ultrasound scans were conducted by certified sonographers.

A limitation of this study is that it was performed in a single tertiary center. The overall PTB rate for singletons in The Netherlands was 5.6% in 2015, of which 1.8% was medically indicated.[22] The overall PTB rate, and especially the iatrogenic PTB rate, in our study population was higher, which is possibly because the selected population in a tertiary hospital in an urban area. Women visiting our hospital for their antenatal care more

often have comorbidities or a complicated previous pregnancy. Furthermore, our clinic is visited by a relatively high number of women with a non-white ethnicity and low socio-economic status, both of which are known as risk factors for PTB.[23]

Preventive measures for spontaneous PTB were offered according to the local guidelines. The national guideline advises the use of progesterone (intramuscular injections) in women with a previous PTB before 34 weeks. Moreover, cervical length measurements are offered to these women between 16 and 24 weeks (every other week). If the cervical length is <25 mm women are counseled for a secondary cerclage.

Since June 2014, nulliparous women or multiparous women without previous PTB before 34 weeks, were offered a cervical length measurement during the fetal anomaly scan. If cervical length was <35 mm (18-22 weeks of gestation) they could be randomized between vaginal progesterone and cervical pessary (Quadruple P trial).

These targeted interventions might have influenced our results. As cervical length is not related to UtA Doppler we expect this influence to be rather limited. Furthermore, we have included a mixed population with both high-risk and low-risk women.

A previous study assessing the relation between second-trimester UtA Doppler and spontaneous PTB reported no significant correlation.[24] This was a historical cohort study performed between 1999 and 2002 in the UK. A total of 234 pregnancies complicated by spontaneous preterm labor were compared with 5472 women who delivered at term. The distribution of the different notches in the spontaneous PTB group was comparable with our cohort (84% no notch, 9.8% unilateral notch and 4.7% bilateral notch in the study by Cobian-Sanchez et al vs 89% no notch, 6.6% unilateral notch and 4.2% bilateral notch in our cohort). No statistical difference in UtA Doppler measurements between the group with a spontaneous PTB and the group with a term birth was found (resistance index >95th centile spontaneous PTB 6% vs 4% in the term group; P = .14). A difference between both studies is the earlier GA at scanning in our group (mean GA 19^{+6} weeks, compared with 21^{+1} weeks in the historical cohort). The study by Cobian-Sanchez et al[24] does not present data for iatrogenic PTB, so we are not able to make a direct comparison between overall PTB rates in the studies. In 2006, Fonseca et al[16] also reported on the relation between second-trimester UtA PI and spontaneous PTB. They showed that UtA PI was higher in women with a spontaneous PTB before 33 weeks. However, compared with maternal characteristics and obstetrical history, measurement of the UtA PI did not result in a better prediction. These results are in line with our data.

Guidelines for estimation of GA were not available at the beginning of our study period, which could have influenced our results. Limiting our analysis to the period 2012-2016 (n = 3809 pregnancies) did not change the results, which indicates that the association is less likely to be to the result of erroneous determination of expected date of delivery.

Identification of UtA Doppler as an influencing factor in the risk for a (spontaneous) PTB may lead to new opportunities for the development of predictive models. Measurement of the UtA Doppler is a relatively quick and low-cost intervention, which can be easily performed during the routine fetal anomaly scan that is embedded in the national screening program. However, although we found an association between UtA Doppler and spontaneous PTB, the predictive capacity was rather limited. A likelihood ratio of 1.9 is usually not enough to justify the use of a test in clinical practice. Indeed, a change in the probability of PTB from 5.5% to 8.0% does not justify general screening. Also, UtA Doppler was not predictive for spontaneous preterm delivery before 34 weeks.

Our results, with a higher risk of late spontaneous PTB in women with an abnormal UtA Doppler, strengthen the hypothesis that impaired placental function has a relation with spontaneous PTB. Current research focuses on antiplatelet therapy as a new strategy in the prevention of PTB.[25-28] Recent studies report a reduction in spontaneous PTB before 34 weeks if antiplatelet therapy is started between 13 and 25 weeks of gestation.[29] We suggest increased surveillance in women with an abnormal UtA Doppler in the second trimester. Further prospective studies should evaluate if abnormal UtA Doppler contributes to the multivariable etiology that is underlying spontaneous PTB.

CONCLUSION

In conclusion, abnormal UtA Doppler indicates not only a higher risk of iatrogenic PTB, but also, though with a weaker association, spontaneous PTB. Further prospective studies are needed to evaluate if UtA Doppler contributes to a multifactorial model that predicts spontaneous PTB.

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SUPPLEMENTARY INFORMATION

Supplementary information is provided here.

SUPPLEMENTAL TABLE 1

	no notch	unilateral notch	bilateral notch	
Outcomes	**N=3 500**	**N= 189**	**N=120**	***P*-value**
GA at delivery	39^{+0}	38^{+2}	37^{+1}	<0.001
Total preterm births				
< 37 weeks	297 (8.5%)	37 (19.6%)	35 (29.2%)	0.001
34-37 weeks	185	21	15	<0.001
32-34 weeks	41	5	5	0.002
22-32 weeks	64	11	15	<0.001
Spontaneous preterm births				
< 37 weeks	186	13	11	0.02
34-37 weeks	114	12	8	0.004
32-34 weeks	30	1	1	0.92
22-32 weeks	35	0	2	0.19
Iatrogenic preterm births				
< 37 weeks	111	24	24	<0.001
34-37 weeks	71	9	7	<0.001
32-34 weeks	11	4	4	<0.001
22-32 weeks	29	11	13	<0.001

CHAPTER 5

Repeated cervical length measurements for the verification of short cervical length

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International Journal of Gynecology & Obstetrics. 2017 Dec;139(3):318-323

ABSTRACT

Objective: To determine if the verification of short cervical length with a repeated measurement improved the identification of patients with short cervical length at increased risk of preterm delivery.

Methods: The present secondary analysis analyzed prospective cohort study data from patients with singleton pregnancies without a history of preterm delivery who presented for obstetric care in the Netherlands and delivered between November 18, 2009, and January 1, 2013. Cervical length was measured during standard anomaly scan and a second measurement was performed if the cervical length was 30 mm of shorter. Logistic regression and Cox proportional hazards modeling were used to evaluate associations between cervical length measurements and spontaneous preterm delivery before 37 weeks of pregnancy.

Results: Cervical length measurements from 12 358 patients were included; 221 (1.8%) had an initial cervical length measurement of 30 mm or shorter. A second cervical length measurement was performed for 167 (75.6%) patients; no differences were identified in the odds of spontaneous preterm delivery when evaluated using the first, second, or a mean of both measurements, regardless of whether cervical length was analyzed as a continuous or dichotomous variable.

Conclusion: Among patients with singleton pregnancies, verification of short cervical length did not improve the identification of short cervical length.

INTRODUCTION

Preterm delivery, defined as delivery before 37 weeks of pregnancy, is a leading contributor to neonatal morbidity and mortality.[1,2] Within the Netherlands, the rate of spontaneous preterm delivery of singleton pregnancies is 5.4%.[3,4] Cervical length is an important predictor of spontaneous preterm delivery[5,6] and studies have demonstrated that mid-trimester asymptomatic short cervical length is associated with an increased risk of spontaneous preterm delivery.[6,7] Cervical-length measurement by transvaginal ultrasonography is normally performed during the standard anomaly scan at approximately 20 weeks of pregnancy.

Preterm delivery-risk assessment, based on mid-trimester cervical length, needs to be reliable in light of the considering the clinical consequences and potential changes in pregnancy management for patients with a short cervical length. Currently, cervical-length measurement is performed only once but repeating this measurement to verify short cervical length could increase precision.

Conversely, although transvaginal ultrasonography examinations are well tolerated by patients and the risk of adverse events is low, the invasive nature of cervical-length measurements is an important consideration. Further, referrals for additional ultrasonography examinations are time consuming and costly; consequently, it should be performed only when there is clear benefit.[8]

The objective of the present study was to determine whether verification of short cervical length with a second cervical-length measurement would improve the identification of patients with a short cervical length, who are at increased risk of preterm delivery.

MATERIALS AND METHODS

The present secondary analysis used data from the Triple P screening study.[9] This nationwide prospective cohort study recruited patients presenting for obstetric care at all settings in the Netherlands, including primary care, between November 18, 2009, and August 1, 2013. This study included asymptomatic, nulliparous and multiparous patients with singleton pregnancies without a history of spontaneous preterm delivery before 34 weeks of pregnancy. The parent study was approved by the medical ethics committee of the Academic Medical Center, Amsterdam, the Netherlands.

In the parent study, eligible patients were invited to participate in a preterm-delivery screening program to have their cervical length measured during a standard anomaly

scan at 16–22 weeks of pregnancy.[9] This program was designed to identify women at risk of preterm delivery based on a short cervical length, defined as 30 mm or shorter. Cervical length measurements were performed at ultrasonography centers in the primary care setting, as well as at obstetric departments of secondary and tertiary referral centers that performed ultrasonography examinations in their regions. Prior to participating in the parent study, sonographers performing initial cervical length measurements completed an e-learning module, received clinical training to perform cervical length measurements, and had to send five cervical length measurements to be judged by an expert panel, as described in detail previously.[10]

All patients who had a cervical length of 30 mm or shorter were offered a second cervical length measurement within 14 days at a secondary or tertiary referral center for verification of short cervical length and for a quality control assessment that was necessary owing to transvaginal cervical length measurement not being incorporated in routine care at the onset of the study. Full protocol details have been published previously.[9,11]

Data from the parent study files were linked to the Netherlands Perinatal Registry (http://www.prn.nl) to obtain pregnancy outcomes for these participants and their children. At the time of the present analysis, all pregnancy outcomes until January 1, 2013, were available in the Netherlands Perinatal Registry. Consequently, all participants who had a cervical length measurement up to August 1, 2012, were selected to avoid any confounding by pregnancy outcomes of patients with an expected due date beyond January 1, 2013.

For the present analyses, all women with a primary cervical length measurement of 30 mm or less at the standard anomaly scan were included. To ensure a heterogeneous population, patients with cervical length measurement made prior to 16 weeks of pregnancy or beyond 22 weeks were included, as were patients with fetuses with congenital anomalies. These data were collected prior to exclusion from the parent study and, consequently, were available for inclusion in the present analysis.

The first and second cervical length measurements, and a mean of both, were analyzed on a continuous scale to prevent loss of information from dichotomization.[12,13] Cervical length was also analyzed as a dichotomous variable to generate a clinical-applicable cut-off value owing to clinical management using continuous information being potentially challenging. The second measurement was categorized as positive verification (≤30 mm) or negative verification (>30 mm); patients who did not receive a second measurement were not excluded but were analyzed as a separate group (classified as verification not performed). These groups of patients were compared with patients who had initial

cervical-length measurements longer than 30 mm. Linear regression analysis was used to determine if the time between the measurements was associated with differences in cervical length measurements.

A logistic regression was used to calculate the predicted risk of spontaneous preterm delivery before 37 weeks of pregnancy using cervical length as a continuous variable. Subsequently, predicted risks were plotted against cervical length and the discriminative ability of cervical length was assessed using the area under the receiver-operating-characteristic curve.

Unadjusted and adjusted logistic models were fitted to assess relations between cervical length as a dichotomous variable and spontaneous preterm delivery before 37 weeks of pregnancy. The adjusted model included the following well-known risk factors for spontaneous preterm delivery: parity, use of assisted reproductive technologies, and hypertensive disorders.[14]

Unadjusted and adjusted time-to-event analyses with Cox proportional hazards models were then used to investigate associations between cervical length and time to delivery. The adjusted model included the same variables as the previously described logistic model. Patients who had iatrogenic onset of labor and those who delivered at or beyond 37 weeks of pregnancy were excluded from this analysis.

A sensitivity analysis was performed by excluding women carrying fetuses with congenital anomalies and women with a cervical length measured prior to 16 weeks of pregnancy or later than 22 weeks. All analyses were performed with SPSS version 21.0 (IBM corporation, Armonk, New York, USA) and $P<0.05$ was considered statistically significant.

RESULTS

A total of 20234 women underwent screening in the Triple P study (between November 1, 2009, and August 1, 2013). Up to January 1, 2013, 12360 of 16204 records with pregnancy outcomes could be linked through the Netherlands Perinatal Registry. There were two patients excluded; one owing to missing cervical length data and one because of an unknown date of delivery, resulting in a cohort of 12358 patients in the present analysis.

The mean ± SD maternal age at initial cervical length measurement was 31±4.7 years, the median cervical length was 43 mm (interquartile range [IQR] 39–49), there were 5919

(47.9%) patients who were nulliparous, the median pregnancy duration at delivery was 39 weeks (IQR 38–40), and 493 (4.0%) patients experienced spontaneous preterm delivery earlier than 37 weeks of pregnancy (**Table 1**).

TABLE 1. Baseline characteristics.

Variable	Total cohort (n=12358)	Patients with a first cervical length ≤30 mm (n=221)
Maternal age, y	31 ± 4.7	30 ± 5.0
Nulliparous	5919 (47.9)	130 (58.8)
White ethnicity	10 290 (83.3)	160 (72.4)
Low social economic status	3214 (26.0)	51 (23.1)
Current smoker	45 (0.4)	1 (0.5)
Assisted reproductive technology-facilitate pregnancy	655 (5.3)	16 (7.2)
Pregnancy duration at first cervical measurement, wk (range)	20.3 ± 0.7 (16–27)	20.3 ± 0.8 (16–23)
First cervical length measurement		
Cervical length, mm	43 (39–49)	28 (25–29)
Cervical length ≤30 mm	221 (1.8)	221 (100.0)
Second cervical length measurement (n=167)		
Measurement interval, d	-	7.0 (4.0–13.0)
Cervical length, mm	-	30 (27–34)
Cervical length ≤30 mm	-	84 (50.3)
Difference between measurements, mm	-	4.0 (2.0–8.0)
Mean cervical length ≤30 mm	-	112 (67.1)
Pregnancy duration at delivery, wk	39 (38–40)	39 (37–40)
Hypertensive disorders	655 (5.3)	19 (8.6)
Spontaneous preterm delivery at <37 wk of pregnancy	493 (4.0)	39 (17.6)

Values are given as mean ± SD, number (percentage), mean ± SD (range), or median (interquartile range).

There were 221 (1.8%) patients with a first cervical length measurement of 30 mm or shorter, and a second measurement was taken for 167 (75.6%) of these patients. The median cervical length among patients with a short cervical measurement was 28 mm (IQR 25–29) for the first measurements made and 30 mm (IQR 27–34) for the second measurements. Among the patients who had second cervical length measurements taken, 84 (50.3%) had second measurements of 30 mm or shorter. The median difference between the first and second cervical length measurements was 4.0 mm (IQR 2.0–8.0) and the median interval between measurements was 7 days (IQR 4.0–13.0).

The predicted risks of spontaneous preterm delivery were plotted against cervical length (**Figure 1**). As demonstrated by the overlying lines in the plots, no differences were found

in predicted risks for spontaneous preterm delivery before 37 weeks of pregnancy when the first, second, or mean of both cervical length measurements were used. This was further confirmed by the comparable discriminative ability of cervical length in predicting preterm delivery using the first, second, or mean of both measurements, with areas under the receiver operating characteristic curves of 0.69 (95% confidence interval [CI] 0.57–0.80), 0.67 (95% CI 0.57–0.78), and 0.70 (95% CI 0.59–0.81), respectively.

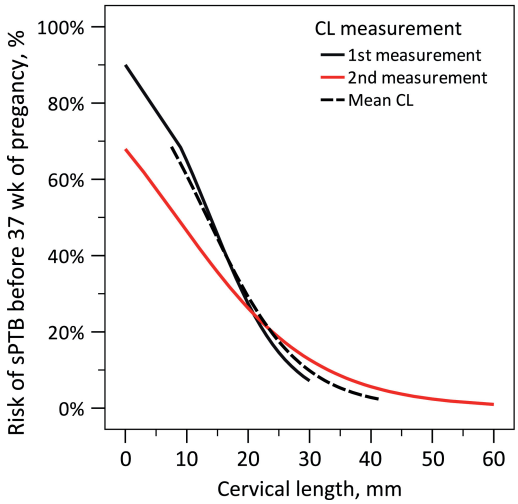

FIGURE 1. Cervical length- predicted risk of sPTD before 37 weeks of pregnancy. **Abbreviations:** sPTD, spontaneous preterm delivery; CL, cervical length.

The odds of spontaneous preterm delivery decreased in line with increasing cervical length for the first, second, or mean of both measurements, with unadjusted odds ratios (ORs) of 0.85 (95% CI 0.79–0.92), 0.91 (95% CI 0.87–0.96), and 0.88 (95% CI 0.82–0.93) per 1-mm increase in cervical length, respectively. Adjustment for known preterm delivery risk factors did not alter these results (**Table 2**).

Comparable associations were observed between cervical length and time-to-delivery with hazard ratios for spontaneous preterm delivery before 37 weeks of 0.86 (95% CI 0.81–0.91), 0.92 (95% CI 0.89–0.96), and 0.88 (95% CI 0.84–0.93) per 1-mm increase in cervical length for the first, second, and mean of both measurements, respectively (**Table 3**). Again, adjustment for known preterm delivery risk factors did not alter these results.

TABLE 2. Association between cervical length and odds of spontaneous preterm delivery before 37 weeks of pregnancy

Cervical length (continuous)	OR (95% CI)	aOR* (95% CI)
First measurement	0.85 (0.79–0.92)	0.85 (0.78–0.93)
Second measurement	0.91 (0.87–0.96)	0.91 (0.86–0.96)
Mean of both measurements	0.88 (0.82–0.93)	0.87 (0.81–0.93)

Abbreviations: *OR, odds ratio; CI, confidence interval; aOR, adjusted odds ratio.*
a Adjusted for parity, use of assisted reproductive technologies, and hypertensive disorders.

TABLE 3. Association between cervical length and time-to-delivery

Cervical length (continuous)	HR (95% CI)	aHR[a] (95% CI)
First measurement	0.86 (0.81–0.91)	0.86 (0.81–0.91)
Second measurement	0.92 (0.89–0.96)	0.91 (0.87–0.95)
Mean of both measurements	0.88 (0.84–0.93)	0.88 (0.83–0.92)

Abbreviations: *HR, hazard ratio; CI, confidence interval; aHR, adjusted hazard ratio.*
a Adjusted for parity, use of assisted reproductive technologies, and hypertensive disorders.

Among patients with an initial cervical measurement of 30 mm or shorter, 39 (17.6%) had spontaneous preterm deliveries prior to 37 weeks of pregnancy. Patients with initial cervical length measurements of 30 mm or shorter had significantly higher odds of spontaneous preterm delivery before 37 weeks of pregnancy compared with patients with an initial cervical length measurement longer than 30 mm (OR 4.0, 95% CI 2.7–5.9; adjusted OR 3.7, 95% CI 2.5–5.6) (**Table 4**).

An increased risk of spontaneous preterm delivery before 37 weeks was identified among both patients with a positive-verification second measurement (OR 5.9, 95% CI 3.0–9.2; adjusted OR 5.2, 95% CI 3.0–9.2) and those with negative-verification second measurements (OR 3.1, 95% CI 1.5–6.1; adjusted OR 3.1, 95% CI 1.5–6.2) in comparison with patients who had a cervical length above 30 mm at the initial measurement.

There were 54 (24.4%) patients with a short cervical length at initial measurement who did not have a second measurement performed. Of these, 5 (9%) had preterm deliveries prior to 37 weeks of pregnancy. This proportion did not differ significantly from women who did have second cervical length measurements (P=0.171). In comparison with patients with initial cervical length measurements above 30 mm, an increased risk of spontaneous preterm delivery before 37 weeks of pregnancy was recorded among patients who did not undergo a second measurement after an initial short measurement (OR 2.6, 95% CI 1.02–6.5). However, after adjustment, this association was not significant (adjusted OR 2.5, 95% CI 0.97–6.2) (**Table 4**).

The time between both measurements was not associated with a difference in length between the measurements (linear correlation coefficient –0.012, 95% CI –0.028 to 0.004; P=0.129) or with the risk of positive second-measurement verification (OR 0.998, 95% CI 0.993–1.003; P=0.444). Sensitivity analyses that excluded patients carrying fetuses with congenital anomalies (n=215) and those who had cervical length measured before 16 weeks of pregnancy of after 22 weeks of pregnancy (n=238) yielded similar results (**Supplemental Tables S1** and **S2**).

TABLE 4. Verification of cervical length measurements and risk of spontaneous preterm delivery before 37 weeks of pregnancy.

		Odds of spontaneous preterm delivery <37 wk of pregnancy[b]	
Cervical length	**Preterm delivery <37 wk of pregnancy[a]**	**OR (95% CI)**	**aOR[c] (95% CI)**
First cervical measurement ≤30 mm (n=221)	30 (13.6)	4.0 (2.7–5.9)	3.7 (2.5–5.6)
Second cervical measurement			
≤30 mm (n=84)	16 (19)[d]	5.9 (3.0–9.2)	5.2 (3.0–9.2)
>30 mm (n=83)	9 (11)[d]	3.1 (1.5–6.2)	3.1 (1.5–6.2)
Not performed (n=54)	5 (9)[d]	2.6 (1.0–6.5)	2.5 (1.0–6.2)

Abbreviations: *OR, odds ratio; CI, confidence interval; aOR, adjusted odds ratio.*
a Values given as number (percentage).
b In comparison with patients who had cervical length >30 mm at first measurement.
c Adjusted for parity, use of assisted reproductive technologies, and hypertensive disorders.
d No significant difference (χ^2 test); P=0.171.

DISCUSSION

In the present secondary analysis of cervical length measurement, a second cervical measurement to verify short cervical length was not necessary in patients with singleton pregnancies undergoing preterm delivery risk assessment.

A strength of the present study was that the data were collected through a nationwide collaboration of primary and secondary care within the Dutch Consortium for Women's Health (www.studies-obsgyn.nl). Consequently, the large high-quality cohort of patients with singleton pregnancies represented the Dutch general obstetric population with a representative preterm-delivery rate.[3] Consequently, it was possible to investigate whether a second cervical length measurement in women with a short cervical length was of added clinical value in risk stratification for preterm delivery. A limitation of the study was that a repeated cervical length measurement was only performed in patients with initial measurements of 30 mm or shorter and that, in the present study population, a 'dip' of cervical length measurements between 20 and 30 mm was observed, probably

as a result of the fact that assessors were not masked.[15] Consequently, partial verification bias could have been present. However, this mainly affected women who were incorrectly classified as low-risk; this could only have led to an underestimation of the effect of cervical length on preterm delivery.[16] Ideally, all primary cervical length measurements would have been repeated; however, this was not the aim of the parent study and, consequently, was logistically impossible in the present analysis. Clinically, the most significant group of patients were those with a short cervical length and it was demonstrated that an additional measurement was not necessary because the risk of preterm delivery was increased in all women with an initial short cervical length, including those who had negative verification and those who did not have a second measurement.

Another issue is whether the interval between measurements could have resulted in differences in cervical length measurements; however, no association was identified between the intervals and measurement differences. Additionally, cervical length shortens throughout pregnancy, primarily during the third trimester, and only approximately 1 mm per week during the mid-trimester period.[17]

To the best of our knowledge, this was the first study to investigate the clinical value of a second cervical length measurement to verify mid-trimester short cervical length in women with singleton pregnancies who had been diagnosed within any obstetric care setting, including primary care.[18,19] In the Netherlands, cervical length measurement in asymptomatic singleton pregnancies was not part of standard care at the time of the study. Consequently, the majority of participating sonographers had to learn to perform cervical length measurements. A potential limitation was that this learning curve could result in greater variation between measurements. Cervical length measurement is considered to be a good reproducible measurement when performed by trained ultrasonographers, with an intra and inter-observer variance of 3–5 mm.[20,21] This is comparable to the present study, where the median difference between measurements was 4 mm. Additionally, participating sonographers completed an e-learning module and were trained in cervical length measurements to improve the quality of the measurements.[11]

In general, whether the reported inter and intra-observer variances are acceptable can be debated; in clinical practice, a smaller change in cervical length than the inter and intra-observer variance can already result in a change in risk classification. This would be most likely to happen when a dichotomous cut-off value (30 mm) is used. Further, when multiple measurements are performed, the phenomenon of regression to the mean—when extreme values tend to change towards their mean—also plays a role. It

is not known if changes in risk stratification are misclassifications; however, it shows that multiple cervical length measurements do not improve accuracy and that a single measurement suffices for this purpose.

Cervical length is an important risk factor for preterm delivery; however, the relative contribution of clinical length to preterm delivery remains unclear, mainly because the prevalence of short cervical length is low and not all women with a short cervical length will deliver preterm. Conversely, promising treatments are available to prevent preterm delivery in women with a short cervical length that stimulate cervical length screening programs for the prevention of preterm delivery.[22,23]

Further research should focus on the properties and dynamics of cervical length throughout pregnancy to determine optimal risk classification to better identify women at risk for preterm delivery based on cervical length. Additionally, attempts to improve the accuracy of cervical length measurements should be made by following the recommended criteria for cervical length measurement more strictly, with the aim of minimizing measurement errors that can lead to the misclassification of patients.[24]

The present study demonstrated that a second cervical length measurement to verify a length of 30 mm or shorter during standard anomaly scan is not currently necessary; it does not further improve the identification of patients who are at increased risk of preterm delivery.

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SUPPLEMENTARY INFORMATION

Supplementary information is provided here.

SUPPLEMENTAL TABLE S1

	Spontaneous preterm delivery before 37 wk of pregnancy	
Cervical length (continuous)	**Odds ratio (95% confidence interval)**	**Adjusted odds ratio (95% confidence interval) [a]**
First measurement	0.85 (0.78–0.93)	0.85 (0.78–0.93)
Second measurement	0.91 (0.87–0.96)	0.91 (0.86–0.96)
Mean of both measurements	0.87 (0.82–0.94)	0.87 (0.81–0.93)

[a] *Adjusted for parity, assisted reproductive technologies, and hypertensive disorders.*

SUPPLEMENTAL TABLE S2

			Odds of spontaneous preterm delivery <37 wk of pregnancy	
Cervical length measurement	**No. (%)**	**Preterm delivery <37 wk of pregnancy [a]**	**OR (95% CI)**	**aOR (95% CI) [b]**
First measurement				
Cervical length ≤30 mm	212 (1.8)	28 (13)	3.9 (2.6–5.9)	3.7 (2.5–5.6)
Second measurement				
Positive verification (≤30 mm)	82 (39)	15 (18) [c]	5.8 (3.0–9.2)	5.1 (2.9–9.1)
Negative verification (>30 mm)	79 (36)	8 (10) [c]	2.9 (.5–6.2)	2.9 (1.4–6.1)
No performed	51 (25)	5 (9.8) [c]	2.8 (1.1–7.1)	2.7 (1.1–6.9)

[a] *Values given as number (percentage).*
[b] *Adjusted for parity, assisted reproductive technologies, and hypertensive disorders.*
[c] *No significant difference (χ^2 test).*

PART II

PREVENTION OF PRETERM BIRTH

CHAPTER 6

The effect of an emergency cerclage to prevent preterm birth: a systematic review

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In preparation

ABSTRACT

Objective: The aim of our review was to systematically review the literature to evaluate the available data on and the effectiveness of emergency cerclage before fetal viability (i.e. before $24^{0/7}$ weeks of gestation).

Method: We performed a comprehensive search in MEDLINE, EMBASE, CENTRAL, Cochrane, PubMed and ClinicalTrials.gov. We included randomized controlled trials, cohort studies and case-control studies comparing emergency cerclage with expectant management in singleton pregnancies with dilation of the cervix ≤5 cm at a gestational age between 14 and 24 weeks.

Results: Four studies met our inclusion criteria and were included in our systematic review which were all non-randomized retrospective studies. The final study population resulted in a total of 215 women, among whom 163 (76%) women underwent cerclage placement and 52 (24%) were expectantly managed. Emergency cerclage was associated with significant lower rates of preterm birth before 37, 34, 32, 28 and 24 weeks of gestation, significant prolongation of the pregnancy and a greater gestational age at delivery compared to expectant management.

Conclusion: The current literature suggests that emergency cerclage before 24 weeks of gestation is associated with improved pregnancy outcomes (i.e., less preterm birth) compared to expectant management. The results are limited by the lack of randomised trials and the potential for bias in the included studies.

INTRODUCTION

Cervical insufficiency is defined as the inability to support a full-term pregnancy due to an incompetent cervix.[1] Cervical insufficiency is clinically characterized by progressive shortening and dilation of the cervix before 24 weeks of gestation without overt signs of preterm labour, leading to mid-trimester pregnancy loss or extreme preterm birth and subsequent neonatal complications related to prematurity.[2, 3] The pathophysiology of cervical insufficiency remains poorly understood. Risk factors associated with cervical insufficiency are equal to those of preterm birth and include cervical surgery, including loop electrosurgical excision procedure (LEEP) and conisation, mechanical dilation of the cervix during pregnancy termination, congenital abnormalities of the cervix/uterus, deficiencies in cervical collagen and elastin, and in utero exposure to diethylstilbestrol.[4] However, in the majority of women with cervical insufficiency, these risk factors may not be present and the cause may therefore remain unknown.[4, 5]

In women with cervical insufficiency, one of the management strategies to prolong pregnancy and prevent preterm birth is the operative insertion of a cervical cerclage. Three different types of cerclage have been described: history indicated cerclage, ultrasound indicated cerclage and emergency cerclage. A history indicated cerclage is advised in several guidelines and can be inserted early in pregnancy, usually between 12 and 14 weeks of gestation, based on a previous history of unexplained very early preterm births or second trimester losses related to painless cervical dilation in the absence of labour.[6] Ultrasound indicated cerclage is performed in women with a history of preterm birth before 34 weeks of gestation and a short cervical length detected on transvaginal ultrasound between 16 and 24 weeks of gestation.[6] An emergency cerclage is generally performed in women who present with cervical effacement and dilatation on physical examination or on transvaginal ultrasound, with or without membranes bulging through the external os, before 24 weeks of gestation. These women generally have no prior history of cervical insufficiency.[6] The safety and efficacy of a cervical cerclage after fetal viability have not been adequately assessed and it is recommend that placement of a cerclage should be limited to pregnancies in the second trimester before fetal viability has been achieved.[7] Premature pre-labour rupture of membranes (PPROM), infection, preterm labor, suture displacement, bleeding and cervical laceration are the most concerning complications associated with cervical cerclage.[8, 9]

There is evidence to support the effect of a history indicated and ultrasound cerclage, mainly in singleton gestations who have a high risk of second trimester losses.[10] An ultrasound indicated cerclage is associated with significant decreases in preterm birth outcomes, as well as improvements in composite neonatal morbidity and mortality.[11]

Only limited data have suggested the possibility of benefit from emergency cerclage. There is only one randomized controlled trial evaluating the use of an emergency cerclage, and this trial included only 23 patients including 7 twin pregnancies.[12] Several nonrandomized studies have compared outcomes of women receiving an emergency cerclage, however, inclusion criteria and results from these studies vary widely. Due to the lack of larger trials that have demonstrated clear benefit, the effect of an emergency cerclage remains inconclusive.

A meta-analysis from 2015 on the efficacy of physical exam-indicated cerclage, which included the randomized trial as well as nine other cohort studies, found that exam-indicated cerclage is associated with improved neonatal survival, significant prolongation of pregnancy, decreased incidence of preterm birth between 24-28 and before 34 weeks.[13] However, this review included studies that compared emergency cerclage to no cerclage between 14 and 28 weeks of gestation. The aim of our review was to systematically review the literature to evaluate the available data on and the effectiveness of emergency cerclage in singleton pregnancies before fetal viability (i.e., before 24 weeks of gestation).

METHOD

This systematic review was reported according the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.[14] The review protocol was registered in the PROSPERO international prospective register of systematic reviews (systematic review record CRD42019137400). This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Identification and selection of studies

A medical librarian (JL) performed a comprehensive search in MEDLINE (OVID MEDLINE(R) In-Process & Other Non-Indexed Citations and OVID MEDLINE(R) 1946 to present), EMBASE, (Embase Classic and Embase from 1947), CENTRAL, Cochrane, PubMed (non-MEDLINE and in-process) and ClinicalTrials.gov. We included MeSH and keyword terms relating to pregnancy and emergency cerclage. No language restrictions were applied. Our search was limited to human studies. The latest update was February 2019. We cross-checked reference lists and citing papers of identified relevant publications via Web of Science. The complete search strategies are shown in Appendix A1. The records retrieved were imported and deduplicated in ENDNOTE X7.5.

Study selection

Two review authors (BK, CvD) independently screened title and abstract of retrieved papers. Any disagreements were resolved by consensus and, where necessary, a third reviewer was consulted (CK). Papers were eligible for screening full-text if they described singleton pregnancies with dilation of the cervix ≤5 cm at a gestational age between 14 and 24 weeks that received an emergency cerclage. Studies that evaluated cervical cerclage based on ultrasound findings with a closed cervix were ineligible for screening. After screening title and abstract, a final decision on inclusion or exclusion was made after reading all remaining articles independently in more detail to determine if the study met inclusion criteria. If the paper did not meet the inclusion criteria of the cervical dilation and/or gestational age at inclusion, we screened whether it was possible to use only a part of the published data of women that met our criteria. We included randomized controlled trials, cohort studies and case-control studies, those without a control group of expectantly managed women were excluded. In addition, we excluded studies with multiple pregnancies, ruptured membranes, regular uterine contractions or signs of labour, identified major fetal abnormalities and signs of fetal distress.

Quality assessment

Two reviewers (BK, CvD) scored all included studies systematically on their methodological quality with the use of the Newcastle-Ottawa Quality Assessment Scale (NOS). The NOS is fitted for quality assessment of analytical studies and is recommended by the Cochrane collaboration for assessing non-randomized studies.[15] The NOS contains eight items, categorised into three dimensions, each of which can be scored: selection (maximum of four stars), comparability (maximum of two stars) and outcome (maximum of three stars). We used the following thresholds for converting the NOS to good, fair and poor standards: good quality was represented by 3 or 4 stars in the selection domain AND 1 or 2 stars in the comparability domain AND 2 or 3 stars in the outcome domain; fair quality as 2 stars in the selection domain AND 1 or 2 stars in the comparability domain AND 2 or 3 stars in the outcome domain; poor quality as 0 or 1 star in the selection domain OR 0 stars in the comparability domain OR 0 or 1 stars in the outcome domain.

Outcome measures

Our primary outcome was preterm birth before 37 weeks of gestation. Additional outcome measures were: preterm birth before 34, 32, 28 and 24 weeks of gestation, composite adverse neonatal outcome, time from diagnosis to delivery, premature pre-labour rupture of membranes (PPROM) and gestational age at delivery.

Data extraction

Data were extracted by one reviewer (BK) using a predesigned extraction form, including author, year, country, study design, cohort size, period of data inclusion, gestational age at inclusion and intervention, dilation of the cervix at the time of inclusion, gestational age at delivery, time from diagnosis to delivery, premature pre-labour rupture of membranes and neonatal outcomes. Authors were contacted for additional data if it was not possible to extract all data.

Statistical analysis

We constructed two-times-two tables (event versus no event) for each included study. Results were assessed using forest plots and presented as odds ratios for the main and secondary outcomes. Odds ratio's and their 95% confidence intervals were calculated by using the Mantel Haenszel method. A p-value of <0.05 was considered to be statistically significant. Heterogeneity among the outcomes of the combined trials was determined using the χ^2 and I^2 tests. Data were pooled and analysed using either the fixed-effects (I^2 <50%) or random-effects (I^2 >50%) model according to the results of the calculation of heterogeneity. If the heterogeneity between the study was judged as substantial before calculating the I^2 and the confidence intervals of the studies overlapped each other, we judged it as most appropriate to pool the results using the random-effects model regardless the calculated heterogeneity. When pooling of results of the included studies was not appropriate, results were reported using narrative synthesis. We analysed the data from all included studies using RStudio.[16]

RESULTS

Study selection and study characteristics

The initial search resulted in 787 articles after removing duplicates, of which 774 articles were excluded after reading the title and abstract (**Figure 1**). The full text of the remaining 13 articles was assessed in more detail and 9 were excluded for the one of the following reasons: did not adequately report results for the control group (n = 1), included twins (n = 1), included pregnancies at a gestational age of more than 24 weeks without reporting subgroups (n = 6), other reasons (n = 1). The remaining 4 studies were eligible to include in the analysis, however in two out of four studies women with a dilation >5 cm were included which was an exclusion criteria in our study. From these two studies we extracted the data from all women with a cervical dilation ≤ 5 cm from the publication[17] or we received the original data if this was not possible.[18] One study reported mean gestational age at delivery only, and we received the data to extract gestational age at

delivery in more detail. This study also included a pessary group which we excluded in our analyses.[19] The final study population for this systematic review resulted in a total of 215 women with a cervical dilation ≤5 cm eligible for an emergency cerclage before 24 weeks of gestational age.[17-20] Among these 215 women, 163 (76%) women underwent cerclage placement and 52 (24%) were expectantly managed. No randomized controlled trial on the effect of emergency cerclage <24 weeks of gestation was found in the medical databases explored, therefore only non-randomized (retrospective) studies were included. Characteristics of each study are described in **Table 1**.

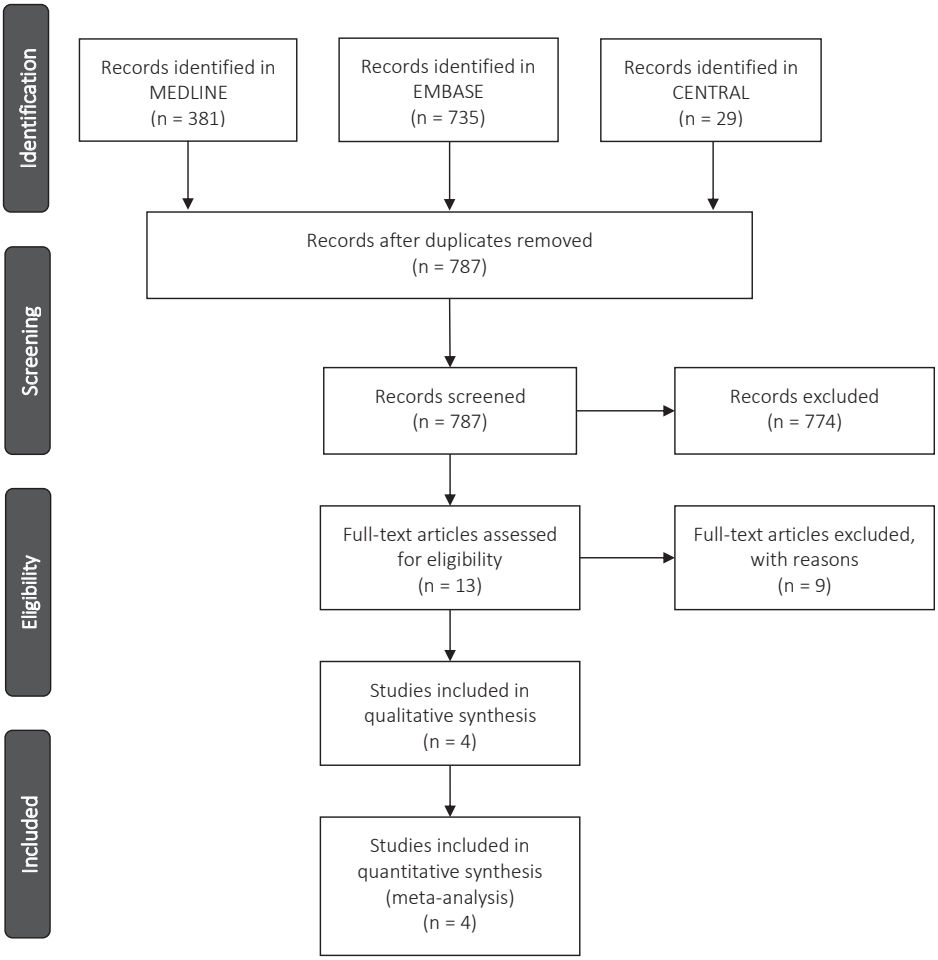


FIGURE 1. PRISMA flowchart

TABLE 1. Characteristics of each study.

Study	Type and setting	Participants	Inclusion criteria	Exclusion criteria	Outcomes	Mean GA at diagnosis/ intervention in weeks	Mean cervical dilation in cm
Ventolini et al, 2009	Retrospective cohort study, single center, United States	56 cerclage, 12 expectant management	Singleton pregnancy, gestational age 18-23 6/7 weeks, cervical dilation 3-5 cm, membranes beyond the external cervical os.	Fetal abnormalities, uterine contractions, uterine bleeding, premature rupture of membranes, clinical symptoms or laboratory findings suggestive of chorioamnionitis, urinary tract infection, bacterial vaginosis	Maternal complications (including pregnanxy loss, rupture of membranes, chorioamnionitis, excessive surgical blood loss >25 ml); gestational age at delivery, time from cerclage placement to delivery, neonatal outcomes (Apgar scores, NICU admissions, neonatal sepsis).	Cerclage group: 19.6 (±3.2); Control group: not available	Not available
Gimovsky et al, 2015	Retrospective cohort study, multicenter, United States	85 cerclage, 18 expectant management; 9 pessary	Singleton pregnancy, gestational age 15-24 weeks, cervical dilation ≥2 and ≤4, exposed membranes	Fetal anatomical or genetic anomaly, bleeding, clinical chorionamnionitis, medically indicated preterm delivery, evidence of progressive preterm labor or miscarriage	GA at delivery, interval between intervention and delivery, rate of PPROM, neonatal survival at discharge	Cerclage group: 20.2 (±2.0); Control group: 21.2 (±4.6)	Cerclage group: 2.5 (±0.6); Control group: 2.6 (±0.72)
Ciavattini et al, 2015	Retrospective cohort study, single center, Italy	18 cerclage, 19 expectant management (after exclusion: 15 cerclage; 13 expectant management)*	Viable singleton pregnancy, cervical dilation ≥1 cm, gestational age 14-24 wks	Multiple pregnancy, PPROM, clinical chorioamnionitis (maternal fever ≥38 degrees, maternal tachycardia >100 bpm, uterine tenderness, foul-smelling amniotic fluid), vaginal bleeding, ative utrine contractions, evidence of life-incompatible fetal anomalies	GA at diagnosis, GA at delivery, time to delivery, late spontaneous miscarriage 13-24 wks, term birth ≥37 wks, PTB <37 wks, PTB <34 wks, PTB <28 wks, rate of SC, neonatal birth weight, Apgar scores 1' and 5', neonatal mortality, neonatal morbidity (BPD, IVH, sepsis, NEC, ROP).	Not available	Cerclage group: 3.1 (±1.3); Control group: 3.2 (±1.6)
Cilingir et al, 2019	Retrospective cohort study, single center, Turkey	9 cerclage, 12 expectant management (after exclusion: 7 cerclage; 9 expectant management)*	Singleton pregnancy, cervical dilation ≥4 cm, protruding membranes	Dilation <4 cm, no bulging membranes, rupture of membranes before or during the procedure, chorioamnionitis (fever, malodorous vaginal discharge), regular uterine contractions, chronic diseases (cardiac pathology, hypertension, diabetes), IUGR, structural/chromosomal abnormalities	Complications of the procedure, pregnancy utcomes (time interval between admission and delivery)	Cerclage group: 18.6 (±1.1); Control group: 21.7 (±1.2)	Cerclage group: 4.4 (±0.5); Control group: 3.7 (±1.2)

** From these studies we selected women with a cervical dilation ≤ 5 cm.*

Abbreviations: *PTB = preterm birth; GA = gestational age; PPROM = premature pre-labour rupture of membranes, wks = weeks, ± = standard deviation*

Critical appraisal

Quality assessment of the included studies performed is shown in **Table 2**. All four included studies showed an overall good rate with regard to the selection and outcome domains of the study groups. The main weaknesses of all studies were their retrospective designs, small sample size and the poor comparability of the intervention and control groups.

TABLE 2. Quality assessment using the New-Castle Ottawa Scale (NOS)

		SELECTION				COMPARABILITY	OUTCOME		
Study	Year	Representativeness exposed cohort	Selection non-exposed cohort	Detection exposure	Absence outcome at start study	Comparability	Outcome assessment	Follow-up	Total
Ciavattini et al.	2015	★	★	★	★	-	★	★★	7
Cilingir et al.	2019	★	★	★	★	-	★	★★	7
Gimovsky et al.	2015	★	★	★	★	-	★	★★	7
Ventolini et al.	2009	★	★	★	★	-	★	★★	7

Outcome measures

Figure 2 shows the meta-analysis of the outcomes that were reported by at least three out of the four included studies.

Preterm birth before 37 weeks of gestation

All included studies reported the outcome preterm birth before 37 weeks of gestation. Of the 163 women who received an emergency cerclage, 116 women (71.2%) delivered before 37 weeks of gestation compared to 49 of the 52 women (94.2%) in the control group (I^2 0%; OR 0.11; 95% CI 0.03-0.35; p-value 0.0002) (**Figure 2A**).

Preterm birth before 34 weeks of gestation

All included studies reported the outcome preterm birth before 34 weeks of gestation. Out of 163 women with an emergency cerclage, 80 women (49.1%) delivered before 34 weeks of gestation compared to 45 out of 52 women (86.5%) in the control group (I^2 0%; OR 0.10; 95% CI 0.03-0.31; p-value <0.0001) (**Figure 2B**).

Preterm birth before 32 weeks of gestation

Data on deliveries before 32 weeks of gestation could be extracted from three out of the four included studies. Out of 107 women with an emergency cerclage, 55 women (51.4%) delivered before 32 weeks of gestation compared to 32 out of 40 women (80.0%) in the control group (I^2 0%; OR 0.13; 95% CI 0.04-0.43; p-value 0.0008) (**Figure 2C**).

Preterm birth before 28 weeks of gestation

Data on deliveries before 28 weeks of gestation could be extracted from three out of the four included studies. Out of 107 women with an emergency cerclage, 46 women (43.0%) delivered before 28 weeks of gestation compared to 30 out of 40 women (75.0%) in the control group (I^2 0%; OR 0.19; 95% CI 0.07-0.51; p-value 0.0010) (**Figure 2D**).

Preterm birth before 24 weeks of gestation

All included studies reported the outcome preterm birth before 24 weeks of gestation. Out of 163 women with an emergency cerclage, 38 women (23.3%) delivered before 24 weeks of gestation compared to 26 out of 52 women (50.0%) in the control group (I^2 39%; OR 0.29; 95% CI 0.13-0.65; p-value 0.0028) (**Figure 2E**).

Neonatal outcome

Data on neonatal outcome were very differently reported and could therefore not be pooled. Ciavattini et al. reported no neonatal mortality in both groups. Neonatal morbidity (described as bronchopulmonary dysplasia, intraventricular haemorrhage, sepsis, necrotizing enterocolitis and retinopathy of prematurity) occurred in 4 out of 13 (30.8%) in the control group compared to none of 15 (0%) in the cerclage group.[17] Ventolini et al reported no neonatal deaths in the cerclage group and neonatal morbidity occurred (defined as neonatal care unit admissions and neonatal sepsis) in 39 out of 56 (69.6%) neonates. Neonatal outcomes in de control group were not reported.[20] The study of Gimovsy et al. reported on neonatal survival at discharge, which was 58 out of 85 (68.2%) in the cerclage group and 7 out of 18 (38.9%) in the control group. No information on neonatal mortality or neonatal morbidity was available.[19] Cilingir et al. reported one out of 7 (14.3%) neonatal deaths in the cerclage group. No information was available for the control group. Neonatal morbidity was also not reported.[18] All neonatal deaths reported did not include mortality rates before 24 weeks of gestation.

Time from diagnosis to delivery

Three out of the four included studies reported data on the time from diagnosis to delivery. The time between diagnosis and delivery was prolonged in the cerclage group compared to the control group with a mean difference of 39.14 days (95% spatie invoegenCI 30.58-47.71; p-value <0.0001) (**Figure 2F**).

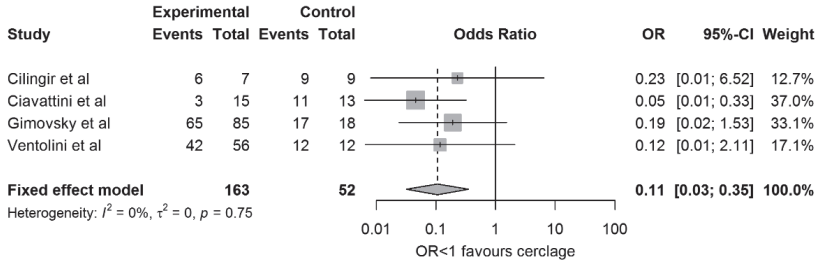
PPROM

Three out of the four included studies reported data on PPROM. Out of 148 women with an emergency cerclage, 30 women (20.3%) had PPROM compared to 11 out of 39 women (28.2%) in the control group (I^2 48%; OR 0.74; 95% CI 0.13-4.28; p-value 0.7348) (**Figure 2G**).

Gestational age at delivery

Three out of the four included studies reported data on the mean gestational age at delivery. The gestational age at delivery was greater in the cerclage group compared to the control group with a mean difference of 4.91 weeks (95% CI 2.32-7.49; p-value 0.0002) (**Figure 2H**).

A. Preterm birth < 37 weeks



B. Preterm birth < 34 weeks

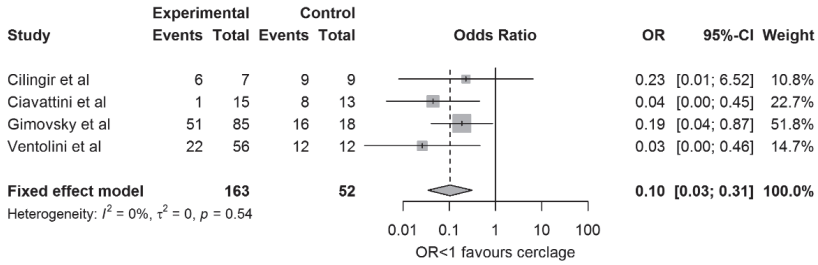
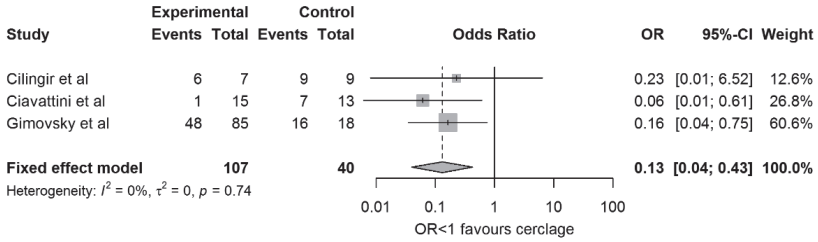
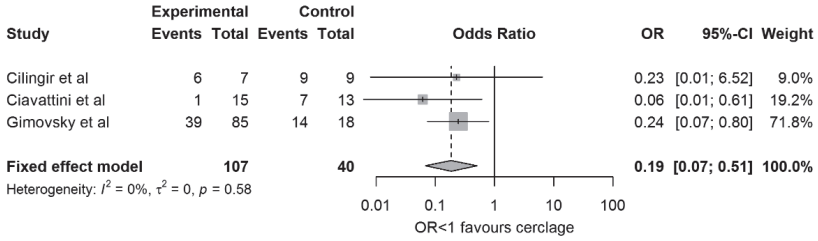


FIGURE 2. Meta-analysis of the outcomes.

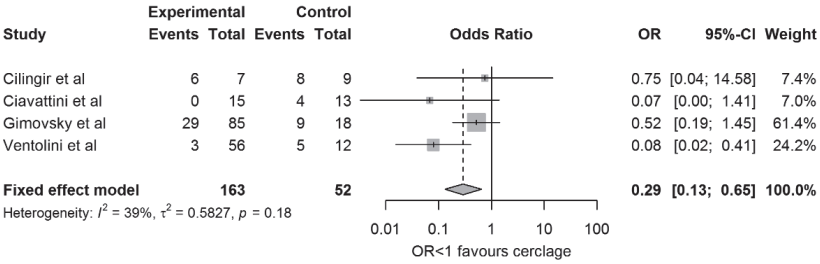
C. Preterm birth < 32 weeks



D. Preterm birth < 28 weeks



E. Preterm birth < spatie invoegen24 weeks



F. Mean time to delivery

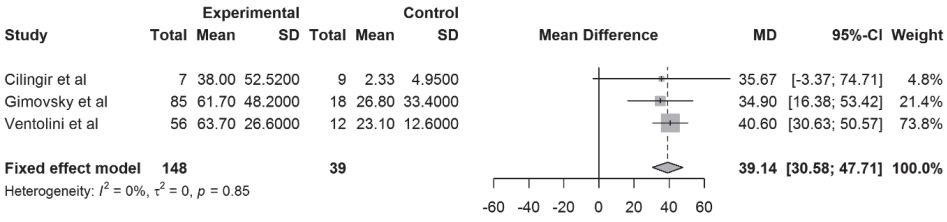
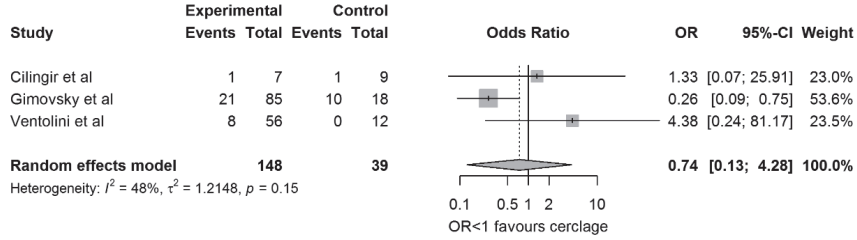


FIGURE 2. Meta-analysis of the outcomes.

G. Premature pre-labour rupture of membranes (PPROM)



H. Mean gestational age at delivery

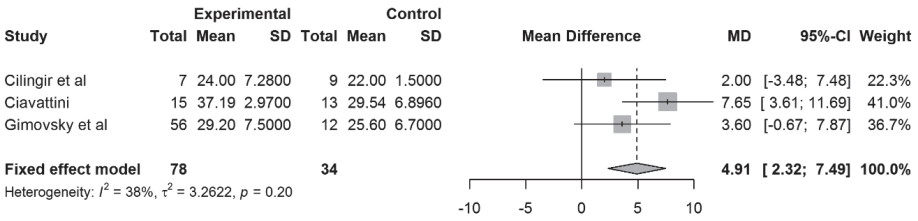


FIGURE 2. Meta-analysis of the outcomes.

6

DISCUSSION

In this systematic review our findings suggest that an emergency cerclage in women with cervical dilation and visible membranes before 24 weeks of gestation is associated with significant lower rates of preterm birth before 37 weeks of gestation compared to expectant management. Similar results were found for preterm birth before 34, 32, 28 and 24 weeks of gestation. In addition, our results suggest a significant prolongation of the pregnancy and a greater gestational age at delivery in women with an emergency cerclage compared to expectant management. There was no significant difference in the occurrence of PPROM between cervical cerclage or expectant management, however these results varied widely between the included studies. Data on neonatal outcome were very differently reported and could therefore not be pooled.

Our results are largely in agreement with the included studies that had consistent findings. A retrospective study of Vaisbuch et al. from 2010 that reported on women with no functional cervical length in the mid-trimester without cerclage shows comparable results for delivery <24 and <37 weeks compared to our results of the expectant management group.[21] In our study, 46.2% in the expectant management group delivered before 24 weeks compared to 45.2% reported by Vaisbuch et el. For delivery before 37 weeks of gestation, the numbers are 94.2% in our study and 97.6% reported by Vaisbuch

et al. A systematic review and meta-analysis from 2015 also evaluating the effect of an emergency cerclage shows similar results, however, this study included pregnancies beyond 24 weeks of gestation which was different compared to our inclusion period of <24 weeks of gestation.

A recent randomised controlled trial evaluating the effect of an emergency cerclage in twin pregnancies with asymptomatic cervical dilation from 1 to 4 cm before 24 weeks found a significant decrease in the incidence of spontaneous preterm birth at all evaluated gestational age (<34, <32, <28 and <24 weeks) and a longer latency period from diagnosis to delivery.[22] Although this trial did not evaluate singleton pregnancies, it shows the potential effect of treatment with an emergency cerclage that is similar to the results of our systematic review.

The strength of this systematic review is that it synthesizes the results of smaller studies on emergency cerclage in singleton pregnancies before 24 weeks of gestation. Although data from randomised controlled trials that evaluate the effect of an emergency cerclage is preferred, evaluating smaller observational studies can add important information to current available evidence on emergency cerclage. Evidence on cerclage treatment after fetal viability is limited and based on current evidence it is not recommended to offer cerclage placement after 24 weeks of gestation only.[6, 7] Our systematic review therefore excluded studies that evaluated the effect of an emergency cerclage >24 weeks. On the other hand, restricting cerclage placement to pregnancies <24 weeks, a subset of women, with a pregnancy >24 weeks of gestation, may not be offered an intervention that they might benefit from which makes gestational age at intervention an interesting question that could be considered to study in a well-designed trial. Furthermore, our systematic review focussed on cervical dilation of ≤5 cm while other studies include advanced stage of dilation as well.

Lack of randomized controlled trials is the most obvious limitation of this systematic review. There is one randomised controlled trial available on emergency cerclage that reports reduced risk of preterm delivery before 24 weeks of gestation and neonatal morbidity.[12] However, this trial included twin pregnancies as well as pregnancies after 24 weeks of gestation and could therefore not be included in our systematic review. Another major limitation is that there is a possibility of both selection and treatment bias in all studies, which exists with non-random allocation and retrospective character of the included studies. Neonatal outcomes were reported very inconsistent by the different studies. Therefore we cannot be absolutely sure that neonatal outcome, which actually should have been the preferred outcome in these studies, is better in the cerclage group, although it seems highly likely with the increase in gestational age in the cerclage group.

CONCLUSION

The current literature suggests that emergency cerclage before 24 weeks of gestation is associated with improved pregnancy outcomes (i.e., less preterm birth) compared to expectant management. However, prospective studies and preferably randomized controlled trials are warranted to assess the effect of an emergency cerclage and to identify the optimal candidates for an emergency cerclage.

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CHAPTER 7

A multi-centre, non-inferiority, randomised controlled trial to compare a cervical pessary with a cervical cerclage in the prevention of preterm delivery in women with short cervical length and a history of preterm birth – PC study

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BMC Pregnancy and Childbirth. 2017 Jul 6;17(1):215

ABSTRACT

Background: Preterm birth is in quantity and in severity the most important contributor of perinatal morbidity and mortality both in well- and low-resource countries. Cervical pessary and cervical cerclage are both considered as preventive treatments in women at risk for preterm birth. We aim to evaluate whether a cervical pessary can replace cervical cerclage for preventing recurrent preterm birth in women with a prior preterm birth due to cervical insufficiency or in women with a prior preterm birth and a short cervix in the current pregnancy.

Methods/design: A nationwide open-label multicentre randomised clinical trial will be set up to study women with a singleton pregnancy and a prior preterm birth before 34 weeks of gestation. Women are eligible in case of previous preterm birth based on cervical insufficiency (primary intervention, <16 weeks) or in case of previous preterm birth and a short cervical length in current pregnancy ≤25 mm (secondary intervention, <24 weeks). Eligible women will be randomised to either cervical pessary or cervical cerclage. Both interventions will be removed at labour or at 36 weeks of gestational age, whatever comes first. The primary outcome will be delivery before 32 weeks. Secondary outcomes will be gestational age at birth, preterm birth rate before 24, 28, 34 and 37 weeks of gestation (overall and stratified by spontaneous or indicated delivery), premature rupture of membranes, use of tocolysis and/or corticosteroids during pregnancy, mode of delivery, maternal infections, maternal side effects, neonatal and maternal hospital admissions, and a composite of adverse perinatal outcomes including both morbidity and mortality. We assume an event rate of 20% preterm birth before 32 weeks for cerclage and use a non-inferiority margin of 10% for the cervical pessary. Using an alpha of 0.05 and power of 0.80 we need 2 groups of 200 women each.

Discussion: The outcome of this study will indicate the effectiveness and the cost-effectiveness of a cervical cerclage and of a cervical pessary.

Trial registration: Netherlands Trial Registry, NTR 4415. Date registered: 29th of January 2014.

INTRODUCTION

Preterm birth is defined as delivery before 37 completed weeks of gestational age (GA). The incidence of preterm birth varies between countries with a range of 5–13% spatie invoegenand results in 15 million preterm deliveries worldwide each year. Preterm birth is a major contributor to perinatal mortality. Of all perinatal mortality, 50–70% is associated with preterm birth.[1]

Approximately 75% of all preterm births occur spontaneously, starting with either contractions or preterm pre-labour rupture of membranes (PPROM). Preterm birth is the leading cause of neonatal morbidity, mostly due to respiratory immaturity, intracranial haemorrhages and infections. These conditions can result in long-term neurodevelopmental sequelae such as intellectual impairment, cerebral palsy, chronic lung disease, deafness and blindness.[2] Thus, prevention of spontaneous preterm birth remains one of the biggest challenges in obstetric care.

An important risk factor for preterm birth is a prior preterm birth. Women with a prior spontaneous preterm birth before 34 weeks have an average risk of 20% (range between 15.8% and 30.2%) of recurrence of spontaneous preterm birth before 37 weeks and 15% before 34 weeks.[3,4] Women with a previous preterm birth before 34 weeks of gestation are usually advised to use progestagens, either 17-hydroxy -progesterone -caproate or vaginal progesterone, in a following pregnancy. Additionally, women with a prior preterm birth due to cervical insufficiency can be offered a primary cervical cerclage, i.e. history based cerclage. Cervical insufficiency is characterized by progressive shortening and dilatation of the cervix before 24 weeks of gestation without signs of preterm labour, and is associated with mid-trimester pregnancy loss and early preterm birth. Screening for cervical shortening by transvaginal ultrasound before 24 weeks of gestation is recommended in women with a prior preterm birth without (clear) diagnosis of cervical insufficiency in prior pregnancies. In case of a short cervix ≤25 mm before 24 weeks of gestational age, these women can be offered a secondary cervical cerclage, i.e. ultrasound indicated cerclage.[5]

A cervical cerclage is a surgical procedure that involves occlusion of the cervix by means of a cervical suture or stitch which is performed under general or spinal anaesthesia proposed by Shirodkar in 1955[6] and by McDonald in 1957.[7] A primary cerclage is considered to be effective in the prevention of preterm birth in women with cervical insufficiency and is usually offered before 16 weeks of gestational age. The largest trial published in 1993 included 1292 women with singleton pregnancies, and showed a significant reduction in preterm birth before 33 weeks of gestation (13% versus 17%; P = 0.03).[8] A meta-

analysis from 2003 demonstrates that primary cervical cerclage has a significant effect in preventing spontaneous preterm birth before 34 weeks of gestation.[9] The effectiveness of a secondary cerclage has been studied in a meta-analysis from 2011. This meta-analysis found that in these women the risk of delivery before 32 weeks' gestation was 19% in women with cerclage as compared to 30% in those without cerclage (RR 0.66 95% CI 0.48–0.91).[10]

Placement of cervical cerclages has proven to be effective in some women at risk for recurrent preterm birth. However, the disadvantage of cervical cerclage is the potential harm. Complications of cervical cerclage include PPROM, preterm labour, infection, suture displacement, and bleeding.[11] In addition, cerclage is associated with an increased risk of cervical laceration, both in nulliparous (OR 3.7, 95% CI 1.1–12.8) and multiparous women (adjusted OR 12.7, 95% CI 5.7–28.2).[12]

The cervical pessary is a soft and flexible silicone device known since 1959 when it was used in women with recurrent miscarriage.[13] Although the exact mechanism of the cervical pessary remains unknown, it has been hypothesised that the pessary relieves direct pressure on the internal cervical ostium by changing the position of the cervical canal and distributing the weight of the pregnant uterus.[14] Another possible mechanism is that the pessary might support the immunological barrier between chorioamnion-extraovular space and the vaginal microbiological flora.[15] Recently, several randomised trials showed that the cervical pessary may be potentially effective as a treatment for preterm birth prevention. The Spanish Pesario Cervical para Evitar Prematuridad (PECEP) trial from 2012 compared treatment with a pessary in women with a short cervix with expectative management and showed a significant decrease in preterm birth before 34 weeks of gestation (OR 0.19; 95% CI 0.12–0.30) and improvement of neonatal outcome (RR 0.14; 95% CI 0.04–0.39) in the intervention (pessary) group. In this study, 11% of 385 women included had at least one prior preterm birth, however, no subgroup analysis was performed for women with a previous preterm birth.[15] The same group performed a similar trial in twin pregnancies and observed a reduction in spontaneous preterm birth before 34 weeks of gestation in the pessary group (16.2% versus 25.7%; p= 0.0001).[16] Another randomized controlled trial performed by Liem et al. in 2014 comparing a pessary with no treatment in twin pregnancies showed similar results in a subgroup with short cervix.[17] Two randomised trials coordinated by the Fetal Medicine Network from the United Kingdom could not confirm these results of the pessary, both in singleton and multiple pregnancies with short cervix.[18,19] The most frequently reported side effect of a pessary is vaginal discharge. Less common reported complications during the use of a pessary are vaginal blood loss or pelvic pain. Cervical laceration as complication is rarely seen in the use of pessary, this chance seems smaller than 0.1%.

Since a cervical pessary can be positioned in an outpatient setting, it is less expensive than placing a surgical cerclage and is therefore potentially more attractive than a cerclage. In addition, a cervical pessary is a non-invasive method contrary to a cervical cerclage which is an invasive procedure. Although both interventions have been available for over 55 years now, both interventions have been compared directly only once. A randomised study performed in 1986 in Germany in women with a prior preterm birth included 242 women and did show comparable outcomes in women using a pessary and women having cerclage (37^{+5} weeks of gestational age at delivery in the cerclage group versus 37^{+1} weeks in the pessary group, p value not significant).[20]

We propose to compare the cervical pessary and cervical cerclage in a head-to-head comparison and hypothesize that the use of a cervical pessary will be equally effective in preventing preterm birth as cervical cerclage. The outcome of the proposed study will indicate the relative effectiveness of cervical pessary for women with a singleton pregnancy with a prior preterm birth due to cervical insufficiency and in women with a prior preterm birth and short cervical length in current pregnancy. In addition, we will be able to compare the costs of both interventions. Since the placement of a pessary is less expensive compared to the surgical application of a cerclage, implementation of this therapy will potentially yield a major cost-reduction.

METHODS/DESIGN

Aim, design and setting

We will perform an international randomised controlled trial under the acronym the PC Study (Pessary or Cerclage to prevent preterm delivery in women with short cervical length and a history of preterm birth; Netherlands Trial Registry NTR 4415, registered at the 29th of January 2014: website http://www.trialregister.nl/trialreg/admin/rctvie-w.asp?TC=4415). The study will assess the effect of a cervical pessary on preterm birth rates and neonatal outcome compared to treatment with a cervical cerclage. The study is set in the Dutch Consortium for Healthcare Evaluation and Research in Obstetrics and Gynaecology - NVOG Consortium 2.0, a collaborative network of all major hospitals in The Netherlands and the Dutch Society of Obstetrics and Gynaecology (NVOG). In addition, international hospitals interested in the trial can participate in this study.

Participants

According to local protocols, asymptomatic women with a singleton pregnancy and a prior spontaneous preterm birth before 34 weeks of gestation are offered the use of

progesterone and cervical length measurements before 24 weeks of gestation. Women with a cervix ≤25 mm before 24 weeks of gestation are eligible to participate in the trial as these women would be eligible for a secondary cerclage. Additionally, women who are considered for a placement of a cerclage before 16 weeks gestation based on their obstetric history of cervical insufficiency (primary cerclage) are eligible.

Eligibility criteria

All women with an indication for a primary or secondary cerclage, as described above, are eligible to participate in this study. Women with placenta praevia, vasa praevia, PPROM, cervical length of less than 2 mm, cervical dilation of 3 cm or more, identified major congenital or chromosomal abnormalities and women with signs of intrauterine infection will be excluded from the study. In addition, maternal age less than 18 years and inability to give informed consent are exclusion criteria.

Procedures, recruitment, randomisation and collection of baseline data

All eligible women will be informed in brief about the clinical trial by the supervising gynaecologist or by the attending resident. Subsequently, the investigator or an authorised member of the investigational staff must explain to potential subjects the aims, methods, reasonably anticipated benefits, and potential hazards of the study. Women will be informed that their participation is voluntary and that they may withdraw consent to participate at any time. They will be informed that choosing not to participate will not affect the care they will receive.

Each woman must give written consent prior to randomisation. The woman will be given sufficient time to read the patient information and the informed consent form and have the opportunity to ask questions. An independent physician will be accessible for any questions the women may have. The consent form must be signed before any study-related activity can take place. A copy of the informed consent form must be given to the participating woman. Patient information is provided in Dutch and English. Women who meet all inclusion criteria but decline to participate are asked to be included in an observational cohort (see **Figure 1**).

Randomisation will be centrally controlled using an online computerised randomisation service made specifically for randomization in clinical trials, ALEA (https://nl.tena-lea.net/amc/ALEA/). Centres will be able to access the randomisation service 24 h/day. Eligible women will be randomized in a 1:1 ratio to cervical cerclage and pessary (see **Figure 1**). Randomisation will be stratified by indication for type of cerclage (primary or secondary) and centre (to prevent any imbalance between groups in aspects of maternal or neonatal

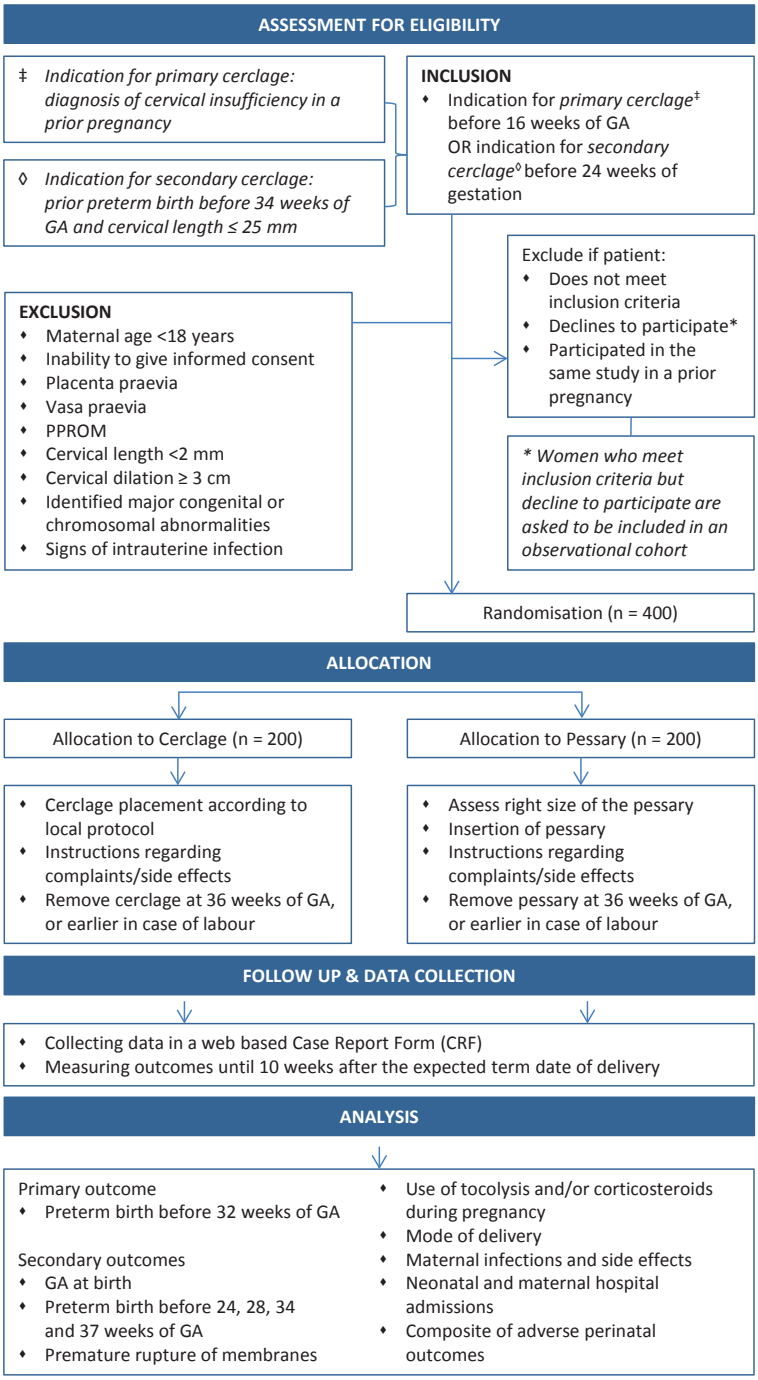

FIGURE 1. Flow diagram PC Study

care that may differ between centres). We will apply block randomisation with a variable block size of 4 and 6. Due to the type of interventions this study will not be blinded. Baseline characteristics (e.g. patient demographics, obstetric and medical history), details of delivery, maternal and neonatal assessments during pregnancy or post-partum will be recorded into a web-based Case Report Form (CRF) that is accessible through a closed part of a web-secured database (see **Figure 1**). We included core outcomes for preterm birth in the CRF.[21] The CRF can be found in Additional file 1.

Confidentiality and data security

Initials of participants as well as year of birth are recorded in the electronic database. Linking personal data with randomisation number can only be done in the local clinics. Each participating clinic receives a login name and password to gain access to the web-secured database. The access is restricted to the database of the clinic to which the password and login name belongs. Full access to the entire database is possible to some members of the research staff, but has to be requested via the trial bureau and data manager of the NVOG Consortium 2.0.

Intervention

Eligible women will be randomly allocated to receive either a cervical cerclage or a cervical pessary (Arabin® pessary). Both will be placed before 24 weeks, or before 16 weeks in case of a primary intervention. Women allocated to a cervical cerclage will be receiving the intervention according to local protocol. Women allocated to a cervical pessary will receive a simple vaginal examination to assess which size pessary fits best. It is important that the pessary is placed by a care giver with expertise to ensure careful placement of the pessary. In case of complaints, (vaginal) examination of the patient is advised to reposition the pessary or to replace the pessary with another size if necessary. Both interventions will stay in place until 36 weeks of gestation or until delivery, whatever comes first. If recurrent or persistent blood loss, premature rupture of the membranes or contractions occur during the use of a pessary, the pessary should be removed. Further management will be according to the national guideline on prevention of preterm birth and local protocols (see **Figure 1**).

Outcome measures

Primary outcome measure

The primary outcome will be delivery before 32 weeks of gestation.

Secondary outcome measures

Secondary outcomes will be time from intervention to delivery, gestational age at birth, preterm birth rate before 24, 28, 34 and 37 weeks of gestation (overall and stratified by spontaneous or indicated delivery), premature rupture of membranes, use of tocolysis and/or corticosteroids during pregnancy, mode of delivery, maternal infections, maternal side effects and both neonatal and maternal hospital admissions. Perinatal outcome will be assessed through a composite of adverse perinatal outcome. This composite outcome contains chronic lung disease, intraventricular haemorrhage (IVH) > grade II, periventricular leucomalacia (PVL) > grade I, necrotising enterocolitis (NEC) > stage I, retinopathy of prematurity (ROP), patent ductus arteriosus (PDA), treated seizures, early and late onset sepsis, neonatal meningitis, (intra-partum) stillbirth, death before discharge from the nursery. The definitions of these outcomes can be found in **Table 1**. All components of the composite outcome will also be assessed individually. In addition, a cost-effectiveness analysis will be performed that will be reported separately from the primary report on the randomised trial.

TABLE 1. Definitions of secondary outcome measures

Outcome	Defined as:
Maternal infections	Two measurements of maternal temperature above 37,8 degrees Celsius at a one hour interval and a maternal pulse >100 beats per minute requiring treatment with antibiotics
Maternal side effects	Vaginal discharge, bleeding, discomfort, dyspareunia, cervical laceration
Chronic lung disease	Babies born before 32 weeks: need for >30% oxygen, with or without positive pressure ventilation or continuous positive pressure at 36 weeks postmenstrual age, or discharge (whichever comes first). Babies born after 32 weeks: need for >30% oxygen with or without positive pressure ventilation or continuous positive pressure at 56 days postnatal age, or discharge (whichever comes first).
IVH > grade II	Haemorrhage in the germinal matrix, ventricles, or cerebral parenchyma; observed on ultrasound examination or MRI
PVL > grade I	Periventricular lucency in the white matter
NEC > stage I	Defined as the presence of the characteristic clinical features of abdominal distension, with or without rectal bleeding, and abdominal radiographic finding associated with pneumatosis intestinalis
Early sepsis	If prior to or at 72 h of life the infant had an infection marked by positive blood, CSF, or urine (catheterised or suprapubic) cultures with or without suspicious clinical findings of infection on physical examination.
Late sepsis	If after 72 h of life the infant had an infection marked by positive blood, CSF, or urine (catheterised or suprapubic) cultures with or without suspicious clinical findings of infection on physical examination OR if there is clinical evidence of cardiovascular collapse or an unequivocal X-ray confirming infection and often cardiovascular decomposition
Neonatal meningitis	Suspected or proven (caused by any pathogen)

Follow up of women and infants

All details of delivery, maternal assessments and admittance during pregnancy will be recorded in an electronic case record form that will be accessible through the web-secured database. In case of admittance of a newborn, details of admittance will also be recorded. The outcome measures, when applicable, will be measured until 10 weeks after the expected term date of delivery.

The possibilities to perform long term follow-up will be assessed and planned, depending on the outcomes of the primary study and granted funding. Permission to approach women for follow-up research will be asked by the initial informed consent.

Statistical issues

Sample size

We plan to evaluate the non-inferiority of a cervical pessary as compared to cervical cerclage. We assume an event rate of 20% for the primary outcome, i.e. delivery before 32 weeks, for cerclage based on current literature. We will use a non-inferiority margin of 10%. If the event rate is 20% for the primary outcome in the cerclage arm, this is equivalent to saying that a pessary is non-inferior to cerclage when the upper limit of the 95% confidence interval of the event rate of the primary outcome in the pessary group is less than 30%. Using a one-sided alpha of 0.05 and power of 0.80 we need 2 groups of 200 women each.

Data analysis

Data will initially be analysed according to the intention to treat method. The primary outcome will be assessed investigating whether the prevalence of the primary outcome, birth before 32 weeks, is not more than 10% higher in the pessary group compared to the cerclage group. Non-inferiority will be concluded when the upper end of a one-sided 95% confidence interval for the risk difference between the prevalence of the primary outcome in the pessary group and the cerclage group is less than 10%.

The secondary outcome time from intervention to delivery will be evaluated by Cox proportional hazard analysis and Kaplan-Meier estimates and plots, with account for different durations of gestation at entry and stratification by indication for intervention (and centre when data allows), and will be tested with the log rank test. Secondary dichotomous outcome measures will be assessed by calculating absolute and relative risks, along with 95% confidence intervals. Differences in continuous outcomes between both strategies will be assessed using a linear mixed model.

In all analyses, stratification by centre will be accounted for with a random intercept for each centre, and by adding the cerclage indication as a covariate to the log-binomial or linear mixed models. If these models fail, A Cochran–Mantel–Haenszel (CMH) approach will be used to take into account that randomisation was stratified on indication for cerclage (and centre, if the data allows). Such a stratified analysis of the estimates the risk differences from each covariate subgroup and uses CMH weights to estimate treatment difference and its standard error. When appropriate, numbers needed to treat will be calculated.

Subgroup analysis

We prespecify three subgroup analyses based on: (1) the indication of the type of cerclage to investigate the effectiveness of the pessary compared to a primary and secondary cerclage separately, (2) the number of previous preterm births (overall and separately for indication for primary or secondary intervention) in which we distinguish those with one previous preterm birth from those with two or more previous preterm births and (3) cervical length ≤15 mm and >15 mm in women with an indication for secondary intervention. Subgroup effects will be investigated for the primary outcome, preterm delivery before 32 weeks of gestation, and for the composite of perinatal outcome. Subgroup effects will be assessed by including an interaction term between the subgrouping variable and treatment allocation as covariate to the regression model. Afterwards, a stratified subgroup analysis will be performed to study the effect of treatment in different strata of the subgroups.

To evaluate the potential of each of the strategies, we will also perform a per protocol analysis, taking into account only those cases that were treated according to protocol.

Safety

(Serious) Adverse Event ([S]AE)

All AEs reported spontaneously by the subject or observed by the investigator or his staff will be recorded. All SAEs will be reported through the web portal ToetsingOnline to the accredited medical ethics committee (MEC) that approved the protocol.

Interim safety review

Safety reviews will be performed after all outcomes of 110 inclusions are available for analysis, and thereafter as determined necessary by the independent data and safety monitoring board (DSMB). The DSMB will be unblinded before making recommendations,

but the researchers are to remain blinded. An extra meeting will be planned if indicated by the safety review. The data and safety monitoring committee can advise to stop the study for safety reasons.

DISCUSSION

To our knowledge there are no other registered ongoing trials comparing the effect of a cervical pessary and a cervical cerclage in women at high risk for preterm birth.

When the pessary was first described in 1959, it was used in women with recurrent late miscarriages and possible cervical insufficiency.[13] The largest randomised controlled trial so far shows no difference between cervical cerclage and pessary in women with previous spontaneous preterm birth and an indication for a cerclage.[20] In addition, the recent PECEP study, a study which compared treatment with a pessary in women with a short cervix with expectative management, included 11% women with at least one prior preterm birth. This study showed an overall significant decrease in preterm birth in the intervention (pessary) group (spontaneous delivery before 34 weeks 12 (6%) in the pessary group vs. 51 (27%) in de control group; OR 0.18; 95% CI 0.08–0.37; $p < 0{\cdot}0001$), however, no subgroup analysis was performed for women with a previous preterm birth.[15] There are clues that a cervical pessary might be as effective as a cerclage in the prevention of preterm birth, however, large recent randomized controlled trials with information on the effectiveness of a pessary in women with a previous preterm birth are lacking.

A cervical cerclage is considered to be effective in the prevention of preterm birth in women with cervical insufficiency and/or short cervix during pregnancy with a previous preterm birth. However, it is associated with serious risks such as premature rupture of membranes, premature contractions, cervical and/or uterine infections, vaginal bleeding and cervical laceration.[11,12] Although the chance of these complications occurring is indeed low, the impact on the course of the pregnancy is major. Additionally, cervical cerclage is a surgical intervention which is usually performed under general anaesthesia, and as such is at risk of surgical complications. A cervical pessary is a non-invasive intervention and can be placed in an outpatient setting. In addition, severe complications related to cerclage are considered to occur more often compared to complications related to the use of pessary. This makes a pessary more attractive as intervention, however, the effect on preterm birth and neonatal outcome should be addressed first.

The outcome of this study will determine whether treatment with a cervical pessary can replace a cerclage to prevent preterm birth in women at high-risk for preterm delivery.

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SUPPLEMENTARY INFORMATION

The supplementary information is provided online with the published version of this chapter:
https://bmcpregnancychildbirth.biomedcentral.com/articles/10.1186/s12884-017-1393-6

PART III

NOVEL INTERVENTIONS

CHAPTER 8

Prevention of preterm birth: Novel interventions for the cervix

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Seminars in Perinatology. ***2017 Dec;41(8):505-510***

ABSTRACT

Preterm birth is the leading cause of neonatal mortality and morbidity worldwide. Spontaneous preterm birth is a complex, multifactorial condition in which cervical dysfunction plays an important role in some women. Current treatment options for cervical dysfunction include cerclage and supplemental progesterone. In addition, cervical pessary is being studied in research protocols. However, cerclage, supplemental progesterone and cervical pessary have well known limitations and there is a strong need for alternate treatment options. In this review, we discuss two novel interventions to treat cervical dysfunction: (1) injectable, silk protein-based biomaterials for cervical tissue augmentation (injectable cerclage) and (2) a patient-specific pessary. Three-dimensional computer simulation of the cervix is performed to provide a biomechanical rationale for the interventions. Further development of these novel interventions could lead to new treatment options for women with cervical dysfunction.

INTRODUCTION

Preterm birth, defined as birth before 37 weeks of gestation, is the major contributor of neonatal mortality and morbidity worldwide.[1] It can cause respiratory immaturity, intracranial hemorrhages and infections, and these conditions can result in a range of long-term complications such as intellectual impairment, cerebral palsy, chronic lung disease, deafness and blindness.[2] The frequency and severity of adverse outcomes of children born preterm increase with decreasing gestational age at birth.[1] Preterm birth is an important complication of both singleton and multifetal pregnancies and preventing preterm birth remains a challenge in clinical obstetric care.[1,2]

The worldwide incidence of preterm birth is 11.1%, which varies between countries within a range of 5–13% and results in approximately 15 million children born preterm each year.[3] The highest rates occur in Southeastern and South Asia where 13.4% of the children are born preterm. Approximately 1.2 million preterm births occur in high-income countries, of which more than 0.5 million occur in the United States where the estimated preterm birth rate is 11–12%.[1]

Although preterm birth is a complex, multifactorial condition, in some women, cervical dysfunction plays an important role.[4] The composition and structure of the cervix controls its ability to remain closed during pregnancy to promote fetal development. In normal delivery, cervical effacement and dilation occurs at term. In preterm delivery, cervical effacement and dilation occurs prior to term, which can lead to a premature birth. Cervical dysfunction is detected by measuring a short cervix with transvaginal ultrasound.[5–7] The risk of preterm birth is inversely proportional to the length of the cervix, with a shorter cervix conferring a higher risk.[6]

Various treatment strategies to prevent preterm birth in women with suspected dysfunctional cervix have been studied, including cervical cerclage, cervical pessary and progesterone.[8] However, these treatment strategies are not effective in all patient populations at risk for preterm birth. There remains a strong need for alternative, effective therapies for preventing preterm birth in women with a dysfunctional cervix. In this review, we discuss two novel interventions to treat cervical dysfunction that are currently being studied; injectable, silk protein-based biomaterials for cervical tissue augmentation (injectable cerclage) and a patient-specific pessary. We also demonstrate a three-dimensional computer simulation of the interventions to provide a biomechanical rationale for efficacy. These complementary interventions aim to address the pathogenesis of cervical dysfunction and to support the native, physiological properties of the cervix.

INJECTABLE BIOMATERIALS FOR CERVICAL AUGMENTATION

Cervical remodeling

In spontaneous preterm birth, the final common event is softening, shortening, and dilation of the cervix, also referred to as cervical remodeling. Cervical remodeling relates to both changes in (1) the material properties of the stroma (i.e., softening) and (2) the anatomical shape of the cervix (i.e., shortening, effacement, and dilation).[4] There is a strong relationship between cervical remodeling and the organization and composition of the cervical extracellular matrix (ECM). The cervical ECM plays a key role in the maintenance of appropriate mechanical function of the cervix.[9] Excessive cervical softening appears to be related to preterm birth.[9] Preventing excessive softening, i.e., reestablishing the normal properties of the stroma, is a promising clinical target for the development of interventions that aim to prevent cervical dysfunction and preterm birth.[4]

Cervical cerclage

Currently, a cervical cerclage is an important treatment option for the prevention of preterm birth in women with suspected cervical dysfunction.[10–13] The fact that cervical cerclage is an effective treatment for a short cervix[13] suggests that cervical dysfunction is causally related to preterm birth in some women. A cervical cerclage is a surgical procedure in which a suture is placed in the stroma to provide added support for the cervix, as proposed by Shirodkar in 1955[14] and by McDonald in 1957.[15] Although a cerclage was efficacious in some studies,[13] no efficacy was seen in other studies.[16] In twins, cerclage may be associated with increased risk of adverse neonatal outcomes.[17] Moreover, placing a cerclage is not without risk. Complications of cerclage include preterm premature rupture of membranes (PPROM), infection, preterm labor, suture displacement, and bleeding.[18] In addition, cerclage is associated with an increased risk of cervical laceration, both in nulliparous and multiparous women.[19]

It is hypothesized that a cervical cerclage prevents premature cervical remodeling by providing support to the cervix. The exact mechanism, however, by which a cerclage provides support and prevents premature cervical remodeling remains unclear. In addition, a cerclage does not address excessive cervical softening, which likely relates to cerclage failure in some women. A comprehensive understanding of the complex process of cervical remodeling and the relationship between biochemical and mechanical properties of the cervix could lead to a more effective intervention to prevent spontaneous preterm birth.[4]

Cervical shortening - cause or consequence of preterm birth

Whether a short cervix causes preterm birth or is a consequence of a different pathophysiology is difficult to determine in individual patients. When a cerclage is not successful, it suggests either (1) the cerclage did not provide adequate support or (2) the cause was unrelated to cervical dysfunction. The most common pathophysiology associated with cervical shortening is infection and inflammation. Among women with clinically diagnosed cervical insufficiency (defined as cervical dilation ≥1.5 cm), the risk of intrauterine infection is 8–51%.[20,21] Among women with a short cervix, the levels of inflammatory cytokines (i.e., MMP-8 and IL-6) are increased.[22–24] In addition, the risk of adverse pregnancy outcomes are increased when cervical dysfunction and intrauterine infection/inflammation are present.[20,21,23,24]

Although infection and inflammation may be present in cases of cervical dysfunction, it is difficult to know the natural history of ascending infection in pregnancy. It is possible that ascending infection leads to subsequent cervical shortening/insufficiency. It is also possible that infection occurs as a consequence of a short cervix. If ascending infection is a consequence of a short cervix, a therapy that prevents cervical shortening could help prevent ascending infection. A hypothesis of this research is that, in some cases, a short cervix leads to an impaired cervical barrier to infection. We advocate for the importance of a detailed study of cervical biomechanics of cervical shortening, which could reveal interventions that provide better support for the cervix compared to present therapies. Improved interventions for the cervix could not only treat cases of cervical insufficiency but also prevent ascending infection in cases where a short cervix is the cause.

Preventing cervical shortening - insights from biomechanical modeling

Although a short cervix plays a central role in the management of women at risk for preterm birth, a detailed understanding of the deformation mechanisms leading to a short cervix is lacking.[4,25] Insight into the key variables that influence cervical deformation can be gained from biomechanical modeling.[26,27] Studies of cervical biomechanics demonstrate that cervical shortening is a complex biomechanical problem influenced by multiple variables including anatomical geometry, cervical loading, and cervical material properties.[26,27] In particular, it is known that the cervical material properties soften during the course of pregnancy[28] and excessive cervical softening likely leads to preterm shortening and dilation. The central hypothesis of our work is that a treatment that improves the functional performance of the cervical tissue could prevent cervical shortening and hence preterm birth. This concept is demonstrated in an initial biomechanical simulation model

showing improving stromal properties reduces the amount of cervical stretching at the internal os (**Figure 1**). As a first attempt to test this hypothesis, we developed a prototype, injectable, silk-based gel to be used for cervical tissue augmentation.[29–31]

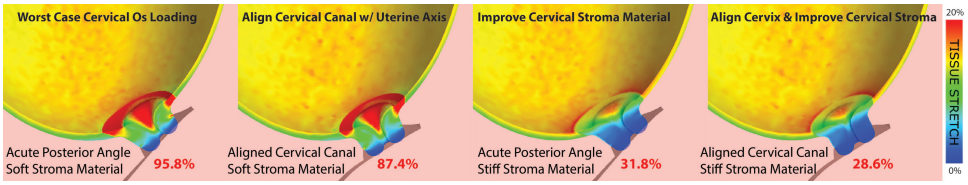

FIGURE 1. Computer simulation results of improving cervical stroma material and cervical angle. The increase in stromal material stiffness and the alignment of the cervical canal with the uterine axis reduces the amount of tissue stretching at the internal os. Percentages indicate the volume ratio of the internal os that is above 8% tensile strain under intrauterine pressure of 8.67 kPA. Details of the finite element model are found in Westervelt et al.[48] A soft and stiff cervix are assigned collagen fiber moduli of 1.71 kPa and 769 kPa, respectively.

Injectable cerclage

Silk fibroin is a fibrous protein that is naturally derived and displays remarkable mechanical properties, chemical flexibility, and biocompatibility.[32] Purified silk protein can be processed into biodegradable gels with tunable mechanical properties, which are important features for a treatment for pregnancy.[33] The physical properties of silk biomaterials can be further modified by blending with other materials to meet functional demands and can therefore suit a wide range of biomedical applications.[34–36] In a recent report, Brown et al.[31] studied a range of injectable silk-based materials for cervical augmentation. In this study, purified silk protein solutions were cross-linked by an enzyme catalyzed reaction to form elastic biomaterials, which were formulated to match the intrinsic properties of cervical tissue during pregnancy.[31] From 108 different silk biomaterial formulations that were screened for mechanical properties in pregnant and non-pregnant tissue, two optimized formulations were further evaluated for biocompatibility, facile injection and in vitro degradation. In vitro degradation of these formulations was studied using concentrated protease solution, which showed tunable control of degradation rate based on the hydrogel formulation. In addition, cervical fibroblasts cultured on these biomaterials were proliferative and metabolically active. Furthermore, in vitro injection of human cervical tissue required low injection force and showed that tissue volume could be increased without significant influence on cervical stiffness. These elastic silk gels are a promising initial prototype for augmentation of cervical tissue during pregnancy.[31]

Preliminary animal study

Biocompatibility and feasibility of cervical injections were studied in timed-pregnant New Zealand White rabbits, which is an accepted animal model to study biochemical changes of the cervix during pregnancy.[37–39] Sterile, sonicated silk was prepared as previously described.[30] Injections were performed via a midline laparotomy approach according to an IACUC approved protocol. Cervical injections were performed on gestational day 15. A laparotomy was performed and the vagina was brought to the abdomen. The wall of the vagina was incised to allow direct visualization of the two cervices (**Figure 2A**). Using a 20-gauge needle, approximately 0.5 mL of sonicated silk hydrogel was injected into the cervical stroma (**Figure 2B**). Outcomes were compared to 4–0 Mersilene suture (**Figure 2C**), which was chosen as a control because it is a common non-absorbable suture used for cervical cerclage. After the cervical procedure, the vagina and abdomen were closed. Cervical treatments were well tolerated; no preterm birth was seen. Silk gel was grossly visualized within the cervical tissue both after injection (**Figure 2B**) and at sacrifice (**Figure 2D**), which occurred at day 27. Histology revealed multifocal deposits of silk gel surrounded by a thin wall of macrophages, lymphocytes, plasma cells, eosinophils, and occasional multinucleated giant cells (**Figure 2E**). A similar response was seen around the suture control (**Figure 2F**). The histological changes appear consistent with a mild foreign body response. These preliminary results in a limited number of animals demonstrate that cervical injections were well tolerated by pregnant rabbits. However, data regarding biodegradation or labor outcomes in vivo are currently lacking and will be assessed in future studies.

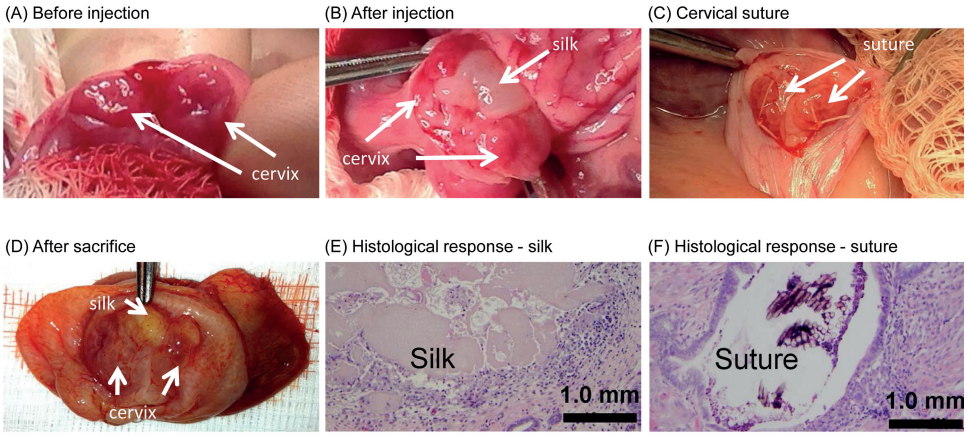

FIGURE 2. Cervical augmentation with silk: (**A**) two separate cervices in rabbit before injection; (**B**) silk immediately after injection, gestational day 15; (**C**) cervical 4–0 Mersilene suture; (**D**) injected silk after sacrifice, gestational day 27; (**E**) mild foreign body inflammatory response to silk biomaterial; and (**F**) mild foreign body inflammatory response to 4–0 Mersilene suture.

PATIENT-SPECIFIC PESSARY

Arabin pessary

The cervical pessary is a flexible silicone device known since 1959 when it was used in women with recurrent miscarriage.[40] The exact mechanism of the cervical pessary remains unknown, yet it has been hypothesized that the pessary relieves the pressure on the internal cervical os by changing the position of the cervical canal and distributing the weight of the uterus and fetus.[41] Hence, the pessary may have the potential to prevent premature shortening and dilatation of the cervix, and premature rupture of the membranes. Another proposed mechanism is that the pessary might support the immunological barrier between chorioamnion-extraovular space and the vaginal microbiological flora.[42] However, the clinical effect of a cervical pessary in the prevention of preterm birth in both singleton and multifetal pregnancies remains unclear since conflicting results have been published so far.[42–46]

A preliminary biomechanical computational analysis of the pessary demonstrates that the device applies contact forces on the outer surface of the cervix to close the cervical canal.[47] This application results in a complex and heterogeneous loading of the cervix that ultimately reduces the amount of tissue stretching at the internal os and increases the amount of tissue compression at the outer surface.[47] In other words, the pessary squeezes the cervix to force closure of the canal. The squeezing action causes tissue compression, and the magnitude of tissue compression depends on the mismatch of pessary and cervix diameters. The biological and functional consequences of this change in tissue loading remain to be determined. Additionally, a biomechanical analysis of multiple cervical canal angle scenarios demonstrates that when the cervical canal is perfectly aligned with the longitudinal uterine axis the mechanical load is better distributed and the tissue stretching at the internal os is at its minimum. Conversely, if the cervical angle is moved posterior, away from the uterine axis, the amount of tissue stretching at the internal os increases with no sizable reduction of pressure on the cervix (**Figure 1**).[48]

Patient-specific pessary

A patient-specific cervical pessary may prevent preterm birth by a similar mechanism to the Arabin pessary, with additional advantages. The basis of a patient-specific pessary is a custom-fit device to maternal anatomy to ensure a reduction of contact pressure on the outer cervix and a reduction of tissue stretching at the internal os by cervical canal alignment with the uterine axis (**Figure 1**).[48] Every pregnancy can have a vastly different anatomy,[49] leading to drastically different mechanical loading patterns on the lower

uterine segment, fetal membranes, and internal os of the cervix.[48] The Arabin pessary currently comes in 9 standard sizes, which may not be an exact fit for all patients, resulting in a pessary that is too loose, tight, short, or tall, leading to discomfort and misplacement of load support. With the acceleration of additive manufacturing processes for flexible surgical-grade materials[50] and computational models of the biomechanical environment of pregnancy,[48] a custom-fit pessary device that beneficially distributes the mechanical load is feasible.

As mentioned previously, the length of the cervix is proportional to one's risk of preterm birth.[6] Two recent studies investigating cervical angle correlation to PTB showed conflicting results. One study found that an extreme posterior angle is associated with PTB,[51] while the other did not find any correlation.[52] The discrepancy in these clinical studies highlights the fact that the mechanisms causing cervical shortening and subsequent preterm birth is multifactorial. A recent biomechanical computational study exploring cervical anatomical and stroma material properties found the largest amount of cervical tissue stretching is found for a soft cervix (i.e., a cervix that has gone through biochemical remodeling) and an acute posterior cervical canal angle (**Figure 1**).[48] Changing the acute angle alone, such that the cervical canal aligns with the uterine longitudinal axis, gives an 8.8% relative reduction in cervical tissue stretching. Changing the stromal material properties alone, such that the cervix is made of a stiffer material, gives a 67% relative reduction in cervical tissue stretching. Changing both the cervical canal angle and the stroma material properties gives a 70% relative reduction in cervical tissue stretching. These computational results suggest a multifactorial approach may be effective to reducing the amount of cervical tissue deformation and stretching and highlight the multifactorial nature of the biomechanical environment of the cervix.

How to design a patient-specific pessary

A patient-specific pessary can be designed by obtaining a patient's anatomy via transabdominal and transvaginal ultrasound and creating a complementary device that appropriately tilts the cervix in the correct direction and fits onto the outer cervix to compress the cervical canal with minimal contact pressure. A custom-fit device requires maternal anatomy dimensions such as the cervical length, anterior uterocervical angle, external cervical diameter, and height of the vaginal canal. A custom pessary should have (1) an inner diameter that matches the outer diameter of the patient, with an inner diameter lip that has a surface area reaching down the cervical length to reduce contact pressure and (2) a device height that accounts for vaginal canal dimensions and cervical canal angle such that the device can align the cervix with the uterus and the device can reach as close to the internal os as possible. The device can then be validated by running

a computational simulation of the device geometry placed within a computer model of the patient's anatomy to verify that it will truly reduce the mechanical loading on the cervix.

CONCLUSION AND FUTURE DIRECTIONS

Greater understanding of the molecular, biochemical and biomechanical function of the cervix will enhance our understanding of the physiological process of cervical function in term and preterm delivery. This multidisciplinary approach opens the field for novel interventions, such as the injectable cerclage and personalized pessary, which aim to interfere with the underlying mechanism of premature cervical softening, shortening and dilation.[4] For these preventive interventions to be useful in clinical care, a clear diagnosis of cervical dysfunction/insufficiency is critical to identify patients at risk for spontaneous preterm birth. Yet, due to the lack of objective findings and criteria for the diagnosis of cervical insufficiency, the identification of women with a dysfunctional cervix remains a challenging task for care givers. This challenge reflects the complexity of preterm birth in which multiple pathways seem to be involved. Multi-disciplinary collaboration involving clinicians, biologists and engineers, could promote a better understanding of the mechanisms underlying cervical dysfunction leading to pre-term birth. In addition, a multidisciplinary approach to the dilemma of preterm birth could lead to novel interventions that could be used in the prevention of preterm birth.

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CHAPTER 9

Cervical augmentation with an injectable silk-based gel: biocompatibility in a rat model of pregnancy

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Reproductive Sciences. 2020 May;27(5):1215-1221

ABSTRACT

The aim of this study was to study the biocompatibility of an injectable silk gel in the cervix in a rat model of pregnancy. The rationale is to study an injectable gel as an alternate treatment for cervical insufficiency. We further aimed to perform cervical injections via a vaginal route to mimic the clinical procedure of a cervical cerclage. We performed an in vivo study in pregnant female Sprague Dawley rats. Cervical procedures were performed using a customized speculum under general anesthesia. Injections were performed on gestational day 16. The responses to silk gel injections were compared to polyethylene terephthalate suture and saline controls on gestational day 19 and postpartum. The inflammatory response was evaluated by histology, PCR for inflammatory gene expression, and ELISA for protein levels of proinflammatory mediators. Silk gel injections were performed on 13 animals. All animals tolerated the procedure. Silk gel occupied 5% of the stroma after injection. Injected silk gel caused neither preterm birth nor prolonged pregnancy and had no effect on the kits. When comparing inflammatory responses, expression of inflammatory genes and proinflammatory proteins in the silk gel group was intermediate between saline (lowest) and cerclage suture (highest). Injectable silk gel was more inflammatory compared to saline injections but less inflammatory compared to the suture material used for cervical cerclage. This study is an important step toward development of an alternative treatment for cervical insufficiency.

INTRODUCTION

Globally, preterm birth is the leading cause of death of children less than 5 years old.[1] The worldwide incidence of preterm birth is 11.1%, which results in nearly 15 million children born preterm each year.[2] Infants that survive preterm birth are at increased risk of long-term complications such as intellectual impairment, cerebral palsy, chronic lung disease, deafness, and blindness.[3]

Cervical insufficiency is a significant cause of preterm birth.[4] In a large study of infants born before 28 weeks, cervical insufficiency was seen in 5% of cases.[5] Also, USA natality records document that cerclage procedures are performed in approximately 15,000 pregnancies per year.[6] Though cerclage can be effective in some patients, it has significant limitations. Cerclage surgery and cerclage removal can be challenging. The procedure risk for ultrasound-indicated and physical examination-indicated cerclage is 0.3% and 0.9%, respectively.[7–9] Also, cerclage does not address the pathogenesis of cervical insufficiency, which is suspected to be impaired mechanical properties of the cervical stroma.[10] The development of an effective alternative treatment for cervical insufficiency would have a significant clinical impact worldwide.

We previously reported on injectable silk-based gels for cervical tissue augmentation as an alternative treatment for cervical insufficiency.[11–13] The rationale for the development of an injectable gel is to provide support to the cervical stroma which could prevent cervical shortening, improve membrane protection, and prevent preterm birth. Injection of a silk gel will increase cervical volume. Augmentation of cervical volume could counteract forces that act to cause funneling and membrane prolapse. Augmentation of cervical stroma will also create a composite tissue with properties that could be stronger than the native stroma.

The silk gel is composed of silk fibroin, which is a fibrous protein with remarkable mechanical properties, chemical flexibility, and biocompatibility.[14] In a previous in vivo study, gelation of silk fibroin was accelerated with sonication. Though sonication promotes silk gelation, an injectable gel that requires sonication is cumbersome for clinical use.[12] In a more recent in vitro study, silk gelation occurred with a crosslinking reaction catalyzed by hydrogen peroxide and horseradish peroxidase (HRP).[13] The HRP-catalyzed silk gel was elastic and flexible. Also, the compressive stress-strain profile and the peak stresses were within the range of native pregnant and nonpregnant tissue.[13,15] The hydrogels were slightly stiffer than pregnant cervical tissue yet softer than nonpregnant tissue, making them ideal for tissue augmentation. However, the biocompatibility of the HRP-catalyzed silk gel was not studied.

The aim of this study was to evaluate the biocompatibility of HRP-catalyzed silk gel in a rat model of pregnancy. We further sought to perform in vivo cervical injections via a vaginal route to mimic clinical use. We hypothesized that the silk gel would show comparable biocompatibility to suture materials used for cervical cerclage surgery. This project is part of a long-term effort to develop an alternative treatment for cervical insufficiency.

METHODS

Preparation of silk fibroin solution and enzymatically cross-linked silk gels

Silk fibroin protein was purified as we have previously described.[13] Briefly, Bombyx mori cocoons were extracted for 60 min to remove the sericin coating from the fibroin fibers. Fibers were rinsed in deionized water, dissolved in a 9.3 M LiBr solution, and dialyzed against water for 72 h to remove LiBr. Enzymatically cross-linked silk gels were prepared as previously described.[15] Silk solutions were diluted to 5% concentration (w/v). Horseradish peroxidase (HRP), type VI lyophilized powder (Sigma-Aldrich, St. Louis, MO), was reconstituted in deionized water and added to the silk solution in a ratio of 10 U HRP to 1 mL of silk solution. Gelation was initiated by adding 10 µL of a 0.5% (v/v in water) hydrogen peroxide solution per 1 mL of silk-HRP (final H_2O_2 molarity 1.63 mM). The parameters of the cross-linking reaction were selected to yield gels which were soft, flexible, and injectable through a 23 gauge needle. Gels were allowed to set in an incubator at 37°C for 2 h. All materials were sterilized by filtration through a 0.22 µm filter before gelation.

Animals

Pregnant female Sprague Dawley rats at gestational day 16 (22 day gestation), obtained from Charles River, were used in all experiments. Day 16 was chosen for hydrogel injection into the tissue because preliminary experiments indicated that hydrogel injection into the rat cervix before day 16 is more difficult because the tissue was stiff. Since the purpose of the study was to study biocompatibility, it was decided to perform hydrogel injection when the tissue was soft. All rats were maintained on a normal chow diet. Animal care and the experimental procedures were carried out in accordance with National Institute of Health guidelines and approved by the Tufts Institutional Animal Care and Use Committee (IACUC protocol B2017-97). General anesthesia was induced with inhaled 3% isoflurane in oxygen and maintained with 1.5% isoflurane. After the pregnant rat was fully anesthetized, the genital area was disinfected with an ethanol wipe 3 times. A customized, conical speculum was placed into the vagina (**Figure 1**). An adjustable light was used to shine into the speculum to optimize visualization of the

cervix (**Figure 1A**). In the silk gel group, a total of approximately 200 µL of silk-gel was injected into four locations (50 µL per injection) using 23 g × 1 ½ needles (**Figure 1B**). To compare the inflammatory response of the silk gel to suture materials used for cerclage surgery, a polyethylene terephthalate suture (5–0 Dacron, Alcon Surgical, Inc., Fort Worth, TX) was sutured in the cervical stroma (**Figure 1C**). Polyethylene terephthalate (PET) suture was chosen because PET suture is the most common suture material used for cerclage. As a negative control, a saline injection was performed in a manner similar to the silk gel injections. The needle and speculum were removed immediately after the procedure. The rats were placed in their cage to recover from the anesthesia. As a second negative control, there was a "no injection" group. The "no injection" group was not exposed to the speculum or anesthesia.

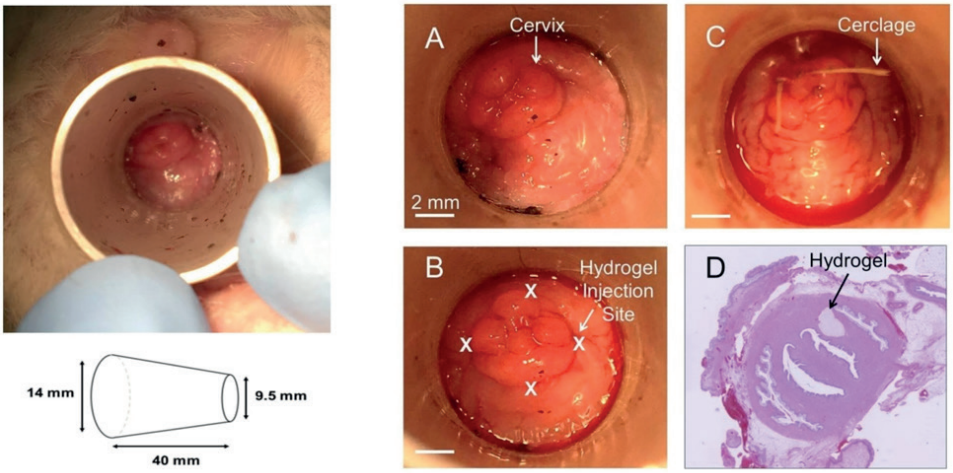

FIGURE 1. Visualization of the cervix using a customized speculum. (**A**) The cervix prior to cervical injection. (**B**) The cervix after silk gel injection. (**C**) Polyethylene terephthalate (PET) 5–0 cerclage suture placed in the cervix. Of note the suture was not placed around the cervix like as in clinical practice due to size constraints. (**D**) Silk gel in the cervical stroma.

The cervix was studied at three time points after surgery. A schematic overview of the different numbers of animals and experiments is presented in **Figure 2**:

- Time point 1: sacrificed immediately after surgery on gestational day 16 to quantify the amount of silk gel in the tissue (N =3)
- Time point 2: sacrificed on gestational day 19 for histology (N = 3 silk gel; N = 2 cerclage) and for RNA and protein assays (N = 4 silk gel; N = 4 cerclage; N = 4 saline injection)

- Time point 3: sacrificed on postpartum day 3, for effect on parturition and histology (N = 3 silk gel; N = 3 saline injection; N = 3 no injection)

Cervical excision was performed using general anesthesia and a laparotomy. The animal was sacrificed after the cervix was excised. The cervix was either snap frozen in liquid nitrogen for RNA and protein assays or fixed in formalin for H&E histology.

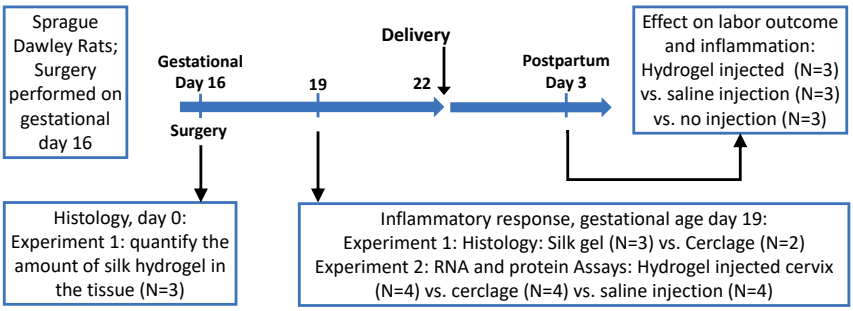

FIGURE 2. Schematic overview of the experiment protocol. Sacrificed animals on gestational day 16 (time point 1) to quantify the amount of silk gel in the tissue; gestational day 19 (time point 2) for histology and for RNA and protein assays; postpartum day 3 (time point 3) for effect on parturition and histology.

Determination of silk gel and inflammatory response in cervical tissue

Formalin-fixed whole cervices were embedded in paraffin, cut in cross-section at 500 µm intervals, and stained with hematoxylin and eosin (H&E). In order to capture an entire cervical specimen at high magnification, image stitching module of Keyence Microscope BZ-X700 was used. Briefly, after the coordinates of its outermost positions were registered, multiple images were joined together without stitch lines, and a single high-resolution image was created. ImageJ (NIH) was used to quantify silk gel areas in cervical histological samples.[16] Twenty-four sections of cervical specimens from three silk gel-injected animals were studied. To assess the inflammatory response, slides of the silk gel injection group and cerclage suture group were reviewed by a veterinary pathologist, blinded to treatment group.

Isolation of RNA and Quantitative RT-PCR

Gene expression of inflammation-related transcripts was compared between saline injection (negative control) group, silk gel injection group, and cerclage suture group. RNA was isolated using an RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. One microgram of RNA sample was used for reverse transcription and

synthesis of cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) following the manufacturer's instructions. Quantitative real-time PCR was performed on a QuantStudio 7 Flex (Applied Biosystems) using SYBR Green PCR Master Mix (Qiagen). All genes were normalized by the housekeeping gene β-actin. Quantification was expressed as fold change relative to gene expression in the saline control group using the 2–(ΔΔCt) method. Predesigned RT-PCR primers for rat inflammatory target genes were purchased from Sigma-Aldrich (Ccr2, R_Ccr2_1; Cd68, R_Cd68_1; Ccr7, R_Ccr7_1; IL6, R_Il6_1; Ccl2, R_Ccl2_1; Tnf, R_Tnf_1; Actb, R_Actb_1).

ELISA

The protein levels of proinflammatory mediators, IL-6 and IL-8, in the tissues were compared between saline injection (negative control) group, silk gel injection group, and cerclage suture group. Native proteins from the cervix were isolated using Total Protein Extraction Kit (BioChain Institute, Inc.). The protein levels of IL-6 and IL-8 in the tissue lysate were determined using commercially available ELISA kits (rat IL-6 ELISA kit from Invitrogen and rat IL-8 ELISA kit from MyBioSource) according to manufacturer's instructions. Protein levels were normalized to the level seen in the saline injection group.

Statistical Analysis

Data are expressed as mean ± SEM. Mean values of protein and RNA assays were calculated from four replicates as indicated. We used the Shapiro-Wilk test to test for normal distribution of the data. Comparisons between means were performed using the one-way ANOVA or Student's t-test and analysis of variance with Bonferroni multiple comparison test where appropriate (GraphPad Prism ver. 5.04, San Diego, California). Results were considered significant at p value <0.05.

RESULTS

Cervical Injections with Silk Gel

A total of 13 animals were injected with silk gel (**Figure 2**). All animals tolerated the injections. No procedure-related complications were seen. Of note, in preliminary experiments, an episiotomy was performed in order to aid visualization of the cervix. However, it was found that with correct sizing of the speculum and blunt dilation of the vagina, an episiotomy was not needed.

Quantification of the Amount of Silk Gel in the Tissue

Histology showed the presence of silk gel in the cervical stroma (**Figure 1D**). Total cervical length was approximately 4 mm. Silk gel was seen in distal 2 mm of cervix; no silk gel was seen in the proximal 2 mm of the cervix (i.e., cervix closest to uterus). Quantification of the percentage of silk gel in the stroma revealed that the mean ± standard deviation was 4.7±1.1% of gel in the stroma area (**Figure 1D; Figure 3**).

Inflammatory Response

At gestation day 19, 3 days after injection, histology of the stroma showed a mild inflammatory response surrounding both the silk gel and the cerclage suture (**Figure 3**). Macrophages, together with smaller numbers of neutrophils and eosinophils, were seen at the interface of the gel and suture.

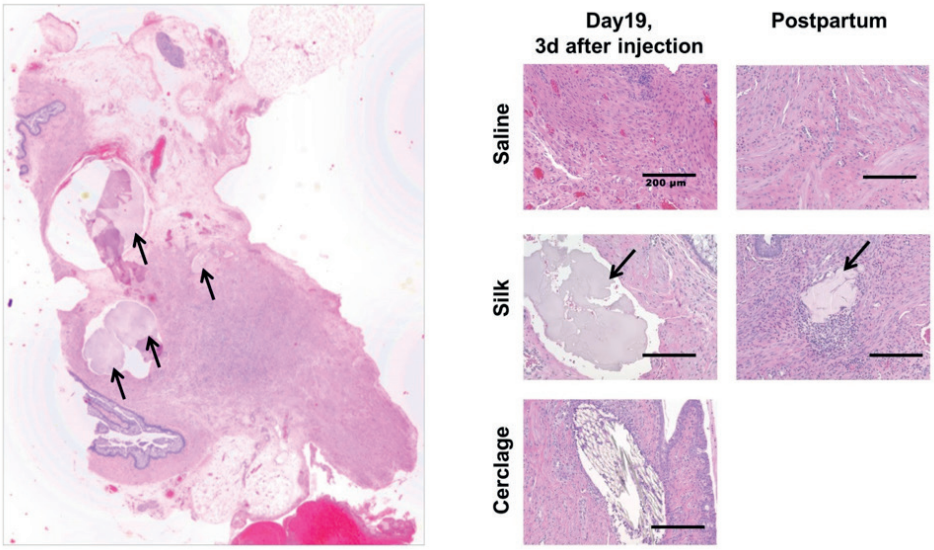

FIGURE 3. Left: Silk gel was quantified in the stroma by visualizing the gel as a homogeneous, pale area separate from the tissue. Silk gel is indicated by the arrows. Silk gel occupied approximately 5% of the tissue area. **Right**: Three days after injection, a mild inflammatory reaction was seen around the silk gel and cerclage suture. A mild, postpartum inflammatory reaction was also seen around the silk gel. The arrows indicate the silk gel. The scale bar is 200 µm.

Three days postpartum (9 days postinjection), silk gel was persistently visualized in the cervical stroma. Again, there was a mild inflammatory response characterized by macrophages, neutrophils, and eosinophils. Thin tendrils of collagen fibers were seen at the gel surface, suggesting an early fibrous response. No postpartum histology data

was available for the cerclage group as delivery with a cervical cerclage in situ is not relevant. Saline control showed minimal inflammatory response on histology 3 days after injection and 3 days postpartum.

Effect on Labor

There was no difference in timing of delivery and number of live pups (**Table 1**) between silk gel, saline injection, and no injection groups ($p = 0.59$).

TABLE 1. Timing of delivery and number of live pups

Rat #	Group	GA at experiment	GA at delivery	No. of pups
1	Silk	E16	E22	14
2	Silk	E16	E22	12
3	Silk	E16	E22	14
4	Saline	E16	E22	11
5	Saline	E16	E22	14
6	Saline	E16	E22	12
7	No injection	E15	E22	12
8	No injection	E15	E22	13
9	No injection	E15	E22	13

Proinflammatory Gene Expression

Expression levels of inflammation-related genes were compared between silk gel, cerclage, and saline groups 3 days after surgery (**Figure 4A**). The data of the proinflammatory gene expression was normally distributed using the Shapiro-Wilk test.

IL-6: The expression level of key pro-inflammatory cytokine interleukin-6 (IL-6) was similar between the silk gel and saline groups but significantly increased in the cerclage group (fold change 1.8 ± 0.22, $p < 0.05$).

Cd68: Cd68 is a marker for tissue macrophages. The expression level was similar between silk gel and saline groups, but significantly increased expression was seen in the cerclage group (fold change 1.4 ± 0.08, $p < 0.05$).

Ccr7: Ccr7 has an important role in the migration of memory T cells to inflamed tissues, as well as stimulating dendritic cell maturation and activation of dendritic cells. The expression level was similar between silk gel and saline groups, but significantly increased expression was seen in the cerclage group (fold change 1.7 ± 0.15, $p < 0.05$).

Ccl2: Ccl2 (monocyte chemoattractant protein 1, MCP1) is a key chemokine that regulates migration and infiltration of monocytes and macrophages to the site of inflammation. Both the silk gel group and the cerclage group showed increased Ccl2 expression level compared to the saline group (fold change 1.7 ± 0.08 and 2.3 ± 0.26, respectively, $p < 0.05$). Ccl2 expression level in the silk gel group was significantly lower compared to the cerclage group. The expression of the receptor for Ccl2 and Ccr2 was similar in all three groups.

TNFα: Tumor necrosis factor alpha is an inflammatory cytokine produced by macrophages and monocytes during acute inflammation and is responsible for a range of signaling events within cells leading to necrosis or apoptosis. The expression level of TNFα in the silk gel and cerclage group was not significantly different. Expression levels of TNFα were significantly increased in both the silk gel and cerclage groups compared to saline (fold change 2.7 ± 0.83 and 2.1 ± 0.37, respectively, $p < 0.05$).

The Protein Level of Proinflammatory Mediators: IL-6 and IL-8

The data for IL-6 and for IL-8 was normally distributed using the Shapiro-Wilk test. ELISA assay showed no significant difference in the level of IL-6 in the silk gel group compared to the saline group (**Figure 4B**). In the cerclage group, significantly increased IL-6 levels were seen compared to the saline and silk gel group. In both silk gel and cerclage groups, increased levels of IL-8 protein were seen compared to the saline group (1.6 ± 0.02 and 2.0 ± 0.16, respectively, vs. saline group, $p < 0.05$). The IL-8 level in the cerclage group was significantly increased compared to the IL-8 level in the silk gel group.

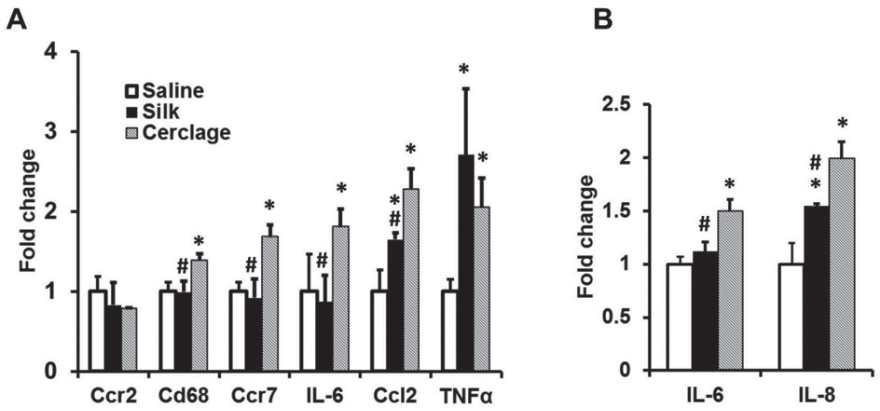

FIGURE 4. (**A**) Expression of inflammatory genes in the silk gel, cerclage, and saline groups. Expression of inflammatory genes in the silk gel group was intermediate between saline (lowest) and cerclage (highest). (**B**) Protein assay for IL-6 and IL-8. Levels of IL-6 were highest in the cerclage group. For IL-8, levels in the silk gel group were intermediate between saline (lowest) and cerclage highest (* $p < 0.05$ vs. saline; # $p < 0.05$ vs. cerclage).

DISCUSSION

Cervical injection of HRP-catalyzed silk gel was biocompatible in a rat model of pregnancy. Vaginal injections were feasible and well tolerated; no adverse reactions were seen. The inflammatory response after cervical injections with silk gel was increased compared to saline injections but less inflammatory compared to the suture material used for cervical cerclage. This study supports further development of an injectable silk gel as an alternative to cerclage in pregnancy.

A pregnant rat model was used to assess the biocompatibility and immune response of the silk gel in vivo. Using a customized speculum, silk gel was injected into the cervix in a manner that mimicked clinical use. Data from histology, gene expression, and protein assays suggest that biocompatibility of silk gel was improved over cerclage controls. The silk gel neither impeded labor nor caused preterm birth. After 3 days postpartum, silk gel was persistently visualized in the stroma, and the inflammatory response appeared mild.

Use of a pregnant rat model for cervical injections is novel, and several limitations were seen. Pilot experiments showed that injection into the cervix prior to day 16 was difficult because the tissue was stiff and visualization of hydrogel in the stroma was inconsistent. Injection of hydrogel at day 16 was more reliable and allowed us to study acute inflammatory effects. Injection at day 16, however, did not permit study of long-term inflammatory changes. A larger animal model will be needed to study long-term inflammatory changes and efficacy of preterm birth prevention. Also, quantification of the silk gel in the stroma showed the gel comprised only 5% of stroma area, which limited studies of efficacy of preterm birth prevention.

The most common cervical suture material in clinical use is nonabsorbable polyethylene terephthalate (PET).[17] Prior studies of suture material focused on the clinical efficacy of PET braided thread (Mersilene™ or Ethibond™) versus PET5-mm tape (Mersilene™).[17] No difference in clinical efficacy was found. However, biocompatibility of PET in the cervix has not been previously studied. Presumably nonabsorbable PET produces a foreign body response in the cervical stroma. Also PET 5-mm tape is associated with vaginal microbiome dysbiosis when compared with a monofilament suture.[18] Whether the foreign body response or vaginal dysbiosis affects clinical efficacy is not known. Biocompatibility of injectable biomaterials (e.g., silk gel) needs to be compared to PET prior to human use. In the present study, biocompatibility of silk gel was less inflammatory compared to PET. However, the effect on the vaginal microbiome of silk gel is still unknown.

The purpose of an injectable gel is to augment cervical tissue to improve cervical function. In addition, the gel will degrade over time (e.g., proteolysis) such that labor will not be impeded.[19] The present study, however, did not assess efficacy of silk gel with respect to cervical augmentation or biodegradation. The amount of silk gel injected into the cervix did not significantly increase cervical volume because the rat cervical target was too small. Thus, the augmentation properties and degradation profile were not studied. A larger animal model will be needed to study cervical augmentation and silk degradation.

The rationale for developing an injectable gel is to improve cervical function in cases of cervical dysfunction. Although a cerclage provides load bearing support, the precise contribution of a cerclage to the biomechanical integrity of the cervix is not known. Preliminary biomechanical studies of cervical integrity demonstrate that cervical shortening is a complex problem influenced by multiple variables including anatomical geometry, cervical loading, and cervical material properties.[20,21] We hypothesize injection of a silk gel that could improve function in two ways. First, cervical augmentation will increase tissue volume, and increased volume could prevent membrane funneling and retain cervical mucus. Second, excessive tissue softening could be countered by a silk gel with increased stiffness.[13] Prior studies have shown that significant cervical softening occurs as early as the first trimester.[22] Cervical softness is clinically important because an abnormally soft cervix is hypothesized to cause cervical insufficiency.[10] While a cerclage provides load bearing support, it does not address the problem of excessive tissue softening. We hypothesize that augmentation of cervical tissue with an injectable gel could stiffen cervical tissue and thus improve function.

Purified silk protein is a promising starting point for an injectable gel for the cervix. The stiffness of the gel can be controlled by modifying the cross-linking reaction and the molecular weight of the protein.[13] The degradation of silk biomaterials can also be controlled. Although silk sutures are categorized as nonabsorbable, biodegradation of silk biomaterials can be accelerated by changing the purification and concentration of silk protein.[19,23] Future studies will be needed to determine the optimum stiffness and degradation profile of the silk gel for clinical use.

CONCLUSION

In conclusion, we report on an HRP-catalyzed silk gel that is biocompatible in vivo with decreased inflammatory response comparable to cervical cerclage. These results are important steps toward the design of an alternative and safe therapy to treat cervical insufficiency. Future work will focus on cervical augmentation properties and biodegradation of silk gel in larger animal models.

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CHAPTER 10

Scaffolds for cervical tissue engineering

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Handbook of Tissue Engineering Scaffolds. Volume 2, chapter 46

ABSTRACT

Normal function of the cervix is critical to a healthy pregnancy. Abnormal cervical function can lead to preterm birth, which is a significant problem in both developing and developed countries. When the cervix fails prematurely, it leads to a clinical condition termed cervical insufficiency. In pregnancies affected by cervical insufficiency, the cervix dilates painlessly, which causes a preterm birth. Treatment options for cervical insufficiency (e.g., cerclage) and cervical shortening (e.g., progesterone supplementation) are not effective in all patients, and many patients with optimal treatment progress to a preterm birth. In this chapter, we discuss tissue engineering scaffolds (1) to study remodeling of the cervical stroma and (2) to repair cervical tissue in pregnancies at risk for preterm birth because of cervical insufficiency. In addition, tissue engineering of the cervix has not been limited to clinical conditions related to pregnancy. Several scaffold-free approaches for generating 3D cancer models of the cervix are reviewed.

INTRODUCTION

Normal function of the cervix is critical to a healthy pregnancy. Abnormal cervical function can lead to preterm birth, which is a significant problem in both developing and developed countries. In healthy pregnancy, the cervix remains closed until the onset of strong uterine contractions at term. In preterm birth, the cervix can dilate prematurely, without uterine contractions. Infants born prematurely are at risk of chronic long-term illnesses including lung disease, eye disease, and neurodevelopmental abnormalities.[1] The cost of caring for preterm newborns is expensive. The Institute of Medicine estimated the cost per preterm newborn in the United States was $51,600.[2]

The cervix is a cylindrical structure with a central canal and forms an anatomical connection between the lower uterus and upper vagina. The central cervical canal connects the uterine cavity to the vaginal canal. The opening of the canal into the uterus is called the internal cervical os. The opening into the vagina is called the external os. The central canal is lined by a single layer of columnar epithelium. The body of the cervix, the stroma, is composed of collagen-rich, fibrous connective tissue with a small amount of smooth muscle.[3] The cervix and upper vagina are supported in the pelvis by the cardinal and uterosacral ligaments, which maintain the position of the cervix during pregnancy.[4,5] The fetal membranes are in direct contact with the superior surface of the cervix at the internal os.

When the cervix fails prematurely, it leads to a clinical condition termed cervical insufficiency.[6] In pregnancies affected by cervical insufficiency, the cervix dilates painlessly, which causes a preterm birth. The diagnosis of cervical insufficiency is made clinically. A typical patient has a history of one or two preterm births caused by marked cervical dilation but minimal uterine contractility. Once the diagnosis is established, the treatment is cervical cerclage. In a cerclage, the cervix is exposed with a speculum and a surgical stitch is placed around the cervix. The rationale of the cerclage is to prevent dilation by providing load-bearing support. A cerclage stitch remains in place until term. At term, the cerclage is removed to permit normal labor. The health burden of cervical insufficiency is significant. United States natality records document that cerclage procedures are performed in 0.3%–0.4% of pregnancies, which is approximately 15,000 procedures per year.[7]

In this chapter, we discuss tissue engineering scaffolds (1) to study remodeling of the cervical stroma and (2) to repair cervical tissue in pregnancies at risk for preterm birth because of cervical insufficiency. In addition, tissue engineering of the cervix has not been limited to clinical conditions related to pregnancy. Several scaffold-free approaches for generating 3D cancer models of the cervix are reviewed.

MECHANICAL ROLE OF THE CERVIX IN PREGNANCY

Biochemical constituents of the extracellular matrix

The cervical stroma is a fibrous connective tissue. The important constituents of the extracellular matrix (ECM) are collagen, water, proteoglycans, hyaluronan (HA), and elastin.

Collagen: Histologic studies show that 80%–85% cervical stroma is ECM and 10%–15% is smooth muscle cells.[8] The ECM plays a key role in the mechanical properties of the cervical stroma, which is the load-bearing region of the cervix.[9] The most important constituent of the ECM is fibrillar collagen. Fibrillar collagens type 1 and 3 are the primary cervical collagens of the cervical stroma. The mechanical properties of the stroma arise from the organization and cross-linking of these collagens.[3,10]

Water: Approximately 75%–80% of cervical tissue is water. By the third trimester, hydration increases an additional 5%. Hydration affects cervical mechanical properties because interstitial fluid flow follows pressure gradients in the tissue and contributes to the transient response to deformation.[11]

Decorin: The proteoglycan content of the cervix is 90% decorin. Decorin is composed of a small core protein covalently linked to a single glycosaminoglycan (GAG) chain. Decorin is known to influence stromal mechanical properties in two ways. First, the GAG chain possesses a fixed negative charge which influences tissue hydration. Second, the core protein of decorin is known to regulate collagen fibril formation.[12] Indeed, changes in the metabolism of decorin at term are postulated to decrease the stability of the collagen network.[13]

Hyaluronan: HA is a large, negatively charged GAG chain. Like decorin, HA regulates tissue water content through its fixed negative charge. HA also increases production of inflammatory cytokines, which promotes migration of inflammatory cells into the cervical stroma at term.[14]

Elastin: Elastin is 0.9%–2% of the cervical dry weight. Elastin fibers permit elastic recoil, which may allow the cervix to recover its shape after the enormous deformation associated with parturition.[15]

Cervical changes in preparation for labor

In normal childbirth, the cervix remains closed until term and then dilates to a diameter of 9–10 cm to allow passage of the fetal head. The amount of cervical deformation is on the scale of orders of magnitude. To prepare for the enormous degree of deformation seen during labor, cervical ECM undergoes a complex sequence of changes, collectively referred to as cervical remodeling. Clinically, cervical remodeling is detected as increasing cervical "softness" on physical examination. Although the temporal sequence of cervical remodeling is not well characterized, a recent report showed that significant cervical softening occurs as early as the first trimester.[16] Cervical remodeling is clinically important because an abnormally soft cervix is hypothesized to cause cervical insufficiency.[17] An abnormally soft cervix is unable to resist the stresses that act to cause cervical dilation during fetal growth.

Studies of cervical remodeling using animal models and human biopsies demonstrate complex changes in the collagen structure of the stroma during pregnancy.[3,18] Studies with biopsies of the human cervix during pregnancy showed that cervical remodeling correlated with increased collagen solubility. Collagen solubility is an indirect measure of the organization and stability of the collagen fibrils.[19] To measure extractable collagen, a solvent system (e.g., 0.5 M acetic acid, pepsin) degrades the cross-linked portion of the collagen fibril allowing extraction of collagen molecules. Remodeled cervical collagen is characterized by fewer cross-links, decreased organization, and increased solubility. Additional insight into mechanisms of altered collagen structure comes from studies with mice.[20] Cervical remodeling in the mouse cervix was characterized by decreased activity of lysyl oxidase (LOX), which catalyzes collagen cross-linking. Decreased LOX resulted in a decline of collagen cross-links and loss of tensile strength. Decline in tensile strength also correlated with decreased expression of matricellular proteins thrombospondin 2 and tenascin C, which have roles in collagen fibrillogenesis.[20] Of particular importance in pregnancy is the recent observation that steroid hormones appear to regulate ECM organization.[21]

SCAFFOLDS TO STUDY CERVICAL REMODELING

A tissue engineering approach to study cervical remodeling was motivated by the limitations of the traditional model systems. Traditional models include using (1) animal models and (2) human cervical tissue. Various animal species have been investigated to study the cervix during pregnancy.[14,22–25] But cervical remodeling in animal models may not reflect changes in the human cervix. Studies with human tissue are performed by obtaining a biopsy of the cervix either during pregnancy or immediately postpartum.

Although studies with human biopsy samples have revealed important information about cervical remodeling[3], many patients decline to participate. In addition, it is challenging to correlate biochemical constituents with tissue mechanical properties from biopsy samples.

The long-term objective of scaffold-based studies of cervical remodeling is to investigate cell–ECM interactions under conditions that mimic pregnancy. Cervical cells are influenced by multiple factors including changes in hormone concentrations, oxygen delivery, inflammatory environment, and mechanical stress state. Previous scaffolds for cervical tissue engineering have been designed to mimic different variables that affect cervical function (**Figure 1**). The idea was to use a tissue engineering paradigm to study aspects of cervical function that are difficult to study with existing model systems.

The principle that guided scaffold design for studies of cervical remodeling was to relate mechanical properties of the synthesized ECM to culture conditions. This principle is important because impaired mechanical properties (e.g., excessive softening) are what presumably cause cervical insufficiency. A tissue engineering system that reproduces ECM softening in vitro could aid mechanistic studies of cervical remodeling. The initial biomaterial chosen for an engineered cervix was a porous silk protein scaffold.[26] Silk was chosen because techniques for controlling the morphological and functional properties of the silk protein scaffolds were well known to the laboratory. The shape was a thin rectangular slab (dimensions 35 × 12 × 1 mm, **Figure 1A**). The rectangular shape was selected to facilitate mechanical testing. The scaffold was thin to promote nutrition delivery to the scaffold interior. The scaffolds were seeded with primary human cervical fibroblasts, which were isolated from nonpregnant hysterectomy specimens using an explant system. The fibroblasts were expanded in culture and seeded on collagen-coated scaffolds by applying a concentrated cell solution (20×10^6 cells/mL) in a drop-wise fashion. It was found that cervical fibroblasts synthesized an ECM with biochemical constituents and morphology similar to cervical tissue.[27] In addition, not surprisingly, a spinner flask system was superior to static culture for ECM production.

In more recent studies, tissue engineering constructs were used to explore relationships between steroid hormones and ECM properties.[28,29] Steroid hormones could be important regulators of ECM remodeling in pregnancy because concentration of estradiol and progesterone rise by several orders of magnitude. In addition, supplemental progesterone is used to prevent preterm birth in women at high risk for preterm birth.[30,31] To study effects of steroid hormones, cervical fibroblasts were seeded on cylindrical, porous silk scaffolds (**Figure 1B**) and cultured in spinner flasks. It was found that estradiol (10^{-8} M) increased production of collagen, and this effect was opposed by progesterone (10^{-7} M or

10^{-6} M). In addition, a progesterone receptor antagonist (RU-486, mifepristone) partially antagonized the effect of progesterone, suggesting the mechanism was mediated by the progesterone receptor.

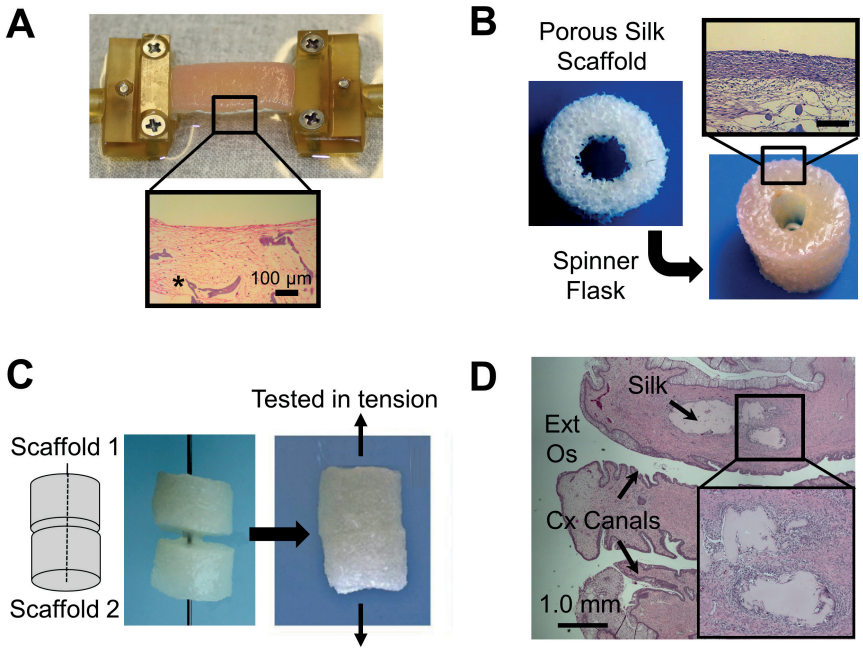

FIGURE 1. Scaffolds for cervical tissue engineering. **A:** A porous silk scaffold was fabricated into a thin slab to facilitate mechanical testing. The asterisk (*) in the hematoxylin and eosin image indicates the silk scaffold (purple). **B:** Scaffolds were also cylindrical in shape (1) for ease of fabrication and (2) to mimic the cervical shape. The pores can be seen on the empty scaffold at the left (size 500–600 µm). The morphology of the newly synthesized mimicked cervical morphology (scale bar 100 µm) is shown. **C:** To test the strength of the newly synthesized tissue independent of the scaffold, two scaffolds were cultured separated by a small gap. After 4 weeks, tissue filled the gap, and the scaffolds were pulled apart in tension. **D:** A prototype tissue scaffold as an alternate treatment for cervical dysfunction is shown. A silk gel is injected into the cervix of pregnant Sprague Dawley rats. The inset shows a mild foreign-body response 4 days of exposure.

To establish an explicit relationship between the mechanical properties of the synthesized ECM and culture conditions, a system was designed to measure the strength of the ECM independent of the properties of the scaffold.[29] When two cylindrical porous silk scaffolds are cultured end to end, newly synthesized ECM will fill the gap and fuse the scaffolds (**Figure 1C**). If the scaffolds are pulled apart in tension, the breaking force is a measure of the strength of ECM, which is independent of the mechanical properties of the scaffold. Using this system, it was shown that progesterone exposure was associated with decreased collagen content and weaker ECM. The study was unique in showing an

explicit relationship between mechanical properties of the ECM and steroid hormone levels. The examples discussed above demonstrate the utility of a tissue engineering approach to study the cervical remodeling in pregnancy.

CERVICAL TISSUE ENGINEERING FOR 3D CANCER MODELS

Tissue engineering for the cervix has not been limited to cervical biology during pregnancy. Groups interested in models of tumor progression in the cervix have used scaffold-free approaches to create organotypic models of the cervical epithelium.[32–36] The squamous epithelium overlying the vaginal portion of the cervix is a site of human papilloma virus (HPV) infection. To study stroma–epithelial interactions in HPV biology, a raft culture strategy has been used.[35,36] Cervical fibroblasts are cultured in collagen matrix to create solid rafts. Cervical epithelial cells are seeded on the surface and cultured at the air–liquid interface. The epithelial cells differentiate into a squamous epithelium with histological features similar to native epithelium. This model has been used to study therapeutic approaches to epithelial lesions[36], HPV life cycle[33,35], and tumor progression[34]. More recently, microtissue precursors were used to fabricate an organotypic model without the need for collagen matrix.[32] It should be noted that stroma–epithelial interactions have also been implicated in cervical remodeling during pregnancy. These coculture techniques could be important for future studies of stroma–epithelial interactions in cervical insufficiency.[37]

INJECTABLE BIOMATERIALS FOR CERVICAL AUGMENTATION

Treatment for cervical dysfunction is offered to two patient populations. First, patients with a clear-cut diagnosis of cervical insufficiency are offered a cerclage.[6] Second, there is a category of patients who have not been diagnosed with cervical insufficiency but who present with cervical shortening, which is a strong risk factor for preterm birth. For patients with cervical shortening, progesterone supplementation is recommended. In this section, cerclage and progesterone supplementation are reviewed and an opportunity for a novel, biomaterial-based treatment for cervical dysfunction is presented.

Cervical cerclage is a well-established treatment for preterm birth prevention.[6] The most common material for a cerclage is a braided, 5 mm, polyethylene terephthalate (PET)

tape suture. The PET suture is placed around the cervix in a circumferential manner and tightened to prevent cervical shortening and dilation. The cerclage is removed when labor is diagnosed or at 37 weeks of gestational age.

Essentially, cervical insufficiency represents biomechanical failure of the cervix. As the fetus grows, there are multiple stresses that act to cause cervical dilation during fetal growth. Stresses influencing cervical deformation include the weight of the fetal sac, the stresses from the uterine wall during uterine growth, and the adhesive contact of the membranes at the internal os.[17] Anatomical geometry of the internal os also influences stress distribution.[38] The applied load on the cervix is resisted by the strength of the connective tissue of the cervical stroma, which continuously remodels as pregnancy advances. It is important to note that patients who present with painless dilation do not always have cervical insufficiency. Patients with placental bleeding and intrauterine infection can present with a clinical phenotype similar to cervical insufficiency. Establishing the primary cause of a preterm birth can be complex when multiple pathologies are present. The decision to recommend cerclage treatment can be challenging, even for senior clinicians.

Patients with a short cervix, without the clear-cut diagnosis of cervical insufficiency, are also offered treatment to prevent preterm birth. The most common treatment for cervical shortening, in the absence of cervical insufficiency, is vaginal progesterone.[31,39] Treatment for cervical shortening is offered because a short cervix is a strong predictor of preterm birth risk with shortest cervix conferring the highest risk.[40] To measure the length, the cervix is visualized with vaginal ultrasound. The length is measured from the internal os to the external os. When cervical shortening occurs, deformation invariably occurs at the internal os.[41,42] Deformation at the internal os is caused by a stress gradient with a stress concentration at the internal os and decreasing stress along the length of the canal.[41]

Treatment options for cervical insufficiency (e.g., cerclage) and cervical shortening (e.g., progesterone supplementation) are not effective in all patients, and many patients with optimal treatment progress to a preterm birth. Although cerclage prevented preterm birth in some studies[43,44], no effect was seen in other studies.[45] In addition, cerclage is a surgical procedure. Complications include premature rupture of membranes, infection, suture displacement, and bleeding.[46] In addition, cervical laceration can occur, which can cause significant hemorrhage.[47] Progesterone supplementation does not have surgical risks, but efficacy in recent controlled trials has been questioned.[48] The limitations of current treatments have prompted a search for an alternate treatment for cervical dysfunction.

Current treatments do not address a critical risk factor for cervical dysfunction, which is excessive softening of the cervical stroma. An alternate treatment that restores the properties of the stroma could resist cervical stresses acting to cause shortening and dilation. One option for an alternate treatment is an injectable biomaterial that augments cervical stroma such that cervical dilation is resisted. Essentially, the injectable biomaterial acts as a tissue scaffold to prevent preterm deformation. Several injectable prototypes have been developed using silk fibroin protein as the starting point.

The clinical case of cervical "change", whether it is described as funneling, effacement, or dilation, is a special case of a more general problem, namely that of solid body deformation. A more precise term for cervical "change" is deformation. Cervical funneling, effacement, and dilation are better described as clinically significant deformation of the cervical stroma, a description that incorporates both clinical significance and solid mechanics. Solid mechanics terminology is a highly technical field. Investigation of clinically significant deformation requires a partnership between engineers and clinicians - the former because of their understanding of how to investigate soft material.

Injectable silk-based biomaterials for cervical augmentation

Silk fibroin is a fibrous protein with remarkable mechanical properties, chemical flexibility, and biocompatibility.[49] Solutions of silk fibroin protein have been processed to form a variety of biomaterials, such as gels, sponges, and films, for medical applications.[50] Blended with other materials, physical properties of silk biomaterials can be further modified to meet functional demands and can therefore suit a wide range of biomedical applications.

A number of design features are important for an injectable treatment for cervical insufficiency including biocompatibility, biodegradation, and a gelation mechanism, which is compatible with the clinical setting.[51–53] The first prototype was a silk solution blended with a two-part polyethylene glycol gelation system.[51] The prototype was able to increase the volume of cervical tissue and was not cytotoxic to cervical fibroblasts. However, the gelation mechanism required exogenous alcohol. To avoid alcohol-based gelation, a follow-up study used a sonicated silk solution.[52] High intensity sonication accelerates spontaneous gelation in a silk solution.[54] In this study, it was found that sonicated silk, injected in pregnant rats, showed a mild foreign-body response similar to the response seen with PET cerclage (**Figure 1D**). However, sonicating a silk solution before injection was clinically cumbersome.

The most recent prototype is enzymatically cross-linked silk hydrogel.[53] Cross-links are created between amino acid phenolic groups on the silk protein using horseradish

peroxidase (HRP) and hydrogen peroxide. The HRP-catalyzed cross-links result in elastic biomaterials with mechanical properties that can be tuned to meet clinical needs.[55] In the study, biomaterials were screened for mechanical properties to match the mechanical properties of cervical tissue. The biomaterials were further evaluated for biocompatibility, facile injection, and in vitro degradation. In vitro degradation was studied using concentrated protease solution, which showed tunable control of degradation rate based on the hydrogel formulation. In addition, cervical fibroblasts cultured on the biomaterials were proliferative and metabolically active. Furthermore, in vitro injection of human cervical tissue required low injection force and showed that tissue volume could be increased without significant influence on cervical stiffness. It is clear that more work is needed before clinical use. But these prototype studies are promising for future development of an alternative treatment for cervical insufficiency and cervical shortening.

SUMMARY

Scaffolds for cervical tissue engineering have been designed to address specific research questions related to cervical biology. To study cervical remodeling, scaffolds were designed to study relationships between the culture environment and the composition of the newly synthesized ECM. For a novel treatment to prevent preterm birth, an injectable biomaterial was formulated, which essentially acts as a tissue scaffold in the cervix. For 3D models of cervical cancer, scaffold-free approaches have been used. Regardless of the research question, tissue engineering methodology has been a valuable tool for novel studies of diseases related to the cervix.

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CHAPTER 11

Summary, general discussion and future perspectives

SUMMARY

Preterm birth is the most important cause of neonatal mortality and morbidity worldwide. The pathogenesis of preterm birth is complex and largely unknown. Despite extensive research, preterm birth remains relatively hard to predict and difficult to prevent. It is a major clinical and scientific challenge in the obstetric healthcare. In this thesis, we aimed (1) to elucidate the role of various risk factors for spontaneous preterm birth and (2) to study preventive management strategies in women at risk for spontaneous preterm birth.

PART I. RISK ASSESSMENT OF SPONTANEOUS PRETERM BIRTH

Multiple risk factors associated with spontaneous preterm birth have been identified that can be categorized in:

1. maternal characteristics, including ethnicity, low socio-economic status, maternal weight, smoking, maternal stress and specific maternal diseases (e.g. depression, periodontitis);
2. obstetric and gynaecological history, including a history of preterm birth which is considered the most important risk factor for preterm birth in subsequent pregnancy, parity, uterus anomaly, cervical excision procedures, one or more curettage treatments and interpregnancy interval;
3. current pregnancy characteristics, including short cervical length or cervical insufficiency, mode of conception, uterine overdistension (e.g. in case of polyhydramnios or multiple pregnancy) and maternal surgery during pregnancy.

In **Chapter 2**, we assessed the effect of interpregnancy interval on adverse neonatal outcomes in the subsequent second and third pregnancy. Previous studies indicate that either a short or a long interval between pregnancies is associated with adverse perinatal outcomes, including preterm birth, low birth weight, and small for gestational age (SGA). This suggests that there is an optimal interval between pregnancies and that spacing pregnancies appropriately could help to prevent these adverse perinatal outcomes. In this study we studied a subgroup of women with three sequential deliveries, after spontaneous preterm birth before 37 weeks in the first pregnancy. We used traditional analysis in which we controlled for known confounders (maternal age, ethnicity, socio-economic status, mode of conception and year of birth). In addition, we used a conditional analysis which enabled us to control for possible unknown confounders by performing

an individual analysis for each mother. After controlling for confounders using traditional analysis, we found that a short interpregnancy interval is associated with a higher risk of preterm birth before 37 weeks, preterm birth before 32 weeks, and low birth weight. However, after controlling for possible confounders using conditional analysis, no effect on preterm birth before 32 weeks was seen, whereas the effect of a short interpregnancy interval on both preterm birth before 37 weeks and low birth weight persisted. For the large intervals, conventional logistic regression analysis revealed an association with preterm birth before 37 weeks, however, this did not persist after applying the conditional method of analysis. Our results may assist clinicians in the counselling of women with a history of spontaneous preterm delivery. We recommend these women to consider an interval of at least 12 months before conception of the next pregnancy.

In **Chapter 3**, we studied the association between parity and the risk of spontaneous preterm birth in a retrospective study including live singleton births (≥22 weeks of gestation) of women with a first, second, third, fourth or fifth pregnancy in The Netherlands using data from the population based Netherlands Perinatal Registry (PERINED). Our data showed that nulliparity (P0) was independently associated with an overall increased risk for spontaneous preterm birth compared to women in their second pregnancy (P1). We also observed an increase in incidence of spontaneous preterm birth <37, <32 and <28 weeks with higher parity in multiparous women, with highest risk for spontaneous preterm birth <28 weeks in women in their fifth pregnancy. These results highlight the importance of the effect of parity on spontaneous preterm birth and may assist in preterm birth risk stratification and counselling.

Chapter 4 describes the utility of mid-trimester uterine artery Doppler in the prediction of spontaneous preterm delivery. We studied uterine artery resistance at the 18-22 weeks anomaly scan in a single center cohort study and we analysed whether the waveform of the uterine artery (no notch, unilateral notch or bilateral notch) was predictive for preterm birth (i.e. delivery before 37 weeks of gestation). Furthermore, we assessed whether the uterine artery pulsatility index (PI) was associated with the risk of preterm birth. The main finding of this study was that women with a higher uterine artery resistance, either manifested in notching or higher PI, are at increased risk for spontaneous preterm birth before 37 weeks of gestation. The risk was particularly present between 34 and 37 weeks of gestation. No statistically significant effect on spontaneous preterm birth before 34 weeks was observed. In conclusion, abnormal uterine artery Doppler is associated with a small increased risk of spontaneous preterm birth.

In **Chapter 5**, we assessed whether verification of short cervical length with a second measurement improves the identification of patients with short cervical length who are

at increased risk of preterm delivery. In this secondary analysis, we analysed prospective cohort study data from patients with singleton pregnancies without a history of preterm delivery who presented for obstetric care in the Netherlands (Triple-P). Cervical length was measured during the standard anomaly scan in the mid-trimester and a second measurement was performed if the cervical length was 30 mm or shorter. No differences were identified in the odds of spontaneous preterm delivery when evaluating using the first, second, or a mean of both measurements. All women with an initial cervical length of 30 mm or shorter, regardless of the outcome of the second measurement, were at higher risk for preterm birth. We concluded that a second measurement does not improve the identification of women with a short cervical length who are at risk of preterm birth.

PART II. PREVENTION OF PRETERM BIRTH

Various treatment strategies to prevent spontaneous preterm birth have been studied, including progesterone, cervical cerclage and cervical pessary. These preventive treatment options are focused on the role of the cervix in pregnancy and both their individual and combined effect is currently being studied in multiple randomized controlled trials worldwide in different study populations (i.e. low-risk, high-risk, singleton and multiple pregnancies).

In **Chapter 6**, we performed a systematic review and meta-analysis to assess the effect of an emergency cerclage in women with asymptomatic dilation of the cervix before 24 weeks of gestation. We included four retrospective cohort studies in this systematic review. Our findings suggest that an emergency cerclage in women with cervical dilation and visible membranes before 24 weeks of gestation is associated with significant lower rates of preterm birth before 37, 34, 32, 28 and 24 weeks of gestation, significant prolongation of the pregnancy and a greater gestational age at delivery compared to expectant management. There was no significant difference in the occurrence of PPROM between cervical cerclage or expectant management, however these results varied widely between the included studies. Data on neonatal outcome were very differently reported and could therefore not be pooled. All studies included were retrospective cohort studies. This gives a high possibility of both selection and treatment bias in all studies, which exists with non-random allocation.

Chapter 7 is the study protocol for a randomized controlled trial (PC-Study) that compares a cervical pessary and cervical cerclage in a head-to-head comparison. We hypothesized that the use of a cervical pessary will be equally effective in preventing preterm birth as cervical cerclage. The outcome of the proposed study will indicate the

relative effectiveness of cervical pessary for women with a singleton pregnancy with a prior preterm birth due to cervical insufficiency and in women with a prior preterm birth and short cervical length in current pregnancy. In addition, the costs of both interventions will be compared and complications of both interventions will be reported. So far, no outcome data are available as this study is currently ongoing.

PART III. NOVEL INTERVENTIONS

Cervical dysfunction plays an important role in spontaneous preterm birth. The composition and structure of the cervix controls its ability to remain closed during pregnancy to promote fetal development. In a normal pregnancy, cervical effacement and dilation occurs at term. If this process of cervical effacement and dilatation occurs prior to term, this can lead to a premature birth. Cervical dysfunction can be detected by measuring a short cervix with transvaginal ultrasound. The risk of preterm birth is inversely proportional to the length of the cervix, with a shorter cervix conferring a higher risk. Although various treatment strategies to prevent preterm birth in women with suspected dysfunctional cervix have been studied, including progesterone, cervical cerclage and cervical pessary, these treatment strategies are not effective in all patient populations at risk for preterm birth. This part of the thesis focused on novel interventions that aim to treat cervical insufficiency.

In **Chapter 8**, we discussed two novel interventions to treat cervical dysfunction that aim to address the pathogenesis of cervical dysfunction and to support the native, physiological properties of the cervix. The first intervention that we discussed was a injectable silk protein-based biomaterial for cervical tissue augmentation (injectable cerclage). The central hypothesis of an injectable biomaterial is that a treatment that improves the functional performance of the cervical tissue could prevent cervical shortening and hence preterm birth. The second intervention we discussed in this chapter was the patient specific pessary. The basis of a patient-specific pessary is a custom-fit device to maternal anatomy to ensure a reduction of contact pressure on the outer cervix and a reduction of tissue stretching at the internal os by cervical canal alignment with the uterine axis.

In **Chapter 9**, we studied the biocompatibility of a silk protein-based injectable gel *in vivo* in a pregnant rat model. We hypothesised that this gel may restore cervical tissue properties and hereby function as an injectable cerclage. We performed cervical injections via a vaginal route to mimic the clinical procedure of a cervical cerclage. We used two control groups, including (1) pregnant rats with cervical injected saline and (2) pregnant rats in which we performed a cervical cerclage using a polyethylene terephthalate suture

(which is also used for cerclage in clinical practice). Cervical procedures were performed using a customized speculum under general anaesthesia. The inflammatory response was evaluated by (1) histology, (2) PCR for inflammatory gene expression (IL-6, Cd68, Ccr7, Ccl2, TNFa) and (3) ELISA for protein levels of proinflammatory mediators (IL-6 and IL-8). We found that injected silk gel caused neither preterm birth nor prolonged pregnancy and had no effect on the number of kits. When comparing inflammatory responses, expression of inflammatory genes and proinflammatory proteins in the silk gel group was intermediate between saline (lowest) and cerclage suture (highest). This study is an important step toward development of an alternative treatment for cervical insufficiency.

In **Chapter 10**, we discussed tissue engineering scaffolds that are used to study cervical tissue. Scaffolds for cervical tissue engineering have been designed to address specific research questions related to cervical biology. To understand the mechanical role of the cervix during pregnancy, we described the biochemical constituents of the extracellular matrix (ECM) of cervical tissue. To prepare for the enormous degree of deformation seen during labour, cervical ECM undergoes a complex sequence of changes, collectively referred to as cervical remodelling. The long-term objective of scaffold-based studies of cervical remodelling is to investigate cell–ECM interactions under conditions that mimic pregnancy. We introduced the use of a porous silk protein scaffold that was used as biomaterial for an engineered cervix. The aim of this scaffold was to study cervical remodelling and to explore relationships between steroid hormones and ECM properties. In addition, we discussed the role of enzymatically cross-linked silk hydrogel as injectable to prevent excessive softening of the cervical stroma and therefore spontaneous preterm birth.

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

Risk assessment

In this thesis, several risk factors for spontaneous preterm birth were discussed and further explored. The most important risk factor for a spontaneous preterm birth is a previous spontaneous preterm birth. Women with a history of spontaneous preterm birth have an average risk of 20% (range 15.8 – 30.2%) of recurrence of spontaneous preterm birth before 37 weeks.[1] The risk increases with a lower gestational age at index pregnancy and the number of spontaneous preterm births.[2] In clinical practice, women with a history of spontaneous preterm birth are considered as high risk and can be counselled preconceptionally about their risk for spontaneous preterm birth in

a subsequent pregnancy. The counselling should include the advice of an appropriate interpregnancy interval, healthy lifestyle, and, if applicable, the advice to stop smoking, lose weight and reduce stress. During pregnancy, these women are offered serial cervical length measurements to further assess their risk of spontaneous preterm birth in the subsequent pregnancy. They are also offered progesterone treatment and, in case of a short cervix <25 mm, a cervical cerclage as preventive interventions.

Women without a history of spontaneous preterm birth, including women with their first pregnancy, are generally considered as a low risk group. However, a significant part of this group will have a spontaneous preterm birth. Considering the large amount of risk factors identified so far, counselling could improve risk assessment for spontaneous preterm birth in all women, including those at low risk. The counselling should focus on the modifiable predictors preconceptionally (e.g. maternal weight, smoking, maternal stress, interpregnancy interval, avoiding curettage treatment in case of first trimester miscarriage or termination of pregnancy) in the context of an awareness programme that focuses on all women in their reproductive age.

During a low-risk pregnancy, cervical length measurements in the mid-trimester can help to identify women at risk for spontaneous preterm birth in an unselected low-risk population. The risk of spontaneous preterm birth is inversely proportional to the size of the cervix, with a shorter cervix predicting a higher risk.[3] Several authors, who critically assessed whether cervical length screening in a low-risk population meets the criteria outlined by the World Health Organization (WHO) of a good screening test, have concluded that universal mid-trimester transvaginal cervical length screening for women with a singleton gestation followed by treatment with vaginal progesterone for those with a short cervix meets all 10 criteria outlined by the WHO.[4] These criteria are globally accepted as requirements that need to be fulfilled for the implementation of a screening programme. Progesterone treatment is associated with a reduced risk of spontaneous preterm birth and adverse perinatal outcomes in patients with a singleton gestation and a mid-trimester short cervix, regardless of the history of spontaneous preterm birth.[5] Based on the evidence available so far, universal transvaginal screening for short cervical length in the mid-trimester in all pregnant women and administration of progesterone treatment in those with a short cervix should be considered.[5-9]

Preventive measures and novel interventions

As described in this thesis, various treatment options to prevent spontaneous preterm birth in different populations of pregnant women have been studied. A previous spontaneous preterm birth is often an indication for the use of progesterone in the next pregnancy as a preventive measure for recurrence of preterm birth. Patients with

a previous preterm birth are considered as high risk and in case of a short cervix, they are also offered a secondary (or ultrasound indicated) cerclage. Patients with a clear-cut diagnosis of cervical insufficiency, based on a previous history of unexplained very early preterm births or second trimester losses related to painless cervical dilation in the absence of labour, are offered a primary (or history indicated) cerclage.[10] Furthermore, there is a category of patients who are considered as low risk and have not been diagnosed with cervical insufficiency but who present with cervical shortening in de mid-trimester. For these patients, progesterone supplementation is recommended.[5] In some cases, a tertiary (or emergency indicated) cerclage is offered to women who present with cervical effacement and dilatation on physical examination or on transvaginal ultrasound, with or without membranes bulging through the external os, before 24 weeks of gestation.[10] In addition, treatment with a cervical pessary is recently being studied widely in patients with a short cervical length in both low-risk and high-risk pregnancies.[11-14]

Currently available treatment options to prevent spontaneous preterm birth (i.e. progesterone, cervical cerclage and cervical pessary) are not effective in all patients since still many patients with optimal treatment will have a spontaneous preterm birth. The limitations of available treatments have inspired a search for an alternate treatment for cervical dysfunction. In this thesis, we focused on an alternate treatment that aimed to treat cervical insufficiency and therefore could prevent spontaneous preterm birth. From a biomechanical view, excessive softening of the cervical stroma is considered as a significant mechanical pathway that leads to spontaneous preterm birth. In this thesis, we described a novel intervention, an injectable cerclage, that aims to restore the native properties of the cervical stroma. The central hypothesis to study an injectable cerclage is that it could improve the functional performance of the cervical tissue. This point of view provides new perspectives in the development of possible novel interventions to prevent spontaneous preterm birth.

Although multiple risk factors for spontaneous preterm birth have been identified, spontaneous preterm birth remains difficult to predict. In addition, the majority of identified risk factors so far are not implemented in the risk assessment of (pregnant) women in current clinical practice. The results from this thesis may help clinicians in improving their risk assessment of spontaneous preterm birth and counselling of women with respect to their risk of preterm birth. A universal awareness programme for both women in their reproductive age and clinicians, including general practitioners and gynaecologists, that focuses on a healthy lifestyle before conception could help to reduce pregnancy complications including spontaneous preterm birth. During pregnancy, a universal risk screening for spontaneous preterm birth should include a prediction model that includes all various variables associated with a higher risk for spontaneous preterm

birth to assess the risk of spontaneous preterm birth for every individual pregnant woman followed by targeted interventions. The main aim of future research is to develop and investigate novel and effective interventions to reduce the incidence of preterm delivery with maximal neonatal and maternal benefit.

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CHAPTER 12

Summary in Dutch (Nederlandse samenvatting)

Vroeggeboorte is wereldwijd de belangrijkste oorzaak van perinatale mortaliteit en neonatale morbiditeit. De pathogenese van vroeggeboorte is complex en grotendeels onbekend. Ondanks veel wetenschappelijk onderzoek blijft spontane vroeggeboorte lastig te voorspellen en te voorkomen. Spontane vroeggeboorte is zowel klinisch als wetenschappelijk een enorme uitdaging binnen de geboortezorg. Dit proefschrift richtte zich op (1) het verklaren van de rol van verschillende risicofactoren voor spontane vroeggeboorte en (2) het bestuderen van preventieve behandelingen voor vrouwen met een verhoogd risico op spontane vroeggeboorte.

DEEL I. RISICO-INSCHATTING VAN SPONTANE VROEGGEBOORTE

Meerdere risicofactoren worden in de literatuur geassocieerd met spontane vroeggeboorte welke ingedeeld kunnen worden in de volgende categorieën:

1. maternale karakteristieken, waaronder etniciteit, lage sociaal economische status, maternaal gewicht, roken, maternale stress en specifieke maternale aandoeningen (bijv. depressie, peridontitis);
2. obstetrische en gynaecologische voorgeschiedenis, waaronder een eerder doorgemaakte vroeggeboorte die tevens de belangrijkste risicofactor vormt voor het krijgen van een spontane vroeggeboorte, pariteit, uterusanomalie, lisexcisies en conisaties van de cervix, één of meerdere curettages en het interval tussen verschillende zwangerschappen;
3. karakteristieken in de huidige zwangerschap, zoals een korte cervixlengte of cervixinsufficiëntie, wijze van conceptie, overdistentie van de uterus (bijv. in het geval van polyhydramnion of meerlingzwangerschap) en maternale chirurgische ingrepen tijdens de zwangerschap.

In **Hoofdstuk 2** hebben wij het effect van het interval tussen zwangerschappen op neonatale uitkomsten in de tweede en derde opeenvolgende zwangerschappen onderzocht. Eerdere studies lieten zien dat een kort of juist lang interval tussen zwangerschappen geassocieerd is met slechtere perinatale uitkomsten in een volgende zwangerschap, waaronder vroeggeboorte, laag geboortegewicht en te klein voor zwangerschapsduur bij geboorte. Dit suggereert dat er een optimaal interval bestaat tussen zwangerschappen en dat het optimaal plannen van zwangerschappen kan helpen om deze slechtere uitkomsten te voorkomen. In onze studie hebben we gekeken naar een subgroep van vrouwen met drie opeenvolgende bevallingen die de eerste keer te vroeg (vóór een zwangerschapsduur van 37 weken) bevallen waren. Om de

associatie tussen het zwangerschapsinterval en de kans op herhaalde vroeggeboorte te onderzoeken, hebben wij gebruik gemaakt van een traditionele analyse waarbij we gecorrigeerd hebben voor bekende confounders (maternale leeftijd, etniciteit, socio-economische klasse, wijze van conceptie en het geboortejaar). Tevens hebben wij een conditionele analyse toegepast die het mogelijk maakt om individuele analyses uit te voeren voor elke moeder om zo te kunnen corrigeren voor eventuele onbekende confounders. Na correctie voor bekende confounders middels de traditionele analyse zagen wij dat een kort zwangerschapsinterval geassocieerd was met een hoger risico op vroeggeboorte <37 weken, <32 weken en een laag geboortegewicht. Na correctie voor eventuele onbekende confounders middels de conditionele analyse viel de associatie met vroeggeboorte <32 weken weg en bleef de associatie met vroeggeboorte <37 en een laag geboortegewicht bestaan. Voor een lang zwangerschapsinterval zagen we een associatie met vroeggeboorte <37 weken na correctie voor bekende confounders, echter viel dit weg na het corrigeren voor eventuele onbekende confounders middels de conditionele analyse. Onze resultaten dragen bij aan de counseling van vrouwen met een eerdere vroeggeboorte, waarbij we op basis van deze resultaten adviseren een interval van minimaal 12 maanden in acht te houden na een eerdere spontane vroeggeboorte.

In **Hoofdstuk 3** hebben wij gekeken naar de associatie tussen pariteit en spontane vroeggeboorte in een retrospectieve studie. We hebben gekeken naar eenlingzwangerschappen (levend geboren neonaten met een zwangerschapsduur ≥22 weken) van vrouwen in hun eerste, tweede, derde, vierde of vijfde zwangerschap. Hiervoor hebben wij gebruik gemaakt van data uit het Nederlandse perinatale registratiesysteem PERINED. Onze resultaten lieten zien dat nulliparitеit onafhankelijk geassocieerd was met een verhoogd risico op spontane vroeggeboorte in vergelijking met vrouwen in hun tweede zwangerschap. Wij zagen tevens een toename in incidentie van spontane vroeggeboorte <37, <32 en <28 weken met het toenemen van de pariteit. Vrouwen in hun vijfde zwangerschap hadden het hoogste risico op vroeggeboorte <28 weken. Deze resultaten benadrukken de rol pariteit in spontane vroeggeboorte en kunnen bijdragen aan de risicostratificatie van vroeggeboorte en de counseling van zwangere vrouwen.

In **Hoofdstuk 4** hebben we onderzocht of het meten van de Doppler in de arteria uterina in het tweede trimester spontane vroeggeboorte kan voorspellen. In deze studie hebben we de weerstand gemeten in de arteria uterina gedurende het structureel echoscopisch onderzoek (SEO) bij een zwangerschapsduur van 18-22 weken. We hebben gekeken of het stroompatroon in de arteria uterina (geen notch, unilaterale notch of bilaterale notch) voorspellend was voor spontane vroeggeboorte (geboorte bij een zwangerschapsduur <37 weken). Daarnaast hebben we onderzocht of er een associatie was tussen de pulsatility index (PI) van de arteria uterina en vroeggeboorte. Het belangrijkste resultaat van deze

studie was dat vrouwen met een verhoogde weerstand in de arteria uterina, gemeten als notch en/of als verhoogde PI, een mild hoger risico hadden op vroeggeboorte <37 weken. Dit effect was met name zichtbaar bij een vroeggeboorte tussen 34-37 weken. We zagen geen effect op vroeggeboorte <34 weken. Deze resultaten laten zien dat het meten van de weerstand in de arteria uterina middels Doppler mogelijk een waarde heeft in het voorspellen van spontane vroeggeboorte.

In **Hoofdstuk 5** hebben we onderzocht of een herhaalde meting van een eerder kort gemeten cervixlengte de identificatie van vrouwen met een verhoogd risico op vroeggeboorte kan verbeteren. We hebben hiervoor een heranalyse verricht waarbij we analyses hebben uitgevoerd in prospectieve data uit een cohort vrouwen met eenlingzwangerschappen zonder eerdere vroeggeboorte in Nederland (Triple-P). De eerste cervixlengte werd gemeten tijdens het SEO en de meting werd herhaald indien de eerste meting 30 mm of korter was. Alle vrouwen waarbij in eerste instantie een korte cervixlengte gemeten was, ongeacht de uitkomst van de tweede meting, hadden een verhoogd risico op vroeggeboorte ten opzichte van vrouwen zonder korte cervixlengte. Hieruit hebben wij geconcludeerd dat een tweede meting om een korte cervixlengte te verifiëren geen toegevoegde waarde heeft in het opsporen van vrouwen met een verhoogd risico op spontane vroeggeboorte.

DEEL II. PREVENTIE VAN VROEGGEBOORTE

Er zijn verschillende behandelingen onderzocht die het risico van vroeggeboorte en de gevolgen daarvan zouden kunnen verminderen, waaronder progesteron, cerclage en pessarium. Deze behandelingen richten zich met name op de rol van de cervix tijdens de zwangerschap. Het effect van zowel de individuele als gecombineerde toepassing van deze behandelingsmethoden wordt momenteel uitgebreid onderzocht in (gerandomiseerde) studies in verschillende subgroepen vrouwen (laag- en hoog-risico vrouwen, eenling- en tweelingzwangerschappen).

In **Hoofdstuk 6** hebben wij een systematische review en meta-analyse uitgevoerd om het effect van een noodcerclage te onderzoeken in vrouwen met asymptomatische ontsluiting van de cervix vóór een zwangerschapsduur van 24 weken. In dit review hebben wij vier retrospectieve studies geïncludeerd. Onze bevindingen laten zien dat een noodcerclage in vrouwen met asymptomatische ontsluiting en zichtbare vliezen vóór de 24 weken geassocieerd is met minder kans op een vroeggeboorte voor een zwangerschapsduur van 37, 34, 32, 28 en 24 weken. Tevens zagen wij een significant langer interval tussen diagnose en bevalling en een langere zwangerschapsduur bij

bevalling in de groep vrouwen met een noodcerclage in vergelijking met expectatief beleid. Er was geen verschil in de incidentie van het vroegtijdig breken van de vliezen tussen beide groepen, echter waren deze resultaten tegenstrijdig tussen de geïncludeerde studies. Neonatale uitkomsten konden door de verschillende manier van rapportage in de individuele geïncludeerde studies niet gepoold worden in de meta-analyse.

Hoofdstuk 7 is het protocol van een gerandomiseerde studie (PC-Study) waarbij een pessarium (interventiegroep) wordt vergeleken met een cerclage (controlegroep) bij vrouwen met een eerdere vroeggeboorte en een verkorte cervixlengte en vrouwen met een indicatie voor een primaire cerclage op basis van de obstetrische voorgeschiedenis. Onze hypothese is dat een pessarium even goed werkt als een cerclage in het voorkomen van vroeggeboorte in deze groep vrouwen. De uitkomsten van deze studie zullen uitwijzen wat het effect is van een pessarium in deze subgroep vrouwen. Daarnaast wordt een kosten-baten analyse uitgevoerd om beide interventies te vergelijken. Momenteel worden er nog patiënten voor deze studie geïncludeerd en zijn er geen resultaten beschikbaar.

DEEL III. NIEUWE INTERVENTIES

Disfunctioneren van de cervix speelt een belangrijke rol in spontane vroeggeboorte. De samenstelling en de structuur van de cervix zorgt ervoor dat deze gesloten blijft gedurende de zwangerschap ten behoeve van de ontwikkeling van de foetus. In een normale zwangerschap zal de cervix in de a-terme periode (dat wil zeggen bij een zwangerschapsduur tussen 37-42 weken) verstrijken en ontsluiten. Als dit proces te vroeg op gang komt (dat wil zeggen bij een zwangerschapsduur <37 weken), kan dat tot een spontane vroeggeboorte leiden. Een niet goed functionerende cervix kan middels transvaginale echografie worden opgespoord waarbij in dat geval een korte cervixlengte gemeten wordt. Hierbij is de kans op een vroeggeboorte gecorreleerd aan de lengte van de cervix: het risico op een vroeggeboorte stijgt met het korter worden van de cervix. Hoewel reeds uitgebreid onderzoek verricht is naar verschillende behandelingsmogelijkheden ter preventie van vroeggeboorte, waaronder progesteron, cerclage en pessarium, weten we dat deze behandelingen niet effectief zijn bij alle subgroepen hoog-risico zwangere vrouwen. Dit deel van het proefschrift richtte zich dan ook op nieuwe interventies om cervixinsufficiëntie te behandelen.

In **Hoofdstuk 8** beschrijven wij twee nieuwe mogelijke behandelingen die beide als doel hebben om de fysiologische functie van de cervix te ondersteunen. De eerste nieuwe interventie die in dit hoofdstuk aan bod komt is een in de cervix injecteerbare gel met als basisstof zijde-proteïne, bedoeld om te gebruiken als een injecteerbare cerclage. De

hypothese van een injecteerbare gel als cerclage is dat deze behandeling het cervicale weefsel kan ondersteunen, waardoor het voortijdig verkorten van de cervix wordt tegen gegaan en daarmee vroeggeboorte voorkomen kan worden. De tweede interventie die besproken werd in dit hoofdstuk is het patiënt-specifieke pessarium. Het principe van een patiënt-specifiek pessarium is een gepersonaliseerd pessarium op basis van de maternale anatomie die op die manier vermindering van de contactdruk op de cervix creëert en hiermee de kans op vroeggeboorte reduceert.

In **Hoofdstuk 9** hebben wij nader onderzoek gedaan naar de weefseltolerantie van een op zijde-proteïne gebaseerde injecteerbare gel in de cervix van zwangere ratten. Onze hypothese was dat deze gel de cervicale weefselstructuur kan versterken en hiermee fungeren als een mogelijke injecteerbare cerclage. Wij hebben transvaginale cervicale injecties verricht in zwangere ratten om de klinische procedure van het plaatsen van een cerclage na te bootsen. Als controle hebben wij twee groepen gebruikt, namelijk (1) ratten waarbij cervicaal fysiologisch zout werd geïnjecteerd en (2) ratten waarbij een cerclage werd uitgevoerd met hechtdraad van polyethyleen tereftalaat (dat tevens gebruikt wordt voor een cerclage in de klinische praktijk). De cervicale procedures werden verricht middels een op maat gemaakt speculum onder algehele anesthesie. De reactie van het cervicale weefsel op de geïnjecteerde gel werd vergeleken met controles waarbij fysiologisch zout werd geïnjecteerd en controles met een cerclage met hechtdraad. De weefselreactie in de drie verschillende groepen werd geëvalueerd middels (1) histologie, (2) PCR voor het bepalen van de inflammatoire genexpressie (IL-6, Cd68, Ccr7, Ccl2, TNFa) en (3) ELISA voor het bepalen van proteïnelevels van pro-inflammatoire mediatoren (IL-6 en IL-8). In deze studie zagen wij dat de op zijde-proteïne gebaseerde injecteerbare gel geen effect had op de zwangerschapsduur of op het aantal geboren jongen. Bij het vergelijken van de weefselreacties zagen wij dat de expressie van inflammatoire genen en pro-inflammatoire proteïnen in de groep die geïnjecteerd was met de gel hoger was vergeleken met de controlegroep die met fysiologisch zout was behandeld, maar lager dan in de cerclagegroep met hechtdraad. Deze studie is een stap richting de ontwikkeling van een alternatieve methode om cervicale insufficiëntie te behandeling en hiermee het risico op vroeggeboorte te reduceren.

Hoofdstuk 10 beschrijft de toepassing van zogenoemde tissue engineering 'scaffolds' om het remodelleren van cervicale stroma en het behandelen van cervicaal weefstel te bestuderen. Een scaffold, vrij vertaald bouwsteiger, is een drie dimensionale structuur ter ondersteuning van weefsel en het nabootsen van weefselstructuur. In de medische wetenschap worden scaffolds gebruikt in vele soorten en maten met verschillende soorten basismaterialen. In dit hoofdstuk beschrijven wij hoe scaffolds gebruikt worden om specifieke vragen te beantwoorden ten aanzien van de biologie van de cervix, met

name tijdens de zwangerschap. Om de mechanische rol van de cervix in de zwangerschap te kunnen begrijpen, hebben wij de biochemische onderdelen van het extracellulaire matrix (ECM) van cervixweefsel beschreven. Ter voorbereiding van de bevalling ondergaat het ECM van de cervix een ingewikkelde verandering dat ook wel cervicale remodelling wordt genoemd. Het lange termijn doel van het gebruik van scaffolds in het bestuderen van cervicale remodelling is om de ingewikkelde veranderingen binnen het ECM te onderzoeken onder omstandigheden die een zwangerschap nabootsen. In dit hoofdstuk beschrijven wij het gebruik van een poreuze scaffold met als basismateriaal het zijde-proteïne dat gebruikt werd als een biomateriaal voor een *in vitro* cervix. Het doel van dit onderzoek was om cervicale remodelling te bestuderen onder invloed van steroïde hormonen (zoals progesteron). Daarnaast beschrijven we hier nogmaals hoe een op het zijde-proteïne gebaseerde gel als injecteerbare cerclage kan fungeren en hoe dit kan leiden tot de ondersteuning van het ECM in cervixweefsel en daarmee zou kunnen bijdragen aan de preventie van vroeggeboorte.

De complexiteit van vroeggeboorte maakt dat, ondanks uitgebreid onderzoek wereldwijd, diverse vraagstukken blijven bestaan. Er is een groot aantal bekende, en mogelijk nog vele onbekende factoren, die bijdragen aan het risico van vroeggeboorte. Wereldwijd loopt er onderzoek, zowel basaal als klinisch, om de etiologie van vroeggeboorte beter te kunnen begrijpen in de hoop preventieve maatregelen te kunnen ontwikkelen. Het onderzoek besproken in deze thesis draagt bij aan het begrijpen van factoren die een rol hebben in het ontstaan van vroeggeboorte en beschrijft bestaande en nieuwe methoden om het risico op vroeggeboorte te verlagen.

APPENDIX

Curriculum vitae
List of publications
Portfolio
Acknowledgements (Dankwoord)

CURRICULUM VITAE

Bouchra Koullali was born on February 20th 1988 in Utrecht, The Netherlands. She graduated from the Blaucapel College (Gymnasium) in Utrecht in 2006. In the same year, she started studying Medicine at the VU University in Amsterdam. In the third year of her bachelor in Medicine, she undertook a research project on the role of T-cells in immunocompromised patients at the VU Cancer Center Amsterdam (CCA) and the Queensland Institute of Medical Research (Brisbane, Australia), supported by a Dutch Cancer Society (KWF) Student Fellowship. She received her M.D. in 2012 and subsequently worked as a resident (ANIOS) in Obstetrics and Gynaecology at the Catharina Hospital (Eindhoven) under the supervision of dr. Simone Kuppens. In 2013, she started her PhD at the department of Maternal Fetal Medicine of the Amsterdam UMC (University of Amsterdam) under supervision of Prof. Eva Pajkrt and Prof. Ben Willem Mol, which resulted in this thesis. During her PhD, she also worked as a fetal sonographer at the outpatient clinic of Maternal Fetal Medicine of the Amsterdam UMC (AMC). In 2017, she conducted a 1-year post-doctoral research fellowship in the group of Dr. Michael House at the Mother Infant Research Institute of the Tufts Medical Center (Boston, US). Her fellowship was supported by the Ter Meulen Grant of the Royal Netherlands Academy of Arts and Sciences (KNAW) and by a scholarship from the Foundation De Drie Lichten. In 2018, she started residency in Obstetrics and Gynaecology at the Amsterdam UMC (AMC) under supervision of Prof. Joris van der Post, and the Zaans Medical Center (Zaandam) under supervision of dr. Neriman Bayram. Bouchra lives with her husband Najim, son Noah (born 2016) and daughter Sara (born 2020) in Utrecht.

LIST OF PUBLICATIONS

2020

- **Koullali B**, van Zijl MD, Kazemier BM, et al. The association between parity and spontaneous preterm birth: a population based study. BMC Pregnancy Childbirth. 2020;20(1):233.
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PORTFOLIO

Name PhD student: Bouchra Koullali
PhD period: November 2013 – December 2020
Supervisors: Prof. dr. Eva Pajkrt, Prof. dr. Ben Willem Mol
Co-supervisor: dr. Martijn Oudijk

1. PhD training	Year
Amsterdam UMC (AMC) Graduate School	
Clinical Epidemiology - Systematic reviews	2016
Basic Course Legislation and Organization (BROK)	2014
Practical Biostatistics	2014
Tufts Medical Center (Boston, US)	
Annual Lab Safety Training	2017
Cell Culture Training	2017
High Hazard Chemical Use Training	2017
Mandatory Animal Care and Use (MACU) Training	2017
Rodent Survival Surgery (RSS) Training	2017
Fetal ultrasonography	
Counselling first trimester screening	2014
Fetal Medicine Foundation: first trimester nuchal translucency	2014
Fetal Medicine Foundation: midtrimester fetal anomaly scan	2014
Practical session in Fetal Cardial Defects	2014
Seminars	
Weekly department seminars (Tufts Medical Center, Boston, US)	2017
Weekly department seminars (AMC)	2013-16
Presentations	
Oral Presentations	
35th Society for Maternal-Fetal Medicine Annual Meeting, San Diego, US	2015
Symposium Prevention of Preterm Birth, Amsterdam	2015
Masterclass Maternal Fetal Medicine - Netherlands meets Germany, Berlin	2014
1st European Spontaneous Preterm Birth Congress, Svendborg, Denmark	2014
Fetal Ultrasonography (WFE) Annual Meeting, Utrecht	2014
Poster Presentations	
36th Society for Maternal-Fetal Medicine Annual Meeting, Atlanta, US	2016
2nd European Spontaneous Preterm Birth Congress, Goteborg, Sweden	2016
1st European Spontaneous Preterm Birth Congress, Svendborg, Denmark	2014

2. Teaching	Year
Supervising Scientific Research Project – Medical bachelor student	2016
Teaching ultrasound and physical examination – 2nd year Medical students	2014-16

3. Grants	Year
Catharina van Tussenbroek	2017
Ter Meulen Grant of the Royal Netherlands Academy of Arts and Sciences (KNAW)	2016
Foundation De Drie Lichten	2016

ACKNOWLEDGEMENTS

Mijn proefschrift is af! Ik heb lang uitgekeken naar dit moment en ik wil graag een aantal mensen in het bijzonder bedanken voor hun bijdrage aan dit proefschrift.

Mijn promotores: Prof. dr. E. Pajkrt en Prof. dr. B.W.J. Mol

Lieve Eva, waar moet ik beginnen om jou te bedanken. Bedankt dat je me de mogelijkheid hebt gegeven om te promoveren en om het echoën te leren. Bedankt voor je steun bij het verkrijgen van een opleidingsplek bij de gynaecologie en verloskunde. Bedankt voor alle leuke trips die we samen gemaakt hebben. Ik zal onze reis samen naar Parijs, en onze musea-marathon van die dag, nooit vergeten! Bedankt voor alle leuke gesprekken 'over het leven'. Ik heb enorm veel respect voor jouw eindeloze inzet voor patiënten. Ik heb veel van je geleerd en ik kijk er naar uit om nog veel meer van je te leren.

Beste Ben Willem, ik kan me ons eerste gesprek in het AMC nog heel goed herinneren. Je zou bijna naar Australië emigreren, maar wilde nog wel mijn promotor zijn. Bedankt voor al je tijd en energie om het onderzoek in dit proefschrift te maken tot wat het is. Ik waardeer je kritische blik en je opbouwende feedback tijdens mijn promotie. Ik bewonder jouw inzet voor het verbeteren van de obstetrische en gynaecologische zorg middels wetenschappelijk onderzoek.

Mijn co-promotor: dr. M.A. Oudijk

Beste Martijn, wat vond ik het leuk dat je mijn co-promotor werd toen je in het AMC kwam werken. Bedankt voor alle brainstorm-sessies, met name tijdens de eindsprint van mijn proefschrift. Ik kijk er naar uit om ook in de kliniek met je samen te werken!

Ik dank de leden van mijn promotiecommissie, prof. dr. J.A.M van der Post, prof. dr. K.W.M. Bloemenkamp, prof. dr. A.H.L.C. van Kaam, prof. dr. D. Oepkes, prof. dr. T.J. Roseboom en dr. M.A. de Boer, voor hun bereidheid zitting te nemen in mijn promotiecommissie.

Mijn dank gaat uit naar alle vrouwen die belangeloos hebben deelgenomen aan de PC-Study waarvan het protocol in dit proefschrift staat. Daarnaast wil ik het Trialbureau, alle researchmedewerkers, arts-assistenten, gynaecologen, verloskundigen en verpleegkundigen binnen het consortium bedanken die hebben bijgedragen aan de inclusies voor de PC-Study.

I would like to express my deepest appreciation to Dr. Michael House. Dear Dr. House, thank you for welcoming me to Boston and making me part of your lab team. You

introduced me in the wonderful world of using bio-engineering approaches for the development of novel therapies for the prevention of preterm birth. Thank you for your profound contribution to my favorite part of this thesis.

I would like to thank Yali Zhang and Nicole Raia. Dear Yali, without your invaluable support in the lab, I would not have had the chance to successfully complete our project. Dear Nicole, thank you for helping me with my first steps in the lab at Tufts. I very much enjoyed working with both of you.

Mijn dank gaat uit naar Anita Ravelli. Beste Anita, ik dank jou voor je enorme bijdrage aan dit proefschrift. Jij wist altijd te bedenken welke analyses de juiste waren voor de vraag die ik stelde. Ik heb veel geleerd van al onze uren samen achter de computer en telefonisch op afstand. Stichting PERINED, dank voor de toestemming voor het gebruik van de PRN data.

Marie-Thérèse, Anouk, Pascale en alle medewerkers van de polikliniek verloskunde en poli prenatale diagnostiek in het AMC, bedankt voor alle hulp tijdens het echoën en de gezelligheid! Angelique, bedankt voor je hulp bij alle administratieve rompslomp.

Laura, bedankt voor de fantastisch leuke tijd die wij samen hebben gehad in het AMC én buiten het AMC. Ik zal al onze echo-dagen, onderzoeksdagen en reizen door de VS nooit vergeten. Wat hebben wij het gezellig gehad! Ook bedankt voor je luisterende oor en het altijd voor mij klaar staan als ik wat kwijt wilde op alle gebieden: echo, onderzoek maar ook privé. Ik vind het heerlijk dat we nog steeds zoveel kunnen praten, lachen en huilen samen. Ik geniet van al onze dates met z'n tweeën én met onze kids. Wat ben ik trots op jou!

Chantal, van samen juco's in het VUmc, naar samen onderzoeker in het AMC en samen AIOS in hetzelfde jaar! Bedankt voor je gezelligheid en voor je steun de afgelopen jaren.

Brenda, mijn dank is groot voor jouw bijdrage aan dit proefschrift. Niet alleen inhoudelijk, maar ook mentaal ben jij een enorme steun geweest gedurende mijn promotietraject, bedankt daarvoor! Ik bewonder jouw kwaliteiten en met name hoe jij je kwaliteiten inzet om anderen te helpen. Bedankt dat ook ik je mocht bellen als ik het even niet meer wist. Je bent een bijzonder mens en ik gun je het allerbeste!

Maud, wat ben ik blij dat we project 2020 succesvol hebben afgerond! Als ik denk aan promoveren, denk ik aan jou. Terugkijkend weet ik eigenlijk niet hoe het gekomen is dat ik je inmiddels (bijna) elke dag spreek. Ik kan niet beschrijven hoeveel waarde ik hecht aan onze eindeloze telefoongesprekken in de auto, thuis achter de laptop, onze

gezelligheid samen als we even uit onze onderzoeksbubbel wilden, onze klaaguurtjes, onze lach- én huilmomenten en inmiddels onze dierbare baby-momenten. Alle keren dat ik er doorheen zat, leerde jij me relativeren. Ik zei voor de grap tegen je dat jouw naam eigenlijk ook op de kaft moet, maar ik zei het eigenlijk ook omdat ik het meende. Zonder jou was dit proefschrift zonder twijfel nooit af gekomen. Voor mij ben je allang niet meer alleen een onderzoeksmaatje, maar een zeer gewaardeerde vriendin. Ik kijk uit naar onze dates waarbij we kunnen genieten dat we dit samen bereikt hebben!

Mijn dank gaat uit naar de vakgroep gynaecologie en verloskunde van het ZMC. Bedankt voor de fantastische eerste 2 jaar van mijn opleiding. Neriman, bedankt voor je onvoorwaardelijke steun tijdens de opleiding, met name tijdens de moeilijke periodes. Ik heb ontzettend genoten van onze gesprekken samen, tijdens en na onze diensten. Ik vind je een fantastisch mens en kijk enorm naar je op. Marianne, bedankt dat je mijn mentor was! Ik heb enorm veel plezier beleefd aan onze etentjes. Ik hoop dat we dat nog eens gaan doen! Alle A(N)IOS, klinisch verloskundigen en verpleegkundigen, bedankt voor de leuke diensten samen.

Asmaa, mijn jeugdvriendin, bedankt voor het luisteren naar mijn eindeloos geklaag over het doen van onderzoek. Met jou kan ik eindeloos praten en is het altijd gezellig!

Kamar, de dag dat wij begonnen als juco in het VUmc werden we al snel maatjes. Met jou kan ik altijd praten over alles in het leven. Ik heb genoten van onze trip in Chicago! Ik hoop op nog vele gezellige momenten met jou.

Mijn paranimfen Dana en Samira. Dana, dat jij mijn paranimf bent kan geen toeval meer zijn. Ik ben dankbaar dat ik jou ken, al sinds het 1e jaar van de studie geneeskunde komen wij altijd samen terecht: werkgroepen, juco's en nu allebei in opleiding in het AMC. Ik ben heel trots op je en ik weet zeker dat je een fantastische kinderarts wordt. Bedankt dat je mijn paranimf wilt zijn!

Sam, lieve zus, eigenlijk ben jij mijn hele leven al een soort paranimf voor mij geweest en ik voel me vereerd dat je me ook nu wilt bijstaan tijdens deze bijzondere mijlpaal. Bedankt voor alles wat je voor mij doet en voor mij betekent. Ik kan je werkelijk altijd bellen en je staat altijd voor me klaar. Dit proefschrift was er niet geweest als jij niet bereid was al die keren op te passen als ik weer eens op mijn vrije dag moest schrijven. Bedankt voor je luisterend oor. Je bent de allerliefste zus die ik me kan wensen!

Ik dank mijn schoonouders, zwagers en schoonzussen voor alle gezelligheid! Ahmed en Jamaa, bedankt dat jullie altijd voor ons klaar staan.

Lieve broers en zussen, dit is waar ik al die tijd mee bezig was en eigenlijk altijd de reden dat ik 'weer eens geen tijd had'. Bedankt voor jullie interesse en jullie steun. Jihane, ik heb genoten van onze vakanties en hoop op meer, ik ben blij dat je mijn zusje bent! Lieve ouders, zonder jullie had ik dit nooit kunnen bereiken. Papa, ik bewonder hoe jij je hele leven zo hard hebt gewerkt voor ons. Mama, jouw inzet om het voor mij mogelijk te maken om te doen wat ik wil is niet in woorden te beschrijven. Bedankt dat ik altijd een beroep op je mag doen. Bedankt dat je de liefste oma bent!

Allerliefste Najim, dit proefschrift is voor jou, van jou en met jou! Bedankt voor nog veel meer dan alleen dit proefschrift. Het leven dat wij samen hebben is mij het meeste waard, samen met onze sterren Noah en Sara. Jullie zijn mijn alles!