Ultrasound and molecular biomarkers for prediction of preterm delivery in different risk groups of singleton pregnancies

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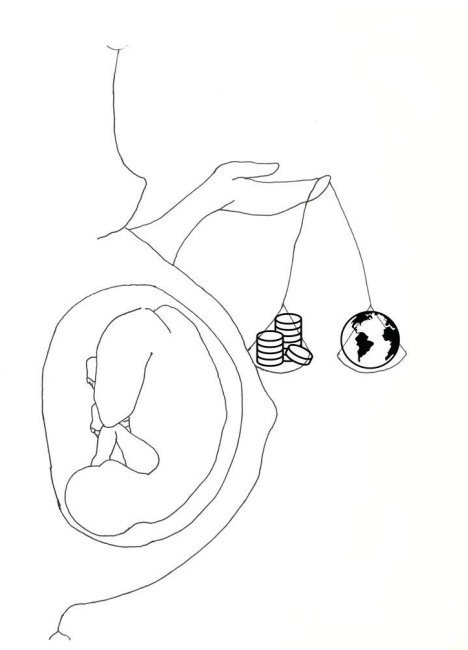

“Price is what you pay; value is what you get”
Warren Buffet
Illustration by Meline Höjer Schou, my beloved friend

To my family,
with endless love

Abstract

Ultrasound and molecular biomarkers for prediction of preterm delivery in women with a singleton pregnancy

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Background: Preterm delivery (PTD) is the leading cause of death in children under five years of age. Spontaneous PTD (sPTD) might be partly preventable by treating asymptomatic women having a short cervical length (CL) with vaginal progesterone. Furthermore, the pathophysiology of spontaneous sPTD is insufficiently understood, limiting the prediction and prevention of the condition.

Aim: To investigate 1) CL as measured by transvaginal ultrasound (TVU) in the second trimester in different risk groups of asymptomatic women and the risk of sPTD; 2) The cost-effectiveness of different strategies to prevent sPTD including CL screening; 3) If there are differences in the vaginal metatranscriptome and human transcriptome between women with sPTD and women with term delivery; 4) Whether a selection of nine specific miRNAs in maternal serum at gestational week 10-14 could serve as biomarkers for sPTD.

Methods: All four studies are based on the CERVIX-study, a prospective blinded multicentre study with the aim to estimate the diagnostic performance of CL (TVU) in asymptomatic women with a singleton pregnancy (n=11 072). In **Paper I** the study population was divided into three main risk groups: women at high risk of sPTD, nulliparous women with no risk factors for sPTD, and parous women with only term deliveries and no risk factors for sPTD. **Paper II** is a decision analytic model to estimate the cost-effectiveness of various CL screening strategies combined with treatment with vaginal progesterone to prevent sPTD. In **Paper III** vaginal sampling was performed prior to TVU at 18 to 20 weeks. Vaginal specimens were subjected to high throughput sequencing of both human and microbial RNA. In **Paper IV**, archived serum samples taken from participants in the CERVIX-study at gestational week 10-14 with a sPTD <34 weeks or who delivered at term were analyzed with RT-qPCR for nine miRNAs previously shown to be associated with sPTD.

Results: Paper I: the effect of CL shortening on the risk of sPTD <33 weeks was similar in all three risk groups, and the discriminatory ability was superior when measurements were performed at 21 to 23 weeks compared to at 18 to 20 weeks. In **Paper II** all suggested interventions gave better health outcomes in terms of less peri/neonatal mortality and more quality adjusted life years in a lifetime perspective when compared to the current situation in Sweden i.e., no screening and no treatment. In **Paper III** 17 human genes were significantly differently expressed in the preterm group when compared to the term group. In **Paper IV** none of the nine analyzed miRNAs was significantly differently expressed in those delivering <34 weeks compared to those delivering at term. Three miRNAs (miR-191-5p, miR-93-5p and miR-15-5p) were overexpressed in those delivering <28 to <32 weeks of gestation.

Conclusion: In asymptomatic women with singleton pregnancies, CL screening by TVU can be used for prediction of sPTD in both high and low risk pregnancies in a population with a low prevalence of sPTD. The method has a moderate discriminatory ability. CL screening by TVU followed by treatment with vaginal progesterone of those at increased risk of sPTD is probably cost-effective in a Swedish setting. Whether the identified human genes in vaginal fluid or miRNAs in maternal serum or plasma could serve as potential biomarkers in the future remains to be shown.

Keywords: cervical length measurement, preterm birth, second trimester, screening, cost-effectiveness analyzes, progesterone, human microbiome, transcriptome, gene expression profiles, microRNA

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Sammanfattning på svenska

Bakgrund: Förtidsbörd, definierat som förlossning innan 37 fullbordade graviditetsveckor, är den enskilt viktigaste orsaken till död hos barn under fem år. Förtidsbörd delas vanligen in i två grupper; spontan förtidsbörd (ca 2/3) vilket innefattar spontan värkstart eller för tidig hinnbristning, eller inducerad förtidsbörd (ca 1/3) där läkare tar beslut om att förlösa i förtid på grund av komplikationer hos den gravida kvinnan eller fostret. Ett flertal studier har visat att spontan förtidsbörd delvis kan förebyggas hos kvinnor med enkelbörd genom att med vaginalt ultraljud identifiera de med en kort livmoderhalslängd, för att därefter behandla dessa kvinnor med vaginalt progesteron. Problemet är att både förekomsten av kort livmoderhals, samt hur väl dess uppmätta längd kan skilja mellan de som föder spontant förtidigt jämfört med de som föder i fullgången tid varierar mycket mellan olika studier och länder. I en stor blindad svensk multicenterstudie fann man att livmoderhalsens längd mätt med vaginalt ultraljud kan användas för att identifiera kvinnor med en ökad risk att föda förtidigt (CERVIX-studien), med reservation för att metoden endast hade en begränsad förmåga att skilja mellan de som föder förtidigt jämfört med de som föder i fullgången tid. Efter studiens avslut kvarstod frågetecken om huruvida förmågan att skilja mellan de som föder spontant förtidigt jämfört med de som föder i fullgången tid skiljer sig mellan olika riskgrupper (tex. hos de med tidigare spontan förtidsbörd, förstföderskor och omföderskor) samt om det skulle vara kostnadseffektivt att införa ett screeningprogram i Sverige för att identifiera kvinnor med en kort livmoder-hals.

En annan viktig aspekt är att vi fortfarande vet för lite om de underliggande mekanismerna till varför vissa kvinnor föder spontant för tidigt och andra inte. För att förbättra diagnostiken, och på sikt även behandlingen, är det viktigt att vi även bedriver forskning avseende de underliggande molekylära skeenden som sannolikt startar flera veckor innan vi kan uppmäta en förkortning av livmoderhalsen.

Syfte med avhandlingen: 1) Undersöka hur väl livmoderhalsens längd uppmätt med vaginalt ultraljud skiljer sig mellan de som föder spontant förtidigt och de som föder i fullgången tid i olika riskgrupper. 2) Huruvida screening av livmoderhalsens längd med vaginalt ultraljud följt av behandling med vaginalt progesteron till de med en kort livmoderhals är kostnadseffektivt i en svensk population. 3) Om det finns

skillnader i genuttrycket i vaginala mikroorganismer eller genuttrycket i humana celler i vagina hos de som föder förtidigt jämfört med de som föder i fullgången tid. 4) Om nio specifikt utvalda mikroRNA (delaktiga i genregleringen av arvsmassan) i framtiden skulle kunna fungera som diagnostiska biomarkörer för spontan förtidsbörd.

Resultat och konklusion: Avhandlingens resultat kan sammanfattas enligt följande: 1) Mätning av livmoderhalsens längd med vaginalt ultraljud under andra trimestern kan användas i en svensk population för att identifiera kvinnor med ökad risk att föda spontant förtidigt i både hög- och lågriskgraviditeter; 2) Det är troligt att screening av livmoderhalsens längd, följt av behandling med vaginalt progesteron till de med en kort livmoderhals skulle vara kostnadseffektivt i en svensk population; 3) Studien gav inga indikationer på att skillnader i det vaginala mikrobiomets genuttryck skulle kunna förklara varför vissa kvinnor föder spontant förtidigt. Sjutton humana gener från vaginala prover visade på ett förhöjt uttryck hos de som födde för tidigt jämfört med de som födde i fullgången tid. Huruvida uttrycket av dessa gener skulle kunna användas som biomarkörer i framtiden återstår att se; 4) Tre av nio undersökta mikroRNA var överuttryckta i graviditetsvecka 10-14 hos kvinnor som sedan födde mycket förtidigt jämfört med de som födde i fullgången tid. Dessa resultat överensstämmer med en tidigare genomförd studie där man undersökte samma nio mikroRNA i en population där risken för förtidsbörd var mycket högre än i vår. Ytterligare studier behövs för att utvärdera om dessa mikroRNA har en roll som biomarkörer i framtiden.

List of papers

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Wikström, T., Hagberg, H., Jacobsson, B., Kuusela, P., Wesström, J., Lindgren, P., Fadl, H., Wennerholm, U. B., & Valentin, L. (2021). Effect of second-trimester sonographic cervical length on the risk of spontaneous preterm delivery in different risk groups: A prospective observational multicenter study. *AOGS, 100*(9), 1644–1655.

II. Wikström, T., Kuusela, P., Jacobsson, B., Hagberg, H., Lindgren, P., Svensson, M., Wennerholm, U. B., & Valentin, L. (2022). Cost-effectiveness of cervical length screening and progesterone treatment to prevent spontaneous preterm delivery in Sweden. *UOG*, *59*(6), 778–792.

III. Wikström, T., Abrahamsson, S., Bengtsson-Palme, J., Ek, J., Kuusela, P., Rekabdar, E., Lindgren, P., Wennerholm, U. B., Jacobsson, B., Valentin, L., & Hagberg, H. (2022). Microbial and human transcriptome in vaginal fluid at midgestation: Association with spontaneous preterm delivery. *Clin Transl Med 12*(9), e1023.

IV. Wikström, T., Kim SH, Leverin AL, Wennerholm UB, Jacobsson B, Valentin L, Bennett PR, Terzidou V, Hagberg H (2023). Association between miRNAs in serum at 10-14 gestational weeks and spontaneous preterm delivery. *In manuscript.*

Contents

List of abbreviations

ASA	Acetylsalicylic acid
ACER	Average cost-effectiveness ratio
ACOG	The American College of Obstetricians and Gynecologists
AUC	Area under the receiver operating characteristic curve
BMI	Body mass index
CCL2	Chemokine ligand 2
CEA	Cost-effectiveness analysis
CI	Confidence interval
CP	Cerebral palsy
CST	Community state type
Cx1	Cervical length measurement at 18+0 to 20+6 gestational weeks
Cx2	Cervical length measurement at 21+0 to 23+6 gestational weeks
eCRF	Electronic Case Record Form
FDA	The U.S. Food and Drug Administration
FDR	False discovery rate
fFN	Fetal fibronectin
FP	False positive
GDP	Gross domestic product
GSEA	Gene set enrichment analysis
HTA	Health Technology Assessment
HPV	Human papilloma virus
ICD	International statistical classification of diseases
ICER	Incremental cost-effectiveness ratio
IL-1	Interleukin 1
IL-8	Interleukin 8
IPD	Individual Patient Data
IPR	Swedish National Inpatient Register
LR+	Positive likelihood ratio
LR-	Negative likelihood ratio
IVF	In vitro fertilization
MBR	The Swedish Medical Birth Register
NNS	Number needed to screen
NPR	The Swedish National Patient Register

NPV	Negative predictive value
PPROM	Preterm prelabor rupture of membranes
PPV	Positive predictive value
PTD	Preterm delivery
PTL	Preterm labor
PDR	The Swedish Prescribed Drug Register
OR	Odds ratio
RCT	Randomized controlled trial
RNA	Ribonucleic acid
ROC	Receiver operating characteristic curve
ROS	Reactive oxygen species
RR	Relative risk
SD	Standard deviation
SE	Standard error
SEM	Standard error of the mean
SEK	Swedish krona
SFOG	Swedish Society of Obstetrics and Gynecology
Spp.	Species (bacterial)
sPTD	Spontaneous preterm delivery
17-OHPC	17-alpha-hydroxyprogesterone caproate
TNFα	Tumor necrosis factor alpha
TP	True positive
TVU	Transvaginal ultrasound
US	United States of America
WHO	World Health Organization
WTP	Willingness to pay
QALY	Quality adjusted life years

Introduction

Definition

Preterm delivery (PTD) is defined by the World Health Organization (WHO) as delivery before 37+0 weeks (37 weeks and 0 days of gestation)[1]. The definition includes singleton and multifetal deliveries resulting in either a liveborn or a stillborn infant[2]. Stillbirth is defined as delivery of a fetus with a birth weight of >500 gram and no signs of life after birth[1]. The reported frequency of stillbirth varies greatly[2, 3] but has been estimated to account for approximately 5 to 10% of all PTDs in high income countries[4, 5]. The lower limit of PTD is often reported as delivery at 22+0 weeks, but there is no international consensus on this issue. This along with a heterogeneous classification of PTD and differences in how gestational age is estimated, complicates international comparison and interpretation of reported PTD rates as well as time trends[2]. In Sweden all live and stillbirths between 22+0 weeks and 36+6 weeks are defined and reported as PTDs since 2008. Before 1 July 2008, the birth of a fetus showing no signs of life <28+0 weeks was classified as a miscarriage.

Subdivision of preterm delivery

PTD is frequently divided in two separate groups based on clinical presentation and mode of onset of delivery. Spontaneous PTD includes spontaneous onset of labor (PTL) and preterm prelabor rupture of fetal membranes (PPROM), whereas indicated PTD is secondary to an obstetric decision to deliver due to maternal or fetal complications. Indicated PTD accounts for 25-30% of all singleton PTDs[6]. This means that approximately 70-75% of all singleton PTDs are of spontaneous onset **(*Figure 1*).**

PTD is often further subdivided according to gestational age at delivery: extreme preterm (<28+0 weeks), very preterm (28+0 to 31+6 weeks) and moderate preterm (32+0 to 36+6 weeks). In recent years, a division of the latter group is increasingly seen, where late PTD (34+0 to 36+6 weeks) is often distinguished from moderate PTD (32+0 to 36+6 weeks)[8]. In Sweden in 2020, 0.2% of all liveborns (including multifetal pregnancies) were born extremely preterm (<28+0 weeks), 0.4% were

born very preterm (28+0 to 31+6 weeks) and 4.5% were born moderately preterm (32+0 to 36+6 weeks). Of all liveborn PTDs, 5.5% occurred <28 weeks (extremely preterm), 9.5% occurred between 28+0 to 31+6 weeks (very preterm) and 85% occurred between 32+0 to 36+6 weeks (moderately preterm) (***Figure 2***)[9].

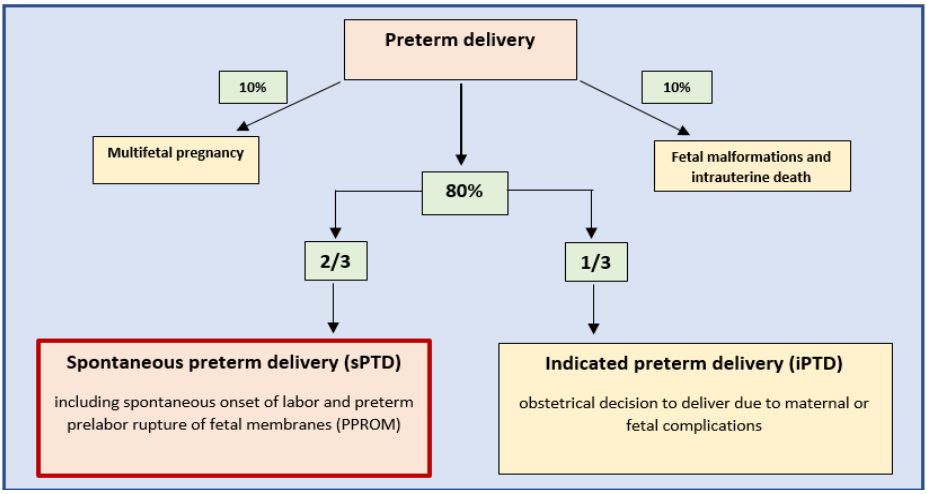

Figure 1. *Illustration of the approximative distribution of spontaneous PTD and indicated PTD*[7].
Illustration by Tove Wikström.

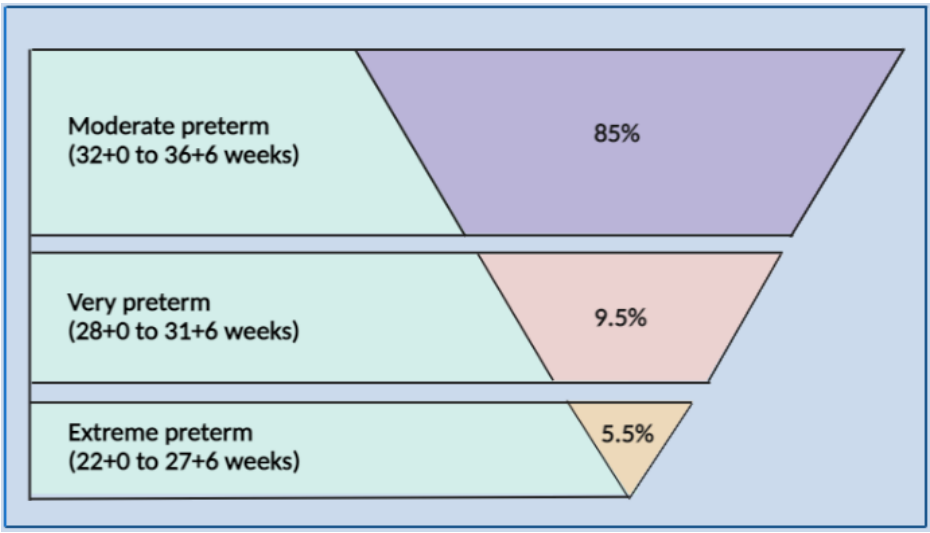

Figure 2. *Proportion of all preterm liveborn babies (including multifetal pregnancies) born extremely preterm (<28 weeks), very preterm (28+0 to 31+6 weeks) and moderately preterm (32+0 to 36+6) in Sweden in 2020. Statistics calculated from official statistics from the Swedish Medical Birth Register from the year 2020*[9].
Illustration by Tove Wikström.

Global epidemiology and consequences of preterm delivery

Globally approximately 15 million babies are estimated to be born preterm, and in 2020 nearly one million babies died due to complications of PTD[10, 11]. In 2020, the global occurrence of PTD varied between 4% and 16%, with the highest rates in South Asia and Sub-Saharan Africa (***Figure 3***)[11]. In later years an undesired trend towards an increase in PTD rates has been noted in several high income countries, mainly due to an increase in late PTDs (34+0 to 36+6 weeks)[10]. In Sweden, the PTD-rate has been stable over the past 40 years with a frequency around 5.4% (including multifetal pregnancies)[9].

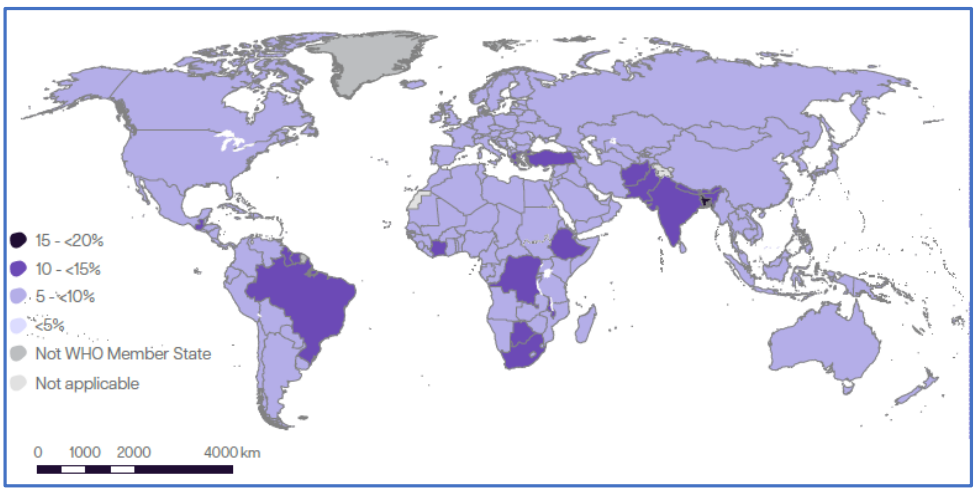

Figure 3. *Estimated preterm birth rates in 2020. World Health Organization, Partnership for Maternal, Newborn and Child Health, United Nations Children's Fund (UNICEF) & United Nations Population Fund. (2023). Born too soon: decade of action on preterm birth. World Health Organization. https://apps.who.int/iris/handle/10665/367620. License: CC BY-NC-SA 3.0 IGO.*
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PTD complications are the leading cause of death among children under five years of age, globally. The survival rates around the world shows great inequality. In low-income settings, more than 90% of extremely preterm babies (born <28+0 weeks) die within the first days of life, compared to less than 10% in high-income countries[12].

Apart from death, PTD is associated with a multitude of short- and long-term morbidities such as respiratory distress syndrome, bronchopulmonary dysplasia, necrotizing enterocolitis, cerebral palsy, neuropsychiatric problems, learning

disabilities as well as hearing and visual impairment[13-15]. Both the risk of death and severe morbidity are inversely correlated to gestational age at birth[15, 16]. Due to improvements in maternal and neonatal care, especially in high-income settings, the numbers of survivors after extreme PTD are increasing[17]. At the same time no decrease in neonatal morbidity has been seen in this group, resulting in increasing numbers of preterm survivors with severe short-and -long term neonatal morbidity. This may have major effects both at an individual and a societal level[18].

Despite the correlation between gestational age at birth and severe morbidity, it is important not to neglect late PTD (babies born 34+0 to 36+6 weeks) and its consequences. Babies born late preterm have significantly higher risks of adverse outcomes compared to babies born at term[19]. The risk of severe adverse outcomes is lower in this group as compared to babies born <34+0 weeks, but because of the high numbers of babies affected, the overall effects of adverse outcomes, both in an individual as well as on a societal level, are substantial. It is also important to consider that the consequences of PTD do not only cause a considerable burden on the affected child, but also on the affected family, as well as on health and social services[20] (***Figure 4***).

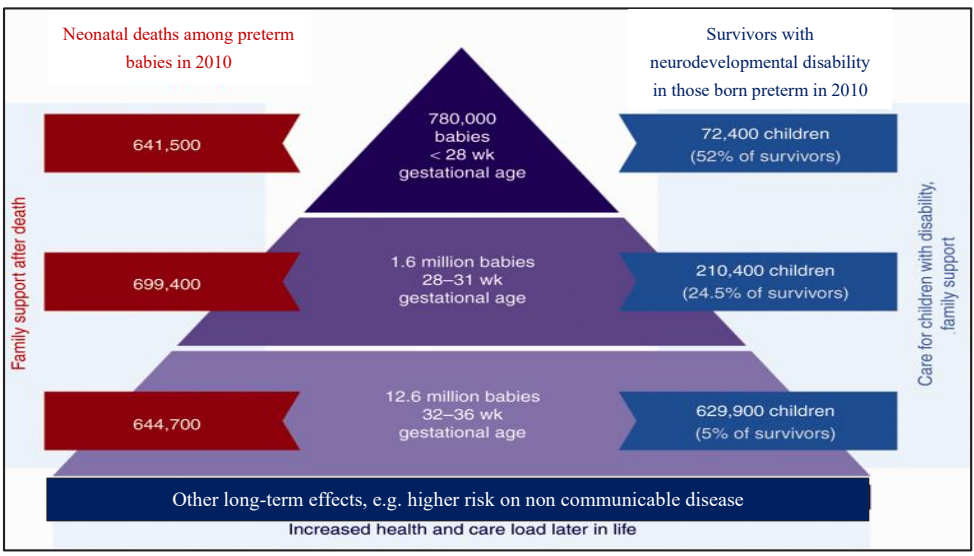

Figure 4. *Global burden of mortality and neurodevelopmental impairment for 15 million babies born in 2010. (Blencowe H, Lee AC, Cousens S, Bahalim A, Narwal R, Zhong N, et al. Preterm birth-associated neurodevelopmental impairment estimates at regional and global levels for 2010. Pediatr. Res. 2013;74 Suppl 1(Suppl 1):17-34.)*
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Risk factors for spontaneous preterm delivery

Despite investigational efforts, the pathogenesis of spontaneous PTD is still poorly understood[21]. The fact that spontaneous PTD is a syndrome rather than a condition complicates the search for a universal diagnostic tool to identify women at risk of spontaneous PTD[6].

There is a plethora of reproductive, maternal and gestational risk factors associated with spontaneous PTD, such as previous PTD, previous spontaneous late miscarriage, heritability, short interval between pregnancies, ethnicity, low or high maternal age, smoking, vascular disorders (e.g. preeclampsia or gestational hypertension), cervical conization, uterine anomalies, low socioeconomic status, assisted reproductive technology, multifetal pregnancy, fetal malformation, polyhydramnios, short cervical length (further discussed below on page 23) and infection during pregnancy[22-24] (***Figure 5***). Importantly, most of these known risk factors carry only a moderately increased risk, and about 50% of all women who deliver spontaneously preterm do not have an identifiable risk factor, limiting the predictive value of clinical risk factors alone[22]. Multiple attempts have been made to create an accurate prediction model for spontaneous PTD using the above-mentioned risk factors. However, two systematic reviews conclude that available risk-scoring systems based on risk factors alone have a low detection rate as well as a high false positive rate[25, 26].

Previous preterm delivery

Of the above-mentioned clinical risk factors, previous PTD has the strongest association with a subsequent PTD in singleton pregnancies. A meta-analysis by Phillips et al. showed an absolute risk of 30% (95% CI 27% to 34%) for a recurrent spontaneous PTD in women with at least one previous spontaneous singleton PTD[27]. Similar results have been reported by Kazemier et al. in a meta-analysis where they compared different pregnancy subtypes (e.g., women pregnant with a singleton pregnancy after a previous spontaneous preterm multifetal pregnancy or women pregnant with a singleton pregnancy after a singleton preterm pregnancy) on the risk of a subsequent spontaneous PTD. In a subgroup-analysis, women with a singleton pregnancy and with a previous singleton spontaneous PTD, showed to have a risk of recurrence of 20.3% (95% CI 19.9% to 20.6%)[28].

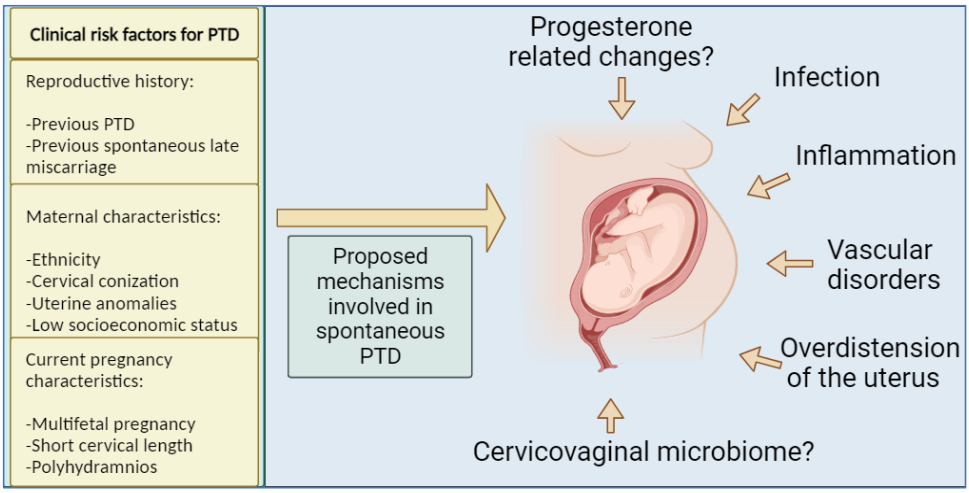


Figure 5. *Examples of clinical risk factors associated with spontaneous PTD and proposed mechanisms resulting in preterm activation of the parturition process.*
Illustration by Tove Wikström.

Cervical conization

Women who have undergone surgical treatment for cervical intraepithelial neoplasia (CIN), i.e., cervical conization, have an increased risk of spontaneous PTD[29]. A Cochrane meta-analysis performed by Kyrgiou et al. showed that women who have undergone any kind of treatment for CIN (excisional or destructive), carry an overall relative risk (RR) of PTD <37+0 weeks of 1.75 (95% CI 1.57 to 1.96). Furthermore, the risk of extreme PTD (<28+0 weeks) was increased with an RR of 2.23 (95% CI 1.55 to 3.22) when compared to women with no treatment. Repeat treatment multiplied the overall risk of PTD <37+0 weeks (RR 3.78, 95% CI 2.65 to 5.39) as compared to women who had not undergone treatment. They also addressed the issue that women with untreated CIN have a higher baseline risk for PTD[30]. The pathological mechanism behind this elevated risk is still not fully understood but several explanatory models have been proposed. One model is that the association has to do with the immune response to the human papilloma virus (HPV) itself, and another is that it is a combination of an underlying vulnerability for spontaneous PTD in women also susceptible for persistent HPV infection due to genetic or socioeconomic factors[30].

Intrauterine infection

Intrauterine infection and inflammation are widely accepted causative factors of spontaneous PTD[6, 22, 31-36], and the main route of infection is thought to be by

ascending microbes from the lower genital tract to the uterus[22]. A key element in the parturition process, whether at term or preterm, is cervical remodeling. The cervical remodeling process is associated with an inflammatory response including up-regulation of prostaglandins, chemokines, cytokines, inflammatory cell infiltration and an increased matrix metalloproteinase activity[37]. This inflammatory process can be activated by infectious microorganisms. When microbes are sensed by toll-like receptors in the decidua and fetal membranes it results in the production of cytokines and chemokines (e.g., IL-1, TNFα and IL-8), inflammatory mediators (e.g., prostaglandins and reactive oxygen radicals) and proteases which can lead to an activation of the immune system, cervical ripening, uterine contractions, and rupture of the fetal membranes[6].

The general perception is that the cervicovaginal microbiota in women of reproductive age, is predominantly dominated by Lactobacillus species (spp.), providing a barrier against genital tract infections. In contrast, an abnormal microbiota is characterized by a low abundance of Lactobacillus spp., resulting in an overgrowth of anaerobic bacteria, such as Gardnerella vaginalis, Prevotella spp., Bacteroides spp. and Mollicutes[38]. This state of imbalance of the vaginal microbiota is commonly referred to as bacterial vaginosis and has been associated with PTD[39, 40]. The diagnosis is traditionally established either clinically, by the Amsel criteria[41] (i.e., presence of clue cells, vaginal pH >4.5, profuse white discharge, and a fishy odor when the vaginal discharge is exposed to potassium hydroxide), or microbiologically with Nugent score[42]. The latter establishes the decrease in Lactobacillus spp. and increase in anaerobes on a Gram-stained vaginal smear. A meta-analysis by Leitich et al. found that bacterial vaginosis increases the risk of PTD <37+0 weeks more than two times (OR 2.19, 95% CI 1.54 to 3.12), and if the diagnosis was made before 20 weeks of gestation the risk quadrupled (OR 4.20, 95% CI 2.11 to 8.39)[43]. Although the precise mechanism through which bacterial vaginosis affects pregnancy remains unclear, it has been suggested that bacterial vaginosis predisposes to ascending infection in early pregnancy[44, 45]. Consequently, it has been hypothesized that early antibiotic treatment of bacterial vaginosis could have a preventive effect on PTD. However, studies have shown conflicting results. The meta-analysis by Lamont et al. showed that women diagnosed with bacterial vaginosis who were treated with clindamycin before 22 gestational weeks had a risk-reduction of PTD by 40%, compared to those who did not receive treatment[46]. In contrast, the meta-analysis by Brocklehurst et al. showed no effect of early intervention with antibiotics to prevent PTD[47]. Thereafter, the PREMEVA trial has been published, a large randomized controlled trial (RCT) performed by Subtil et al.,

where 84 530 women were screened for bacterial vaginosis before 14 weeks of gestation. In the trial 2869 women with bacterial vaginosis (diagnosis based on Nugent score), were randomly allocated to receive clindamycin or placebo. The trial showed no reduction in either PTD or late miscarriage[48].

In later years the techniques to investigate the microbiome has developed drastically, enabling sequencing of the whole vaginal genome/transcriptome. Multiple studies have tried to characterize the relationship between the cervicovaginal microbiome and spontaneous PTD, with the aim of finding a microbial signature that could predict spontaneous PTD[49-59]. Most studies have applied 16s rRNA gene sequencing, providing information on the taxonomic composition of the bacterial communities, but not on whether the bacteria are active members of the microbiome[60]. Interpretation of these studies are difficult as both the microbiome itself, and the relationship between the microbiome and spontaneous PTD, seems to differ depending on particularly race and ethnicity[51, 52, 61]. For example, it has been proposed that Lactobacillus depletion, as well as an altered species diversity, is more common in African American women than in Caucasian women[50, 51] , but that the observed deficit of lactobacillus and higher species diversity serves as risk factors for spontaneous PTD primarily in Caucasian women and not in African American women[51, 57]. This illustrates the complexity of the syndrome of spontaneous PTD, as well as the difficulty of translating the findings and associations of spontaneous PTD in one study population to other populations.

Hypertensive disorders of pregnancy

Women with hypertensive disorders of pregnancy such as gestational hypertension or preeclampsia have an increased risk for spontaneous PTD[22]. Interestingly, it has been shown that approximately 30% of patients with PTD have placental disorders consistent with vascular hypoperfusion of the placenta with a histological picture of underdeveloped myometrial spiral arteries, usually associated with preeclampsia[62]. Furthermore, Rasmusen et al. found that women with a PTD in their first pregnancy without preeclampsia, had a four to seven times higher risk of preterm preeclampsia (onset <37+0 weeks) in the second pregnancy compared to those who had a term delivery[63]. This suggests that PTD and vascular disorders in pregnancy share pathophysiological mechanisms and deserves further investigation.

Ethnicity

A metanalysis by Schaaf et al. demonstrated an increased risk (pooled OR 2.0; 95% CI 1.8 to 2.2) of PTD among women of black ethnicity compared to women of white ethnicity[64]. The reason for this disparity is still poorly understood. Manuck concludes in her review that the differences in the risk of PTD linked to ethnicity cannot solely be explained by sociodemographic factors[65]. As described previously, PTD is rather a syndrome than a single condition, and many of the risk factors associated with PTD are difficult to quantify because of their interrelations[65]. In conclusion, it is important to consider the study population when interpreting the results of a study, as this may affect the generalizability of the results.

Prediction of spontaneous preterm delivery

Cervical length

Multiple studies have shown that a short cervix (often defined as ≤25 mm[66]) measured by transvaginal ultrasound in the second trimester, is associated with spontaneous PTD in asymptomatic women with a singleton pregnancy[67-72]. Treatment with vaginal progesterone to those at risk for spontaneous PTD has been shown to possibly reduce the risk of PTD and improve neonatal outcome (further discussed on page 28)[73]. Therefore, universal second trimester cervical length screening with transvaginal ultrasound has been proposed[66]. Over the years, published studies reporting on the prevalence of a short cervix, as well as on the ability of cervical length to correctly predict spontaneous PTD, have shown highly inconsistent results (**Table 1 and 2**)[68 67, 69, 70, 74-80]. This variation may be explained by differences in the characteristics of the study population (e.g., obstetric risk factors and ethnicity), cervical length measurement technique, the level of experience of the person performing the transvaginal ultrasound assessment of cervical length, and whether the study was blinded or not.

Table 1. Large observational studies presenting the prevalence of short cervix as measured by transvaginal ultrasound in asymptomatic women with a singleton pregnancy in the second trimester.

Author, journal, year of publication	No. women	Country/population	Measurement timepoint	Prevalence of short cervix				
				≤15 mm	10-20 mm	≤20 mm	≤25 mm	≤30 mm
Iams et al.[68] *NEJM* 1996*	2915	Multicentre, USA, 63% African American women	24 weeks	-	-	3.4%	8%	25%
Heath et al.[81] *UOG* 1998	2702	Single center, UK, 52% non- Caucasian women	23 weeks	1.7%	-	3.4%	8%	18%
Taipale et al.[67] *Obstet Gynecol* 1998*	3694	Single center, Finland, 99% Caucasian women	18-22 weeks	-	-	-	0.3%	3%
Leung et al.[69] *UOG* 2005*	2880	Single center, Hong Kong, 100% Chinese women	18-22 weeks	-	-	0.2%	1.8%	10%
Hassan et al.[75] *UOG* 2011	458	Multicentre, international, 69% non-Caucasian women	19-23 weeks	-	2.3%	-	-	-
Grobman et al.[76] *AJOG* 2012	657	Single center, USA, 68% non-Caucasian women	16-22 weeks	-	-	-	-	10.3%
Van Os et al.[70] *UOG* 2017	20 234	Multicentre, The Netherlands, ethnicity not reported	approx. 20 weeks	-	-	-	-	1.8%
Wulff et al.[77] *UOG* 2018	3477	Multicentre, Denmark, 97% Caucasian women	19-21 weeks	-	-	0.30%	0.78%	-
	3299		23-24 weeks	-	-	0.85%	1.79%	-
Kuusela et al.[80] *BJOG* 2021*	11 072	Multicentre, Sweden, 89% Caucasian women	18-20 weeks	0.14%	0.55%	0.61%	3.98%	19.60%
	6288	at 18-20 weeks and 92% at 21-23 weeks	21-23 weeks	0.22%	1.03%	1.13%	4.36%	18.60%

**Blinded studies, i.e., the assessor as well as the participant were blinded to the results of the cervical length measurements.*

Table 2. Summary of blinded studies (measurement results not provided either to pregnant woman or to medical staff) estimating the ability of cervical length as measured by transvaginal ultrasound to predict spontaneous preterm delivery in asymptomatic women with a singleton pregnancy.

					Cervical length					
					≤20 mm		≤25 mm		≤30 mm	
Author, journal, year of publication	**No. women**	**Country/ population**	**Measurement time-point**	**Outcome predicted by short cervix (prevalence of outcome)**	**Sens.**	**Spec.**	**Sens.**	**Spec.**	**Sens.**	**Spec.**
Iams et al.[68] *NEJM* 1996	2915	Multicenter, USA, 63% African American women	22-24 weeks	sPTD <35 weeks (4.3%)	23%	97%	37%	92%	54%	75%
Taipale et al.[67] *Obstet Gynecol* 1998	3694	Single center, Finland, 99% Caucasian	18–22 weeks	sPTD <35 weeks (0.8%)	-	-	7%	100%	-	-
				sPTD <37 weeks (2.4%)	-	-	6%	100%	-	-
Carvalho et al.[78] *UOG* 2003	529	Single center, Brazil, 65% non-Caucasian	22–24 weeks	sPTD <33 weeks (1.9%)	40%	97%	-	-	-	-
				sPTD <35 weeks (NA)	42.3%	96.7%				
Leung et al.[69] *UOG* 2005	2880	Single center, Hong Kong, 100% Chinese women	18–22 weeks	sPTD <34 weeks (3.7%)	10.5%	99.9%	26.3%	98.3%	36.8%	90.1%
Davies et al.[79] *JOGC* 2008	964	Single center, Canada, ethnicity not reported	24 weeks	sPTD <35 weeks (1.7%)	6.3%	99.6%	25%	97.1%	50%	85.9%
				sPTD <37 weeks (4.8%)	2.2%	99.8%	13%	97.3%	26.1%	85.8%
Kuusela et al.[80] *BJOG* 2021 (the CERVIX-study)	11 072	Multicenter, Sweden, 89% Caucasian women at 18-20 weeks and 92% at 21-23 weeks	18–20 weeks	sPTD <33 weeks (1.0%)	11.1%	99.5%	27.0%	96.1%	46.0%	80.5%
				sPTD <37 weeks (5.3%)	3.1%	99.5%	9.1%	96.2%	26.8%	80.8%
	6288		21–23 weeks	sPTD <33 weeks (1.0%)	34.6%	99.0%	38.5%	95.8%	57.7%	81.6%
				sPTD <37 weeks (5.1%)	8.0%	99.1%	15.1%	96.0%	32.4%	81.9%

Sens., sensitivity; Spec., specificity; sPTD, spontaneous preterm delivery; NA, not available

The differences in the reported sensitivity and specificity of cervical length as measured with ultrasound to correctly predict spontaneous PTD has revived the question of whether cervical length screening for prediction of spontaneous PTD is suitable for all populations despite differences in ethnicity and prevalence of spontaneous PTD. Consequently, Kuusela et al[80] performed a prospective blinded multicenter diagnostic study at seven hospitals in Sweden, to investigate the ability

of second trimester cervical length measurement to discriminate between asymptomatic women with a singleton pregnancy delivering preterm *vs* at term (the CERVIX-study)[80].

The CERVIX-study included 11 072 - mainly Caucasian - women with transvaginal ultrasound cervical length measurement at 18+0 to 20+6 weeks (Cx1), and 6288 women with cervical length measurement at 21+0 to 23+6 weeks (Cx2), all with complete delivery data. Of the 11 072 women, 6179 had cervical length measurement at both Cx1 and Cx2 with at least 14 days between measurements. The CERVIX-study is further described under the section "methods". In brief, the study showed that second trimester cervical length measurement can identify women at high risk of spontaneous PTD in a predominantly Caucasian population with a low prevalence of spontaneous PTD, and that the diagnostic performance was at best moderate (**Table 2**). The discriminative ability of cervical length measurement was better for early spontaneous PTD (<33+0 weeks) and was substantially better if measurements were performed at 21 to 23 weeks compared to at 18 to 20 weeks. The best definition (cut-off) of "short" cervical length has been debated, and in the CERVIX-study[80] it was shown to be 29 mm for cervical length measurement at 18 to 20 weeks (Cx1), and 27 mm for measurements at 21 to 23 weeks (Cx2). Problematically, there is uncertainty regarding the effect of vaginal progesterone in women with a cervical length between 26 and 30 mm[82]. The main questions remaining after completion of the CERVIX-study were: 1) does the discriminative ability of second trimester transvaginal cervical length measurement differ between different obstetric risk groups, and 2) would it be cost-effective to introduce a cervical length screening program, followed by treatment with vaginal progesterone to women considered at increased risk of spontaneous PTD (short cervix) in Sweden, and if so, to whom should it be directed? This thesis addresses the above questions based on the study population in the CERVIX-study[80].

Biomarkers

Despite that second trimester cervical length measurement has been shown to be reasonably good at discriminating between those who deliver preterm *vs* at term[67-69, 78-80], cervical length screening has its limitations: it is expensive, has a moderate discriminative ability and requires ultrasound expertise. Due to this, it would be of great economic and practical value to find an easily measured biomarker to predict spontaneous PTD, a marker that could be used alone or as a complement to cervical length screening to sharpen its diagnostic capacity.

The most studied biomarker to date is fetal fibronectin (fFN), an extracellular matrix glycoprotein found at the maternal-fetal interface of the amniotic membranes. Elevated concentrations of fFN in cervicovaginal fluids have been shown to be associated with PTD[83, 84]. The test is taken as a bedside test on vaginal or cervical secretions[85]. fFN has been evaluated in women with and without symptoms of threatening PTD. In the metanalysis by Honest et al. the area under the receiver operating characteristic curve (AUC) (for discrimination between those giving birth within the following seven days *vs* later) was 0.84 in women presenting with symptoms of threatening PTD (i.e., contractions and cervical length shortening). The corresponding AUC-value in asymptomatic women for discrimination between those who gave birth <34+0 weeks *vs* later was 0.61[86]. The use of fFN to predict spontaneous PTD in asymptomatic women is not recommended due to its poor discriminative ability, but it is used in symptomatic women at many centres over the world.

In recent years it has been suggested that microRNAs (miRNAs) have the potential of functioning as biomarkers in several pathological processes, e.g., cancer and neurodegenerative disease[87]. In short, miRNAs are small, non-protein-coding RNAs with an important posttranscriptional gene-regulating function. They predominantly repress gene expression by inhibiting the translation of target messenger-RNAs (mRNAs), resulting in reduced protein synthesis (***Figure 6***)[88]. Most data suggest that miRNAs do not require perfect base pairing with targeted mRNA and can therefore have a pleiotropic effect on the regulation of gene expression. This means that one miRNA can target a multitude of different mRNAs, and another mRNA can be repressed by multiple miRNAs[89]. Even though several miRNAs share targets, the levels of individual miRNAs have been shown to differ substantially between different tissues[87]. MiRNAs are considered to be stable molecules and are therefore relatively easy to detect in human body fluids, enhancing their potential as diagnostic and prognostic biomarkers[90]. Interestingly, Cook et al. recently reported on an association between the expression of nine specific miRNAs and spontaneous PTD <34+0 weeks[91], emphasizing the potential possibility of using miRNA-profiles as either stand-alone or as complementary biomarkers for spontaneous PTD in the future.

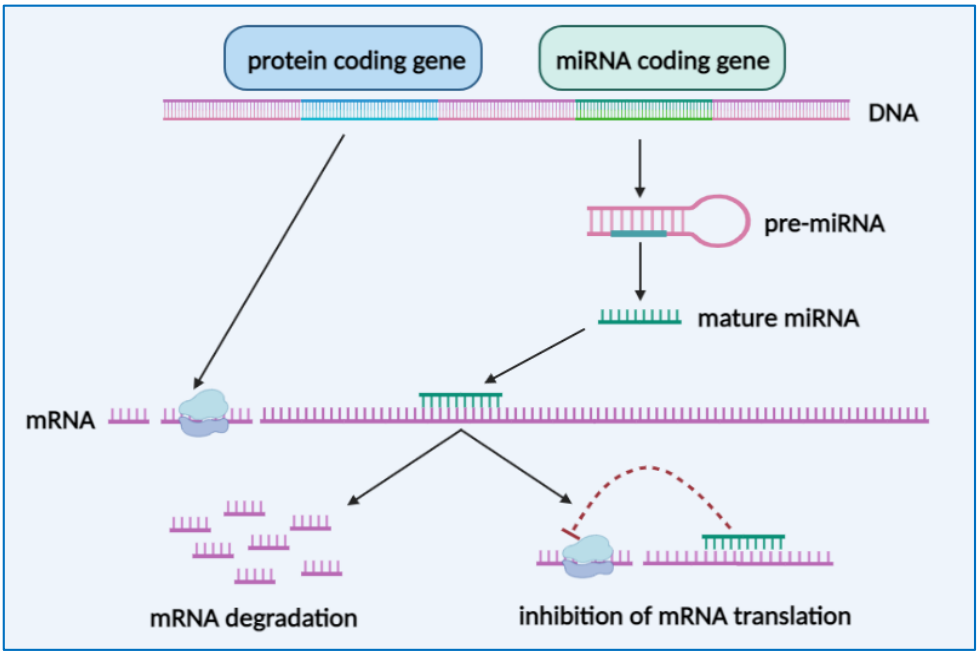


Figure 6. *Schematic illustration of miRNA biogenesis and function. Binding of a miRNA will lead to either target mRNA degradation or inhibition of its translation.*
Illustration by Tove Wikström.

Prevention of spontaneous preterm delivery

A range of interventions to prevent spontaneous PTD has been suggested and are in use to varying extents over the world. The most discussed interventions (apart from lifestyle and behavioral changes) are treatments with progesterone (vaginal, intramuscular, or oral), cerclage and more recently acetylsalicylic acid (ASA) and cervical pessary. There is currently insufficient evidence supporting the use of cervical pessary to prevent spontaneous PTD, and it will therefore not be discussed here[92].

Progesterone

Progesterone is essential for the maintenance of a pregnancy until term. One of its critical roles is to induce cellular changes to facilitate embryo implantation and endometrial decidualization in early pregnancy. It has also been shown to have immunomodulatory effects by exerting an immune tolerogenic effect on trophoblasts and to increase the amount of anti-inflammatory mediators[93]. In addition,

progesterone is suggested to maintain uterus quiescence by inhibiting the production of prostaglandins as well as the expression of contraction-associated proteins in the myometrium (**Figure 7**)[94].

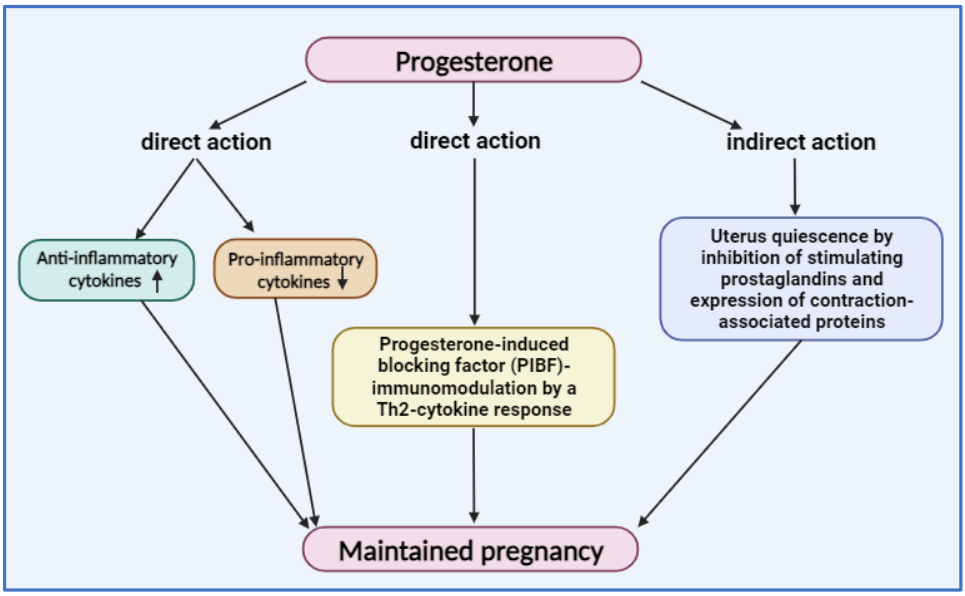


Figure 7. *Schematic illustration of the proposed effects of progesterone in maintaining pregnancy.*
Illustration by Tove Wikström.

Two main forms of non-endogenous progesterone are available as medical products: natural micronized progesterone and semisynthetic progestogen. Natural micronized progesterone is similar to endogenous progesterone whereas semisynthetic progestogen including 17-alpha-hydroxyprogesterone caproate (17-OHPC), has a different chemical structure[95]. Natural micronized progesterone is most commonly administered as daily vaginal gel or suppositories, whereas 17-OHPC is administered as a weekly intramuscular injection. In Sweden, intramuscular injection of 17-OHPC is not approved by the Swedish Medical Products Agency for prevention of spontaneous PTD.

Two large individual patient data meta-analyses (IPD meta-analyses)[73, 82] as well as a recently published Health Technology Assessment (HTA) report based on a systematic review[92], concludes that vaginal progesterone has a favorable effect in

reducing the number of PTDs in asymptomatic women at high risk of PTD (**Table 3**). In addition, progesterone appears to be a safe treatment with few and mild side effects for both the mother and the baby. Importantly no differences on the child's long-term outcome have been demonstrated[82, 92].

Table 3. Estimated effect of vaginal progesterone to prevent PTD in two IPD-meta analyses and one HTA-report. *Statistically significant results are indicated in bold.*

Outcome (weeks)	Romero et al.[73]* *AJOG* 2018		EPPPIC-study[82] *Lancet* 2021		HTA-report 2022[92]	
	No. trials/ participants	RR (95% CI)	No. trials/ participants	RR (95% CI)	No. trials/ participants	RR (95% CI)
PTD† <37	5/974	0.90 (0.77-1.05) ‡	9/3769	0.92 (0.84-1.00) §	8/2416	0.85 (0.71-1.02) §
PTD† <34	5/974	**0.65 (0.51-0.83)** ‡	9/3769	**0.78 (0.68–0.90)** §	10/3800	**0.77 (0.62-0.94)** §
sPTD <34	5/974	**0.72 (0.55-0.95)** ‡	NA	NA	2/330	**0.57 (0.38-0.86)** §
PTD† <33	5/974	**0.62 (0.47-0.81)** ‡	NA	NA	5/974	**0.63 (0.48-0.83)** §
sPTD <33	5/974	**0.70 (0.51-0.95)** ‡	NA	NA	NA	NA
PTD† <28	5/974	**0.67 (0.45-0.99)** ‡	9/3769	0.81 (0.62-1.06) §	NA	NA

HTA, health technology assessment; RR, relative risk; CI, confidence interval; PTD, preterm delivery; sPTD, spontaneous preterm delivery, NA, not applicable
**Including only women with a cervical length ≤25 mm*
†Including any PTD (spontaneous and indicated)
‡Fixed effect model reported
§Random effect model reported

These results are confirmed in a recent systematic review and network meta-analysis by Care et al., in which the authors conclude that vaginal progesterone should be the treatment of choice in women with either a history of PTD or short cervical length as measured with ultrasound. They recognize that 17-OHPC and cervical cerclage have shown potential of reducing spontaneous PTD <34+0 weeks as well as neonatal death, but that it is not superior to vaginal progesterone[96]. The effect of vaginal progesterone to prevent PTD is best documented in women with a short cervix (≤25 mm)[73, 82, 92], but there is evidence that it could also be effective in women with a history of previous PTD or late miscarriage with unknown cervical length[97].

Cerclage

Cerclage offers mechanical support to the cervix by placing a stitch around the cervix, either by a vaginal (most common) or a transabdominal approach, with the purpose of preventing preterm dilation of the cervix[98]. In Sweden, the frequency of administered cerclages differs between centra, and reliable statistics are difficult to access. Most probably it is a rare procedure used for very specific risk groups. In the CERVIX-study, 1 of 11 072 women received a cerclage after inclusion in the study[80], and in 2020, 10 of 18 920 women (0.05%) who gave birth in Region Västra Götaland in Sweden had a cerclage placement during pregnancy[92].

Cerclage is often categorized by its indication for insertion, either as a "history-indicated cerclage" placed in asymptomatic women based on obstetric history, or as an "ultrasound-indicated cerclage" placed in asymptomatic women with a cervical shortening, or as a "rescue-cerclage" when the cervix is already open and fetal membranes are exposed[99]. In the HTA-report published in 2022, vaginal cerclage was shown to have a potential reductive effect on the risk of any PTD (spontaneous or indicated) in women with a singleton pregnancy. Statistically significant results were found for PTD <37+0, <34+0, <33+0 and <28+0 weeks **(Table 4)**[92].

Table 4. Table modified from the HTA-report published in 2022[92] showing the effect of cerclage on the risk of any PTD (spontaneous or indicated) in women with a singleton pregnancy.

Outcome	No. trials/ participants	Relative effect RR (95% CI) *	Absolute effect (%)
PTD <37 weeks	4/1919	**0.78 (0.69 to 0.88)**	**29.0 *vs* 37.6**
PTD <35 weeks	1/301	0.76 (0.56 to 1.03)	31.8 *vs* 41.8
PTD <34 weeks	4/1919	**0.79 (0.66 to 0.94)**	**20 .0 *vs* 22.3**
PTD <33 weeks	2/1517	**0.79 (0.63 to 0.99)**	**14.4 *vs* 18.3**
PTD <32 weeks	1/101	0.85 (0.27 to 2.70)	10.3 *vs* 12.1
PTD <28 weeks	4/1915	0.77 (0.60 to 1.00)	9.1 vs 12.2
Perinatal mortality	3/1818	**0.72 (0.54 to 0.97)**	**8.0 *vs* 12.2**

Statistically significant results are indicated in bold. HTA, health technology assessment; RR, relative risk; CI, confidence interval; PTD, preterm delivery
** Random effects model reported*

The International Federation of Gynecology and Obstetrics (FIGO) recommends that "history-indicated cerclage" should be offered to women who have had three or more PTDs or late miscarriages, and that an "ultrasound-indicated cerclage" should be offered to women with a cervical length <25 mm if they have had one or more PTDs or late miscarriages[99]. This limits its use as the majority of women do not meet these criteria and are therefore not considered to benefit from the treatment. Another important aspect is that cerclage is an invasive procedure, performed under general or regional anesthesia, requiring both access to an operating theatre and anesthesiologic staff, implying high costs. Because of this, a general screening of cervical length followed by insertion of cerclage in women considered at risk for PTD would probably not be considered cost-effective in a Swedish setting[92]. Importantly, this does not mean that cerclage does not have an important role in the strive for prolongation of pregnancy in some women. However, the indication for (either cervical or abdominal) cerclage needs further investigation.

ASA

ASA is an established treatment to prevent preeclampsia for women at risk, although the mechanism behind its preventive effect is still unknown[100]. In later years the question on whether ASA could be used to reduce the rate of spontaneous PTD has been raised, though studies have shown conflicting results regarding its effect[101-103]. Hypothetically, the protective effect of ASA on the risk of spontaneous PTD could be through decreasing the uterine contractility via inhibition of cyclooxygenase (COX)-dependent prostaglandin synthesis[104]. In the recently published HTA report based on a systematic review, no evidence was found for ASA to have a favorable effect on the rate of PTD, perinatal mortality or morbidity, though the analysis is based on only one trial (n= 387 women)[92].

Health economic evaluations

Health economic evaluations can be used to show the economic impact and relative value of money to gain health of the intervention. Health care systems, as well as social care systems are generally resource limited. Simply measuring the costs of an intervention will not give information about whether an intervention is cost-effective or not. A cheap intervention may represent poor value for money if it has little effect on the outcome. An economic evaluation is the process of measuring cost-effectiveness, and a common way to do this is to perform a cost-effectiveness analysis (CEA). A CEA will measure two parameters, cost and outcome, to obtain

an incremental cost effectiveness ratio (ICER). The ICER is calculated by dividing the difference in costs for the intervention group compared to the control group with the difference in health gained or lost. The most common measure of gained or lost health is quality adjusted life years (QALY) which includes both a quality and quantity aspect of health, where the quality scale ranges from 0 (dead) to 1 (perfect health) and the quantity consists of the time spent in the condition (**Figure 8**)[105].

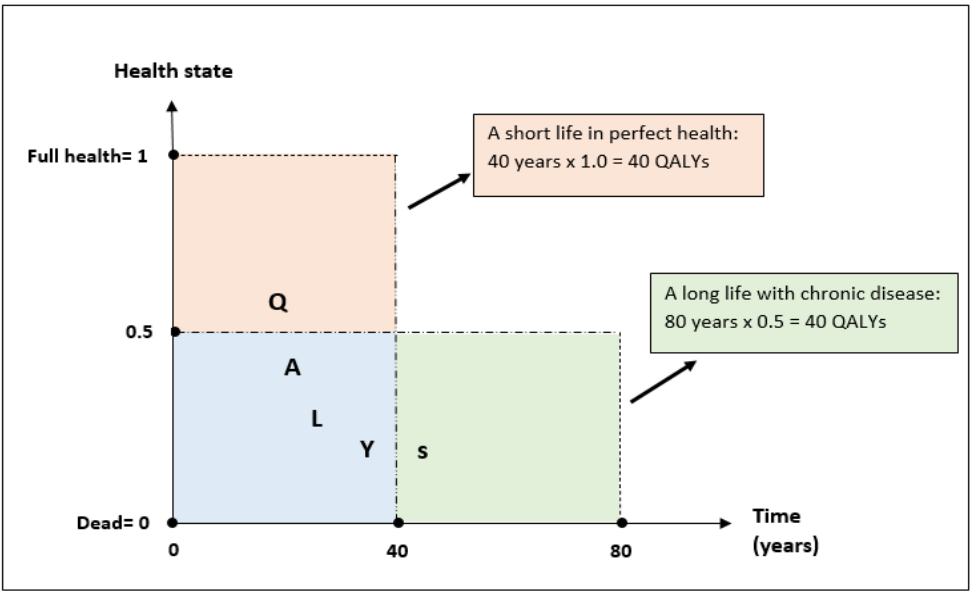


Figure 8. *A simplified illustration of how a QALY is calculated and the relationship between the estimated health state and time. On the y-axis health state is shown (expressed as QALY-weights where 0 represents death and 1 represents full health). On the x-axis time (in years) is shown.*
Illustration by Tove Wikström, modified from the article: Arnesen T, Trommald M. Roughly right or precisely wrong? Systematic review of quality-of-life weights elicited with the time trade-off method. J Health Serv Res Policy. 2004;9(1):43-50. QALY, Quality adjusted life years.

If the less costly alternative in a health economic evaluation is also the most effective alternative, it will be described as *dominant*. However, if the least costly alternative is not the most effective, the decision of which intervention to choose is less clear. In this situation the ICER plays an important role as it represent the additional costs per gained QALY (**Figure 9**). Thus, a health economic evaluation can help to clarify whether differences in costs between one alternative and another is justifiable according to the recommendations made by the health care administration of a country, i.e., willingness to pay (WTP)[106]. The Swedish National Board of Health

and Welfare have recommended a WTP per gained QALY of about 56 000 USD (corresponding to approximately 500 000 Swedish krona (SEK)), although it is important to take into account that this value is not written in stone[107]. The WTP can in certain cases be both lower and higher depending on e.g., the severity and frequency of the disease or condition, as well as the treatment effect achieved. Also, there is no general international agreement on WTP. A published review analyzing WTP from 333 unique health economy studies from different countries summarizes that the average value is about 75 000 EUROS (corresponding to about 700 000 SEK and 79 000 USD) per QALY. It has been suggested that this value should be used as an approximate threshold for WTP depending on the condition or illness evaluated[108].

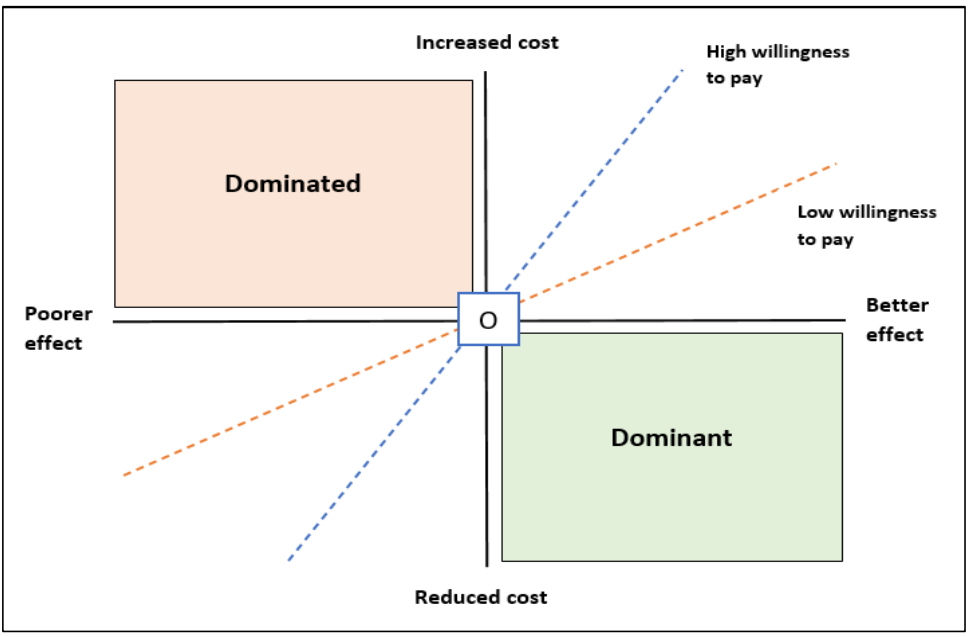


Figure 9. *Illustration of the cost-effectiveness (CE) plane, with the reference strategy occupying the origo of the graph (O). The difference in cost (incremental cost) is shown on the y-axis and the benefit of an alternative strategy is shown on the x-axis. The area above the horizontal line is cost increasing, and the area to the right of the vertical line gives better health outcome compared to "O". If an alternative is both less expensive and generates better health compared to "O" it is classified as "dominant". Correspondingly an alternative that is more costly with poorer effect compared to "O" is classified as "dominated". When a new strategy adds both benefits and costs (upper right-hand quadrant) a CE-ratio must be calculated to judge the benefits relative to its costs.*
Illustration by Tove Wikström, modified from the report on "Health Economic Evaluations" by the Public Health Agency of Sweden.

In the systematic literature search conducted as a basis for the health economic analysis in this thesis (Paper II), five studies were identified to evaluate the cost-effectiveness of cervical length screening followed by treatment with vaginal progesterone to those considered at high risk for PTD[109]. All five studies were from the United States (US)[110-114]. Four of the studies reported cervical length screening, followed by vaginal progesterone in case of a short cervix, to be cost-effective[110-113]. Only one study questioned its cost-effectiveness[114]. However, the results from these studies are difficult to apply to a Swedish context since there are substantial differences between the target populations and the organization of the health care system. To exemplify this, the PTD rate in singleton pregnancies was 4.4% in Sweden in 2018[115] and 10.2% in the US[116] while the health care expenditure made up 11% of the gross domestic product (GDP) in Sweden[117] compared to 18% in the US[118]. This emphasizes the importance of evaluating the underlying context when interpreting a health economic evaluation, and to realize that the results are not necessarily transferable to all populations.

Aim

In a population with a low prevalence of spontaneous PTD this thesis aims to evaluate:

- the influence of cervical length, as measured with transvaginal ultrasound, on the risk of spontaneous PTD, in different risk groups of asymptomatic women with a singleton pregnancy (Paper I)

- the cost-effectiveness of different strategies to prevent spontaneous PTD, including second trimester cervical length screening and treatment with vaginal progesterone of women considered at increased risk of spontaneous PTD (Paper II)

- whether there are differences in the active vaginal microbiome and human transcriptome in the second trimester between asymptomatic women with a singleton pregnancy who subsequently deliver spontaneously preterm *vs* at term (Paper III)

- nine specific miRNAs in serum as biomarkers in early second trimester for spontaneous PTD in asymptomatic women with a singleton pregnancy (Paper IV)

Patients and methods

Setting and Study design

All four studies are based on the study population of the CERVIX-study, a prospective blinded multicentre study conducted at six university hospitals and one regional hospital in Sweden (https://doi.org/10.1186/ISRCTN18093885). Inclusion took place between May 2014 and June 2017[80]. In short, asymptomatic women (i.e., without signs of preterm labor) with a singleton pregnancy were consecutively recruited to the CERVIX-study at their routine second trimester fetal ultrasound examination. The examination included fetal biometry for estimation of gestational age and fetal anatomy scanning. Women ≥18 years old with a live singleton pregnancy between 18 gestational weeks+0 days (18+0 weeks) and 20+6 gestational weeks were invited to participate. An overview of the study design of the CERVIX-study, including inclusion and exclusion criteria is shown in **Figure 10**.

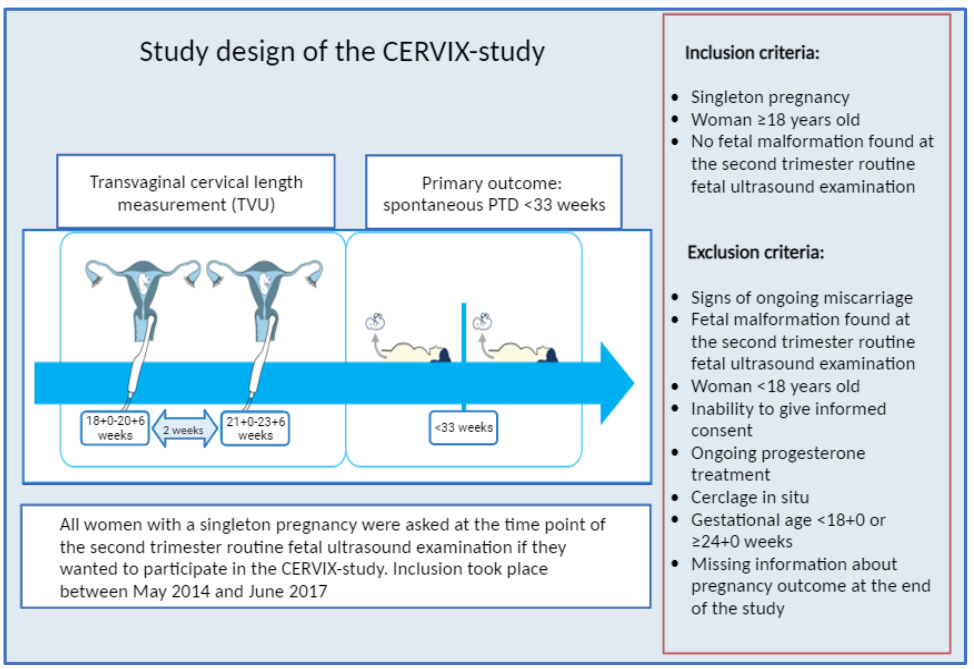


***Figure 10**. Overview of the study design of the CERVIX-study[80].*
Illustration modified from a figure by Professor Bo Jacobsson, printed with permission.

The study protocol included two transvaginal ultrasound (TVU) measurements of cervical length: one between 18+0 and 20+6 weeks (Cx1), performed on the day of the routine fetal ultrasound examination, and a second one between 21+0 and 23+6 weeks (Cx2; optional) with at least 14 days between the two measurements. Participants and staff were blinded to the cervical length measurements, and results were only disclosed to staff and participants if the ultrasound showed signs of an unavoidable miscarriage. Spontaneous PTD was defined as delivery <37+0 weeks (including late miscarriages occurring after inclusion in the study), either after spontaneous onset of labor or after preterm pre-labor rupture of membranes, the latter regardless of whether labor was induced or not.

Information on participant characteristics and pregnancy outcome was retrieved from either the electronic case record form (eCRF; standardized anamnestic information obtained from the women at enrollment), the Swedish Pregnancy Register[119], the Swedish Prescribed Drug Register, or the Swedish National Patient Register[120]. Information on previous spontaneous PTD (singleton or multifetal) was obtained from the Swedish Medical Birth Register[121] as described below (page 42, **Figure 11**). An overview of the design of each of the four studies in the thesis is seen in **Table 5**.

Table 5. Overview of study design of paper I-IV.

The CERVIX-study[80]: a prospective blinded observational multicentre study including: 1) 11 072 asymptomatic women with a singleton pregnancy with cervical length measurement at 18+0 to 20+6 weeks (Cx1) with accessible delivery data 2) 6288 asymptomatic women with a singleton pregnancy and cervical length measurement at 21+0 to 23+6 weeks (Cx2) with accessible delivery data				
	Paper I	**Paper II**	**Paper III**	**Paper IV**
Study period	2014 to 2017		2016 to 2017	2014 to 2017
Country	Sweden, seven centres		Sweden, three centres	Sweden, two centres
Study design and aim	Pre-planned exploratory analysis of the CERVIX-study to evaluate the discriminative ability of cervical length as measured with TVU in asymptomatic women with a singleton pregnancy in different risk groups	Decision analytic model based on the CERVIX-study to estimate the cost-effectiveness of various strategies to prevent sPTD in asymptomatic women with a singleton pregnancy	Nested case-control study to evaluate potential differences in the vaginal microbial and human transcriptome in asymptomatic women with a singleton pregnancy delivering preterm *vs* at term	Nested case-control study to evaluate the association between nine specific miRNAs in maternal serum and sPTD, alone or in combination with cervical length (TVU). This is an external validation of the results published by Cook et al.[91]
Sample size	11 072 women Analyses were performed separately for the Cx1-population (11 072 women) and for the Cx2-population (6288 women)		1219 women from the Cx1-population were sampled for vaginal fluid before cervical length measurement by TVU Cases: 48 sPTD (48/1219) Controls: 96 term deliveries (39+0-40+6 weeks) chosen by simple random selection	Cases: 61 women from the CERVIX-study (Gothenburg/Malmö) who delivered spontaneously <34 weeks with routinely taken serological samples for infectious screening (HIV, Syphilis and Hepatitis C) at 10-14 weeks Controls: 207 women with term deliveries (39+0-40+6 weeks) with available serum samples chosen by simple random selection
Data sources	The electronic Case Record Form, the Swedish Medical Birth Register, The Swedish Pregnancy Register, the Swedish National Patient Register, the Swedish Prescribed Drug Register, Obstetrix* and Statistics Sweden†			
Outcome	sPTD <33 weeks sPTD <37 weeks		sPTD <37 weeks	sPTD <34 weeks
Analyses of biospecimen material	NA		RNA extraction from vaginal specimens followed by high throughput sequencing. Expression analysis of both human and microbial transcripts	RNA extraction from serum samples followed by expression analysis of miRNAs by RT-qPCR. miRNA analysed in Gothenburg in cooperation with Kings College London

sPTD, spontaneous preterm delivery; TVU, transvaginal ultrasound; RNA, ribonucleic acid; NA, not applicable; RT-qPCR, real-time quantitative reverse transcription polymerase chain reaction; miRNA, micro ribonucleic acid

**Electronic medical record system used in most obstetrical facilities in Sweden (Cerner AB Sweden)*

† Only used in Paper II

Data sources

Figure 11 is an overview of the data sources used for maternal characteristics and pregnancy outcome in Paper I-IV. All data sources are described in detail in the text below. Linkages between the registers and electronic medical records are possible via the unique personal identification number assigned to each permanent resident in Sweden. This process is highly regulated and requires ethical approval from the ethical committee, as well as from the Swedish National Board of Health and Welfare.

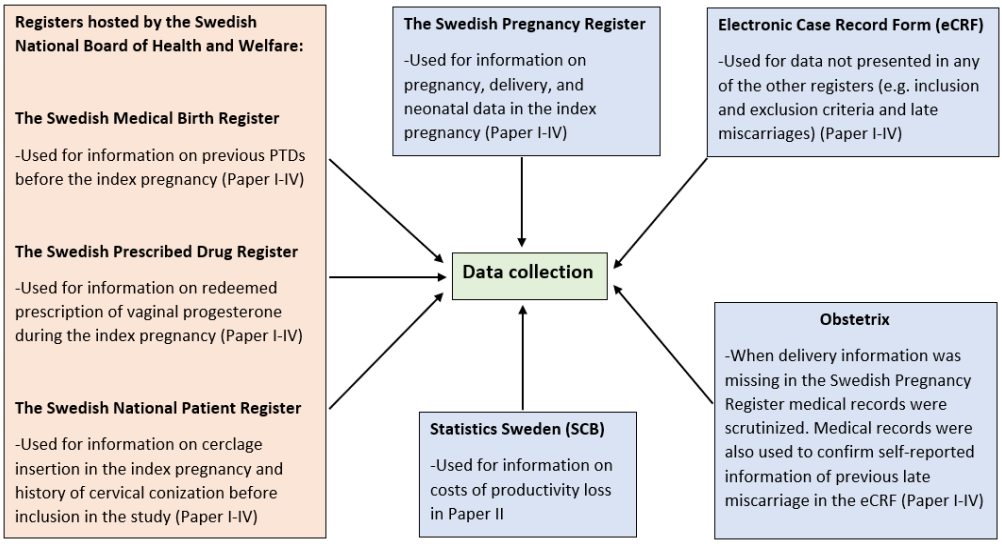


***Figure 11**. Overview of registers and other data sources used in Paper I-IV.*

The Swedish Pregnancy Register

The Swedish Pregnancy Register is a national quality register, initiated by Swedish health professionals in 2013. The register is hosted by The Swedish Association of Local Authorities and Regions. It is a merge of two pre-existing registers, the Maternal Healthcare Register (started in 1999) and the National Quality Register for Prenatal Diagnosis (started in 2006). The register collects data with start at the first antenatal visit and stops at the post-partum follow-up visit. Collected data include maternal demographics, reproductive and maternal health information, prenatal ultrasound examinations, delivery outcome as well as maternal and neonatal outcomes. In 2019, the Swedish Pregnancy Register covered 91% of all deliveries in

Sweden. Unlike the registers hosted by the Swedish National Board of Health and Welfare, patients can decline to participate in data collection. The register is considered to have very good validity (>95%) for more than 50% of the included variables and good validity (70 to 90%) for the remaining variables[122]. The register has also been externally validated by Stephansson et al. in 2015, estimating the coverage of the register to be more than 90%[119].

Data for the Swedish Pregnancy Register are collected from three different sources:

1) Manually entered data by antenatal care midwives at the first antenatal visit and at the follow-up visit post-partum (variables not entered in the electronic medical record e.g., country of birth, level of education, main occupation etc.).

2) Directly transferred data from the first trimester combined ultrasound and biochemistry screening for the risk of fetal chromosomal abnormalities.

3) Automatic transfer of data from patient electronic medical records (Obstetrix and similar) including data from antenatal care, biometry from ultrasound examinations performed during pregnancy, delivery outcome, and post-partum care including international classification of disease (ICD) codes and codes for procedures (e.g., surgery, cardiotocography (CTG) and administration of drugs).

The Medical Birth Register

The Medical Birth Register (MBR) was founded in 1973 and is hosted by the Swedish National Board of Health and Welfare. It is compulsory for all health care providers providing maternity services to report to the register, and the register includes data on about 99% of all deliveries in Sweden. The information is obtained from medical records of antenatal, delivery, and neonatal care units. The register was validated by Cnattingius in 1988 and 2001 and is considered to have good validity[123]. By linkage to registers provided by Statistics Sweden (described below), information on personal identification number of the newborn, the nationality of the parents as well as the mother's country of birth are added to the register.

The Swedish National Patient Register

The Swedish National Inpatient Register (IPR) was established in 1964 and has complete coverage since 1987. Today IPR is part of the Swedish National Patient register and is hosted by the Swedish National Board of Health and Welfare. Since

2001 the register also includes data from specialized outpatient care and day surgery. Primary care is not yet included in the register. Data is collected based on in- and out-patient diagnoses according to the ICD-code system. Information to the register is delivered to the Swedish National Board of Health and Welfare once a month from each of the 21 county councils in Sweden and from private caregivers. Quality controls of the register are performed continuously, where control checks of specific variables are performed. If the data is suspected to contain incorrect or invalid data points, new data are requested from the caregivers. The register was validated by Ludvigsson et al. in 2011 and validity was considered good[120].

The Swedish Prescribed Drug Register

The register was established in 2005 and is hosted by the Swedish National Board of Health and Welfare. The register provides the basis of the official statistics of redeemed prescriptions of drugs in Sweden. It contains information on Anatomic Therapeutical Chemical codes (ATC-codes), prescription amount, date of prescription and when the product was redeemed. The register is updated once a month with data from the Swedish eHealth Agency based on the monthly billing of the pharmacies. Since all data is based on electronically collected data, the proportion of missing data is small. Drugs used during hospitalization are not recorded in the register.

Statistics Sweden (SCB)

Statistics Sweden is a national administrative authority responsible for collecting and presenting official statistics on areas such as level of education, income, population changes, socioeconomic conditions, immigration, emigration, family relations, and vital statistics such as childbirth and deaths. In Paper II data from Statistics Sweden are used to estimate the indirect cost for absence from work, assuming production loss to be valued at market price (i.e., productivity loss).

Case Record Form (eCRF) for the CERVIX-study

At enrolment in the study standardized anamnestic information was obtained from the women and recorded in a web-based electronic case record form (MedSciNet AB, www.medscinet.se) by specially certified midwife sonographers (further described below). Examples of variables included in the eCRF were variables related to inclusion and exclusion criteria for the study such as, history of late miscarriage, cervical surgery, and self-reported ethnicity.

Obstetrix

Obstetrix is an electronic case record system used by most antenatal and delivery care units in Sweden. Data from Obstetrix are reported and transferred to several registers as described above. Obstetrix was also used for validation of the diagnoses spontaneous PTD in the index pregnancy, information on history of spontaneous and indicated PTD in case of contradictory information in the Medical Birth Register, as well as for validation of previous spontaneous late miscarriage by scrutiny of medical records.

Cervical length measurement and certification

Cervical length was measured with the study participants in the lithotomy position with an empty urinary bladder. The transvaginal probe was introduced into the vagina to obtain a sagittal view of the cervix. Measurements were taken without fundal or suprapubic pressure. A 5-9 MHz transvaginal probe connected to a GE Healthcare Voluson E8 Expert or E6 ultrasound system (GE Corporate) was used. In paper I, II and IV results are presented for the shortest of three measurements of endocervical length taken over at least 3 minutes. Measurements had to fulfill five quality criteria: 1) the cervix occupies at least 75% of the screen, 2) the anterior and posterior lip of the cervix are of equal thickness, 3) the full length of the endocervical canal is clearly seen, 4) the inner and outer cervical os are clearly seen as well as the virtual inner os if isthmus is present, 5) callipers are positioned correctly at the internal and external os and at the virtual inner os if isthmus is present (***Figure 12)***.

The transvaginal cervical length measurements were performed by 25 midwife sonographers certified to perform cervical length measurements in the study after theoretical and practical training. The theoretical training consisted of two lectures, one from The Fetal Medicine Foundation, (https://fetalmedicine.org/cervical-assessment-1) and one created by the steering committee of the CERVIX-study. The practical training was given as local hands-on training by a physician or midwife certified to perform cervix measurements in the study. After certification, quality controls were performed four times a year. A midwife sonographer who failed three subsequent quality checks could no longer continue to examine study participants.

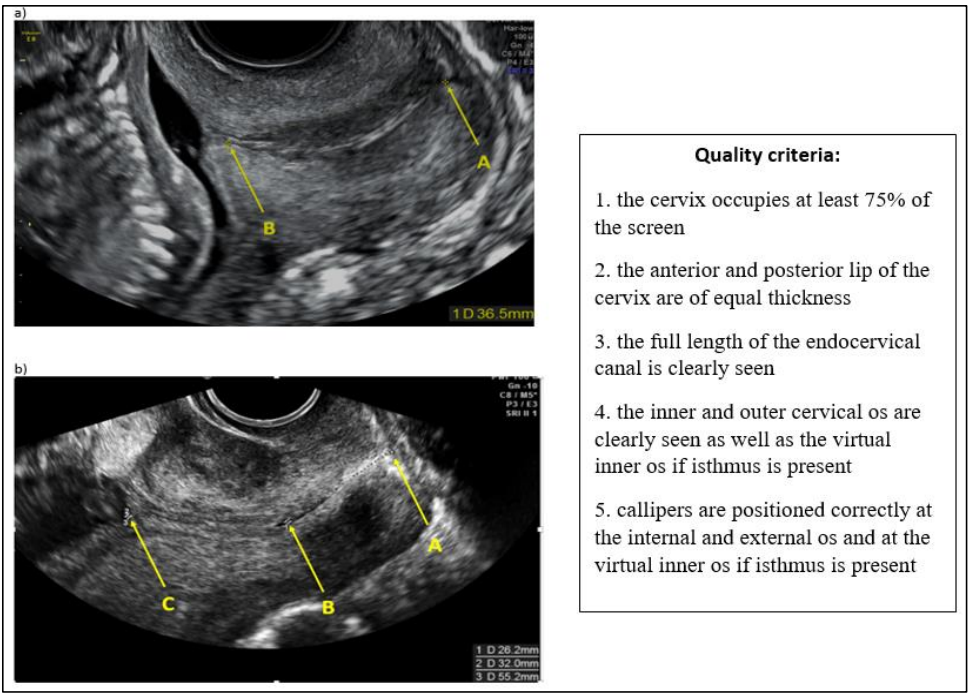


Figure 12. *Measurement of cervical length when isthmus is absent (a) or present (b). Isthmus is the lowest part of the uterine corpus that develops into the lower uterine segment as pregnancy progresses. A denotes the external os, B denotes the internal os, and C denotes the inner os if isthmus is present. Measurements were taken as a straight line from A to B (endocervical length), B to C (isthmus length), and A to C. Three measurements of each distance were taken during at least 3 min, each measurement being taken on a new image. All images had to fulfill the five quality criteria*[80].

Paper I

The study is a pre-planned exploratory analysis of the CERVIX-study[80]. Complementary data collection was performed to obtain reliable information on previous late miscarriage by scrutiny of medical records of participants with a self-reported previous late miscarriage. Late miscarriage was defined as: 1) spontaneous miscarriage at 16+0 to 21+6 weeks according to last menstrual period, 2) missed abortion if fetal size measured with ultrasound corresponded to 16+0 to 21+6 weeks, 3) self-reported miscarriage between 16+0 and 21+6 weeks if no information was found in the medical records.

Spontaneous PTD <33+0 weeks (including stillbirths and late miscarriages) was chosen as primary outcome because delivery <33+0 weeks is associated with high risk of short-term and long-term complications[124, 125]. The secondary outcome was set to spontaneous PTD <37+0 weeks. The study population was divided into three main risk groups: women at high risk of spontaneous PTD, nulliparous women without risk factors for spontaneous PTD, and parous women with only previous term deliveries and no risk factors for spontaneous PTD. The high-risk group included women with previous spontaneous PTD <37+0 weeks (singleton or multifetal)[126-128], previous late miscarriage[126, 129] or cervical conization before inclusion in the study (based on the existence of any of the following surgical procedure codes: LCD00, LCD03 or LCD 10)[130, 131] ***(Figure 13a and b)***.

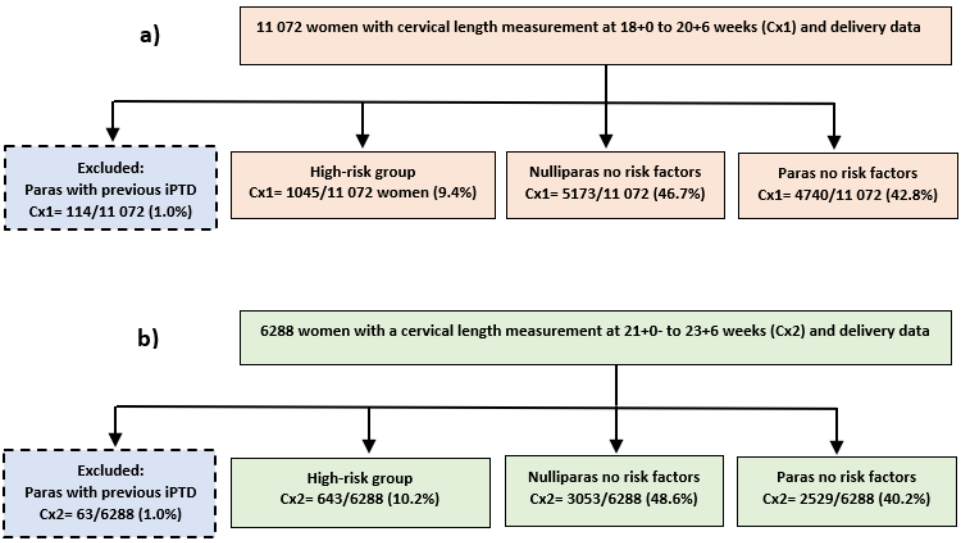


Figure 13. *Overview of the study population in Paper I and its risk groups. a) The study population with cervical length measurement at 18+0 to 20+6 weeks (Cx1), b) the study population with cervical length measurement at 21+0 to 23+6 weeks (Cx2). The high-risk group includes women with previous spontaneous PTD, previous late miscarriage and/or cervical conization. Nulliparas with no risk factors include nulliparous women with no cervical conization and no late miscarriages. Paras with no risk factors include parous women with only previous term deliveries, no cervical conization and no late miscarriages. Indicated preterm deliveries were excluded because the association between indicated preterm delivery and the risk of subsequent spontaneous preterm delivery is insufficiently known[128].*
sPTD, spontaneous preterm delivery; iPTD, indicated preterm delivery

The decision to define the "high risk-group" as described above, was based on the existing literature. The three risk factors for spontaneous PTD in singleton pregnancies were chosen, because these risk factors have the highest reported

association with spontaneous PTD[132]. In addition, a univariate analysis was performed to estimate the effect of these three risk factors in the study population. The results of this analysis were in concordance with the literature (***Figure 14***).

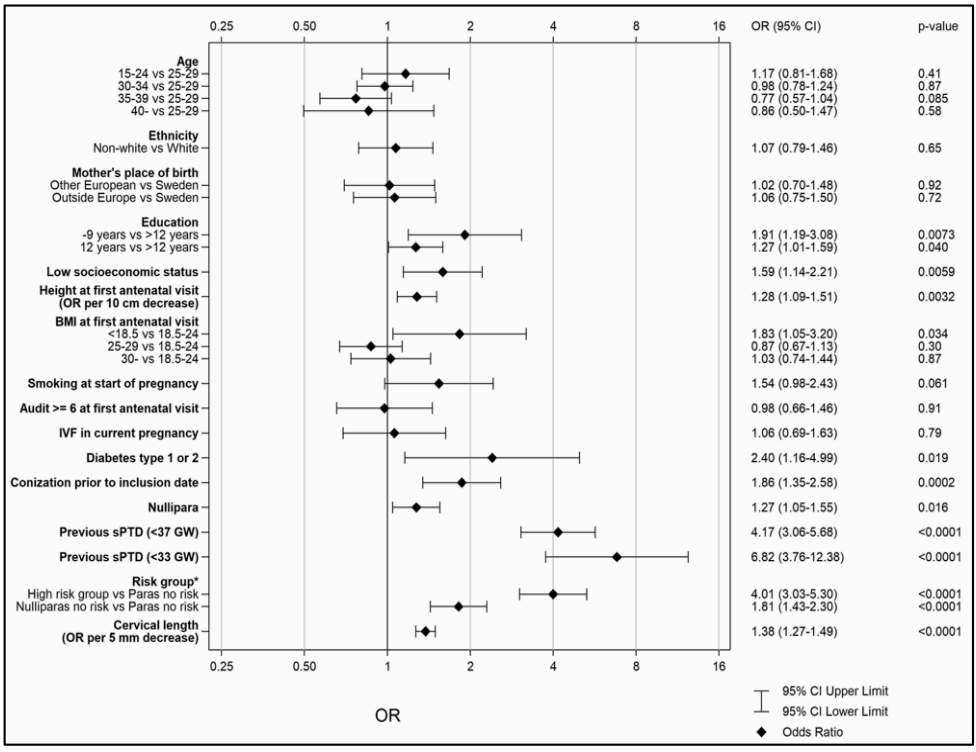


Figure 14. *Univariate analysis of risk factors in the CERVIX-study population at Cx1 (i.e., cervical length measurement at 18+0 to 20+6 weeks, n= 11 072).*
OR, odds ratio; IVF, in vitro fertilization; sPTD, spontaneous preterm delivery

Paper II

The study is a decision analytic model based on the Swedish CERVIX-study[80] to estimate the cost-effectiveness of various strategies to prevent spontaneous PTD in asymptomatic women with a singleton pregnancy. Analyses were performed separately for cervical length screening at 18+0 to 20+6 weeks (Cx1) and for cervical length screening at 21+0 to 23+6 weeks (Cx2).

Four strategies (universal screening, low-risk based screening, high-risk based screening and nullipara screening) included second trimester cervical length screening with transvaginal ultrasound followed by vaginal progesterone treatment

in case of a short cervix. A fifth strategy implied vaginal progesterone treatment of women with previous spontaneous PTD or late miscarriage but no cervical length screening (No screening, treat high-risk group). For comparison a sixth strategy was used, implying no specific intervention to prevent spontaneous PTD, reflecting the current situation in Sweden (No screening). In the base-case scenario cervix was defined as short if ≤25 mm. Other suggested definitions of short cervical length were also evaluated and are shown in ***Table 6***.

Table 6. Strategies for prevention of spontaneous preterm delivery in asymptomatic women with a singleton pregnancy. The red box indicates the evaluated strategies, and the blue box denotes the strategies involving cervical length measurement.

Strategy	Screening population	No screening population	Measurement at Cx1 (18+0 to 20+6 weeks) Definition of short cervical length (CL)	Measurement at Cx2 (21+0 to 23+6 weeks) Definition of short cervical length (CL)	Treatment
1. No screening	NA	All women	NA	NA	
2. No screening, treat High-risk group	NA	All women	NA	NA	Women with a previous sPTD or late miscarriage are treated with vaginal progesterone without screening
3. Universal screening	All women	NA	≤25 mm (base-case) ≤29 mm ≤20 mm	≤25 mm (base-case) ≤27 mm ≤20 mm	Screened women with a short cervix are treated with vaginal progesterone
4. Risk-based screening	Women with a previous PTD, previous late miscarriage or cervical conization	All women except those with a previous PTD, previous late miscarriage or cervical conization	NA	≤25 mm (base-case) ≤27 mm ≤20 mm	Screened women (previous PTD, late miscarriage or cervical conization) with a short cervix are treated with vaginal progesterone
5. Low-risk based screening	All women except those with a previous sPTD or previous late miscarriage	Women with a previous sPTD or previous late miscarriage	≤25 mm (base-case) ≤29 mm ≤20 mm	≤25 mm (base-case) ≤27 mm ≤20 mm	Screened women with a short cervix are treated with vaginal progesterone. Unscreened women with a previous sPTD or late miscarriage are treated with vaginal progesterone
6. Nullipara screening	All nulliparous women (except those with a previous late miscarriage)	All women except screened nulliparous women	≤25 mm (base-case) ≤29 mm ≤20 mm	≤25 mm (base-case) ≤27 mm ≤20 mm	Screened women with a short cervix are treated with vaginal progesterone. Unscreened women with a previous sPTD or late miscarriage are treated with vaginal progesterone

CL, cervical length; NA, not applicable; sPTD, spontaneous preterm delivery; PTD, preterm delivery

Probabilities for short cervix, as well as for spontaneous PTD at <33+0 weeks and at 33+0 to 36+6 weeks were derived from the CERVIX-study[80, 133]. The sources for probabilities of stillbirth, neonatal mortality, long-term morbidity (defined as cerebral palsy (CP)) as well as estimated costs are summarized in ***Table 7.*** All costs were deflated to July 2021 prices using a consumer price index[108]. Vaginal progesterone was assumed to reduce spontaneous PTD at <33+0 weeks with 30% and spontaneous PTD at 33+0 to 36+6 weeks by 10% based on the reported estimates in the IPD meta-analysis by Romero et al.[73].

Table 7. Summary of sources for estimates of mortality, long term morbidity, and costs.

Variable	Data source
Estimates of mortality and long term morbidity	
Intrauterine fetal death Neonatal mortality	The Swedish Medical Birth Register (year 2014, 2015, 2016, and 2017)
Long term morbidity classified as cerebral palsy (CP)	Himmelman et al. 2015[134], and personal communication with Kate Himmelman
Costs	
Vaginal progesterone (Utrogestan®)	Pharmaceutical specialities in Sweden (FASS)
Education of midwife sonographers	Romosan et al. 2020[135] and Skåne University Hospital, Sweden (2019)
Implementation and quality control of a screening program	Gross salary costs (including payroll taxes) are based on information from Skåne University Hospital, Sweden (2019) The costs for a quality control were approximated by using the model for the existing Swedish quality assurance program for prenatal screening for chromosomal abnormalities
Transvaginal sonogram Visit to physician	Based on information from Skåne University Hospital, Sweden (2019)
Cost of delivery	Derived from costs reported by Sahlgrenska University Hospital, Sweden (2017-2019) Estimation of the proportion of vaginal *vs* cesarean delivery in each gestational age-category is based on data from the CERVIX-study[80]
Productivity loss (temporal parental leave) in case of preterm delivery	Statistics Sweden
Neonatal care	Derived from costs reported by Sahlgrenska University Hospital, Sweden (2017-2019)
Long term disability	Kruse et al. 2009[136], Kruse 2006[137]

Paper III

The study is a nested case-control study within the CERVIX-study[80]. Between March 2016 and June 2017, women who accepted to participate in the CERVIX-study at Sahlgrenska University Hospital, Skåne University Hospital and Karolinska

University Hospital were also asked to agree to sampling of vaginal fluid. The study protocol included collection of vaginal fluid from the posterior vaginal fornix prior to transvaginal ultrasound measurement of cervical length at 18+0 to 20+6 gestational weeks. Women who had had vaginal intercourse within the past 48 hours were not sampled.

Primary outcome was set to spontaneous PTD <37+0 weeks (including spontaneous late miscarriages). For each case of spontaneous PTD or late miscarriage two controls were selected. The controls were chosen using simple random selection of women who had completed the study with spontaneous start of delivery at 39+0 to 40+6 gestational weeks, either after spontaneous onset of labor or after prelabor rupture of membranes. The interval 39+0 to 40+6 weeks was chosen because both delivery at early term and late term or post term is associated with a slightly higher risk of complications than delivery at 39+0 to 40+6 weeks[138-142]. Vaginal specimens were subject to Next Generation Sequencing (NGS) of both human and microbial RNA. Patient flow is shown in ***Figure 15.***

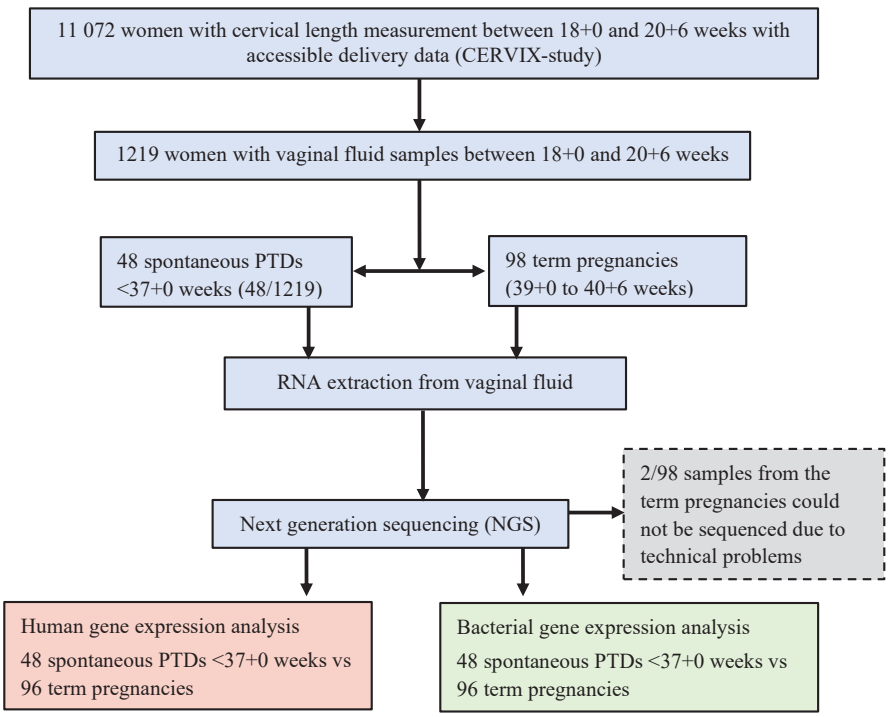


Figure 15. *Flowchart showing the study design and group composition in paper III. PTD, preterm delivery. † Includes two spontaneous late miscarriages after inclusion in the study at 18+0 to 21+6 weeks.*

NGS is a technology capable of sequencing multiple DNA/RNA molecules in parallel[143]. This enables hundreds of millions of DNA/RNA molecules to be sequenced at a time in contrast to the more conventional Sanger method where a single molecule is read at a time[144]. This allows for generation of large datasets and can provide an overall picture of the genomic and transcriptomic gene expression. The transcriptome is the transcriptionally active part of the genome[145] ***(Figure 16)***.

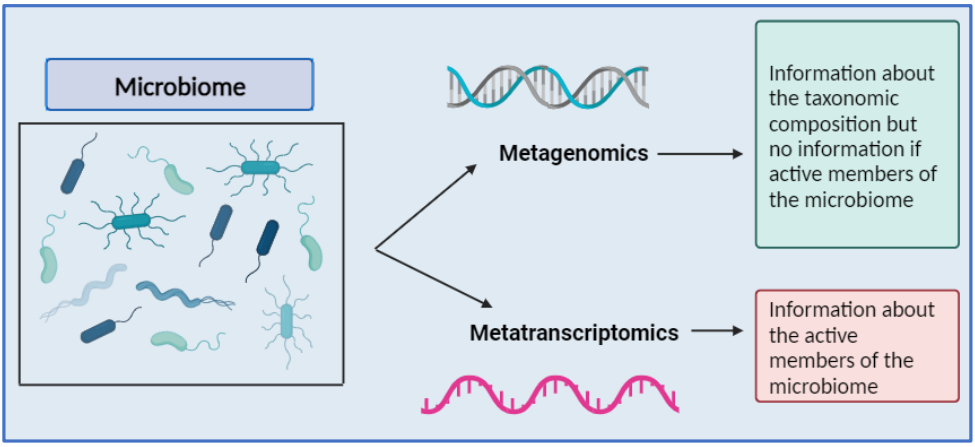


Figure 16. *Illustration of the difference of metagenomics and metatranscriptomics. Illustration by Tove Wikström.*

Figure 17 illustrates the technical procedure of NGS used in the analysis to examine whether the active vaginal microbiome and human transcriptome differ between women who subsequently deliver preterm *vs* at term.

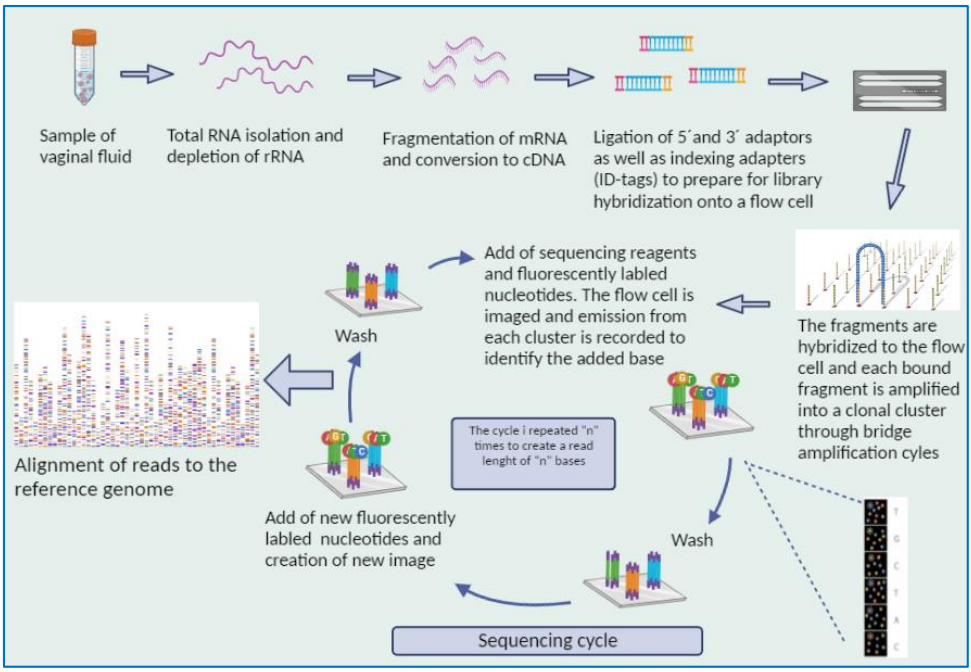


Figure 17*. Simplified illustration of the main steps in NGS, adapted from Illumina.com*[146]*. The sequencing library is prepared by random fragmentation of the mRNA, followed by conversion to cDNA and ligation of 5´and 3´adapters, as well as indexing adapters. The sequencing library is thereafter loaded into a flow cell and the fragments are captured on a lawn of surface-bound oligonucleotides complementary to the library adapters and amplified through bridge amplification. The templates are thereafter ready for sequencing, performed by using a reversible terminator-based method that detects single bases as they are incorporated into the reads. The sequence reads consisting of "n" bases are thereafter aligned to the chosen reference genome.*
Illustration by Tove Wikström.

Paper IV

The study is a nested case-control study within the CERVIX-study[80]. Primary outcome was set to spontaneous PTD <34+0 gestational weeks (including spontaneous late miscarriages). Secondary outcomes were set to spontaneous PTD <32+0 weeks, spontaneous PTD <30+0 weeks and spontaneous PTD <28+0 weeks. The diagnosis of spontaneous PTD in the index pregnancy was validated by scrutiny

of medical records. For each case of spontaneous PTD or late miscarriage three controls were selected. The controls were chosen using simple random selection of women who had completed the study with spontaneous start of delivery at 39+0 to 40+6 gestational weeks, either after spontaneous onset of labor or after prelabor rupture of membranes. The interval 39+0 to 40+6 weeks was chosen because both delivery at early term and late term or post term confer a slightly higher risk of complications than delivery at 39+0 to 40+6 weeks[138-142].

Serological samples were taken and handled in a routine setting, primarily to screen for HIV, syphilis, and hepatitis C at gestational week 10 -14 as recommended by the Swedish National Board of Health and Welfare[147]. The serological specimens were subject to reverse transcriptome real-time quantitative polymerase chain reaction (RT-qPCR) to accesses the relative difference in gene expression of let-7a-5p, miR-374a-5p, miR-15b-5p, miR-19b-3p, miR-23a-3p, miR-93-5p, miR-150-5p, miR-185-5p and miR-191-5p between those women who delivered preterm and those who delivered at term[91]. Patient flow is shown in **Figure 18.**

To be certain that the variations in Cq-values are due to real biological changes and not to technical issues in the performance of the PCR reaction, the Cq-values need to be normalized. Commonly the “delta-delta Cq method” is used (also called the Livak method)[151]. This means that the target sample Cq-values are compared to two or more reference genes of the relative expression of the target miRNA in relation to the reference genes (described in the method section in paper IV). Thereafter the difference in fold change between the normalized case and control samples can be calculated. Fold change is defined as the ratio between two measurements, where a fold change above 1 indicates an upregulation of the gene of interest, while a fold change below 1 indicates a downregulation of the gene of interest in relation to the control group.

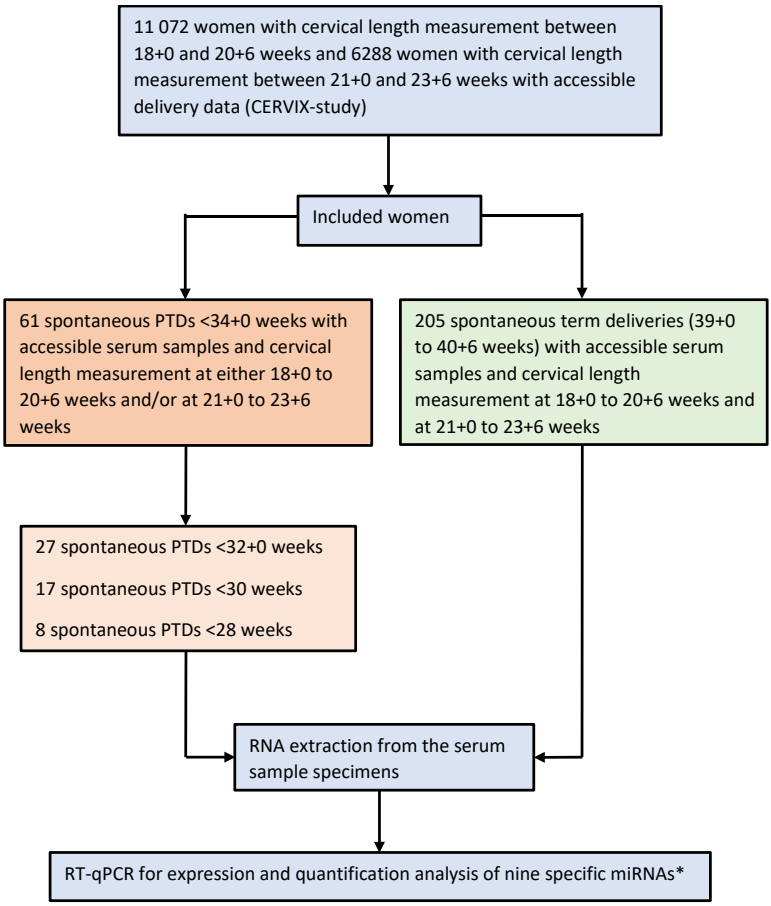


Figure 18. *Flowchart showing the study design and group composition in paper IV. PTD, preterm delivery; RT-qPCR, reverse transcriptome real-time quantitative polymerase chain reaction; miRNA, microRNA.*
**Examined miRNAs: let-7a-5p, miR-374a-5p, miR-15b-5p, miR-19b-3p, miR-23a-3p, miR-93-5p, miR-150-5p, miR-185-5p and miR-191-5p*[91].

RT-qPCR is used to quantify the absolute amount of a specific target sequence or to compare relative amounts of a target sequence between samples. The amplification of the target sequence is monitored in real-time by a target-specific fluorescent signal emitted during amplification[148, 149]. RT-qPCR probes and dyes are designed to be sequence-specific, nevertheless there will always be background fluorescence in all RT-qPCR experiments. To gain accurate information about the intended target gene it is critical to account for this background signal in the analysis of the sampled data. This issue is addressed by taking two important values into account[150]:

1. The threshold line: the point at which a reaction reaches a fluorescent intensity above background levels. This level is set before starting the RT-qPCR.
2. The Quantification Cycle value (Cq-value) is the PCR cycle number at which the sample reaction curve intersects the threshold line, i.e., it tells us how many cycles it took to detect a real signal from the sample. Cq-values are inversely related to the amount of target nucleic acids in the sample and correlates with the target copies in the sample. Low Cq-values indicate high amounts of the target sequence, and high Cq values indicate low amounts of the target sequence (**Figure 19**).

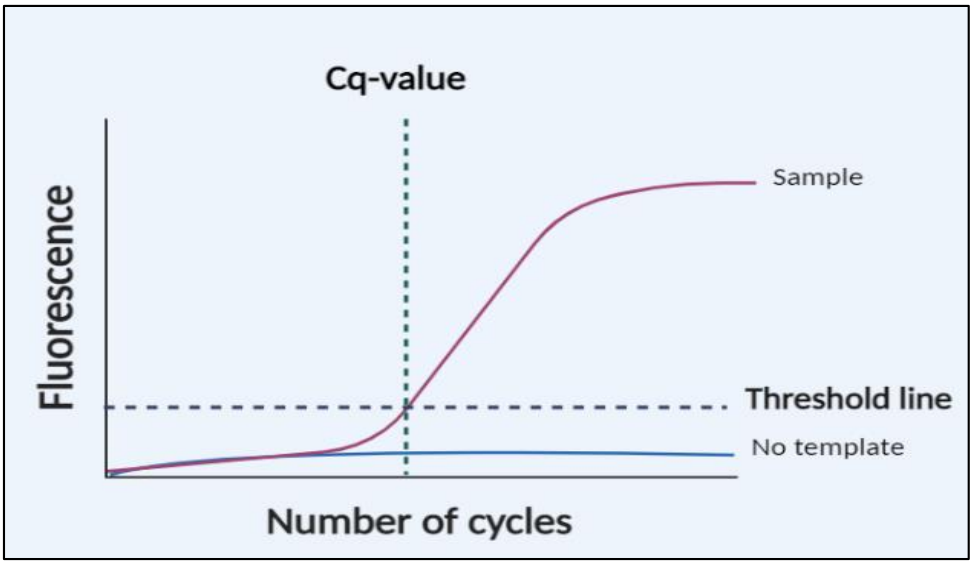


Figure 19. *Illustration of the point at which the polymerase chain reaction reaches a fluorescent reaction above the background level (threshold line) and the Quantification Cycle value (Cq-value) where sample reaction curve intersects the threshold line.*
Illustration by Tove Wikström

Statistical analyses

Paper I

To test if the effect of cervical length on the risk of spontaneous PTD (odds ratio per 5 mm decrease in cervical length) was similar in different risk groups, interaction analyses were performed using logistic regression with spontaneous PTD as the dependent variable and cervical length, risk-group, and risk-group in combination with cervical length as independent variables. A *p*-value of <0.05 was used to indicate a statistically significant interaction.

The discriminative ability of cervical length, to differentiate between women who delivered preterm *vs* at term is described as AUC, sensitivity, specificity, positive and negative predictive value, positive and negative likelihood ratio, number of false positive test results per one true positive test result, and number needed to screen to identify one spontaneous PTD. The discriminative ability was estimated for the following cervical length cut-offs: ≤20 mm, ≤25 mm, ≤29 mm ("best cut-off" at Cx1 in the CERVIX-study) and ≤27 mm ("best cut-off" at Cx2 in the CERVIX-study)[66-69, 73, 78, 80].

Paper II

A decision analytic model was constructed as a combined decision-tree (during screening year) and a Markov model with three health states (healthy, long-term morbidity (CP), or death) conducted with annual cycles with a time horizon of 100 years (***Figure 20***).

All analyses were performed from a societal perspective, including both direct costs within the health and social-care systems as well as productivity loss. The effectiveness of each strategy is expressed as QALYs, and the cost-effectiveness of a strategy is presented as the either average cost per gained QALY as compared to "No screening" (ACER) or as ICER. (***Figure 21***).

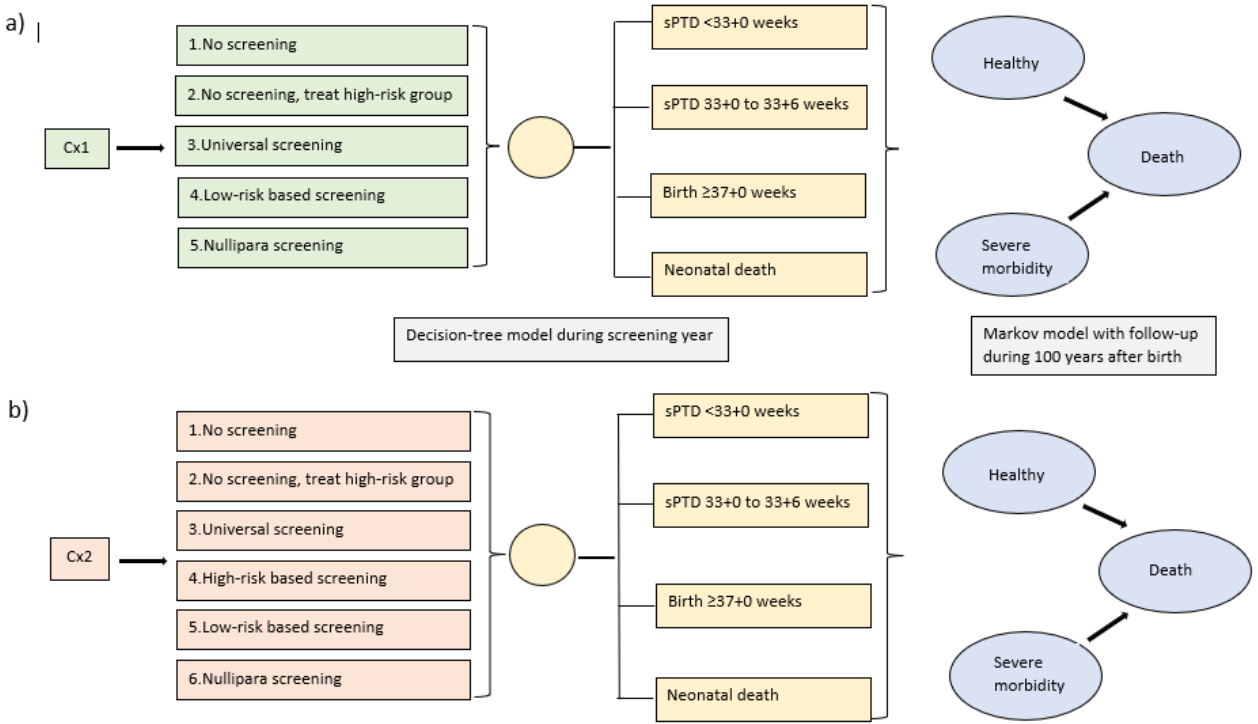


Figure 20. *Overview of investigated strategies for prevention of spontaneous PTD based on cervical length screening and vaginal progesterone treatment. a) denotes cervical length measurement at 18+0 to 20+6weeks (Cx1) i.e. on the day of the routine fetal scan, and b) denotes cervical length measurement at 21+0 to 23+6weeks (Cx2), i.e., at an additional appointment. The decision analytic model was constructed as a combined decision-tree model (screening year) and a Markov model with three health states (healthy, long-term morbidity or death) conducted with annual cycles for a time horizon of 100 years.*

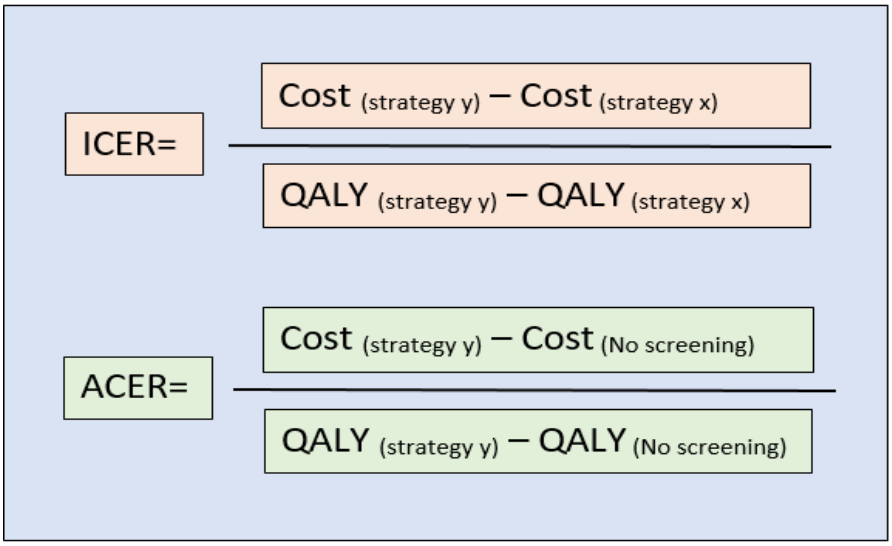


Figure 21. *Incremental cost-effectiveness ratio (ICER) and average cost-effectiveness ratio (ACER).*
Illustration by Tove Wikström.

For base-case strategies (cervical length ≤25 mm), deterministic sensitivity analyses were performed to estimate the impact of each variable. The model inputs were varied one at a time, keeping other variables fixed. Analyses were performed separately for Cx1 and Cx2 strategies. Also, a threshold analysis was performed for the most cost-effective base-case strategies in Cx1 and Cx2 to investigate the minimum required effect of progesterone for the strategy to remain cost-effective.

For the most cost-effective base-case strategies, a probabilistic sensitivity analysis was performed. In this analysis all prevalence probabilities, effectiveness of progesterone, costs and QALY weights were varied by using a Monte Carlo simulation with 1000 simulations. A Monte Carlo simulation is a method used to predict the probability of a variety of outcomes by constantly repeating random samples, i.e., a method to explain the impact of uncertainty[152]. The results of the simulations are shown as cost-effectiveness acceptability curves. The probabilistic sensitivity analyses were performed separately for Cx1 and Cx2 strategies.

Paper III

Figure 22 summarizes the bioinformatic analysis performed. For statistical analysis DESeq2 was used based on its advantages in analyzing differential expression of high throughput data. DESeq2 assumes a negative binominal distribution of the data,

thought to be preferable when handling count-based, multimodal, and dispersed data. The dispersion is a function of the mean and the variance, which helps to estimate the variance for a given mean value, critical for identification of differentially expressed genes in high throughput data. This is done in DESeq2 by first estimating the dispersion value for each gene (using data only from that gene), followed by a second step where DESeq2 assumes that genes with similar expression levels have similar dispersion, i.e., using information from all genes[153]. A full description of the methodology used to assess the difference in the vaginal metatranscriptome and the human transcriptome in women delivering preterm *vs* at term is described under the section "method" in Paper III.

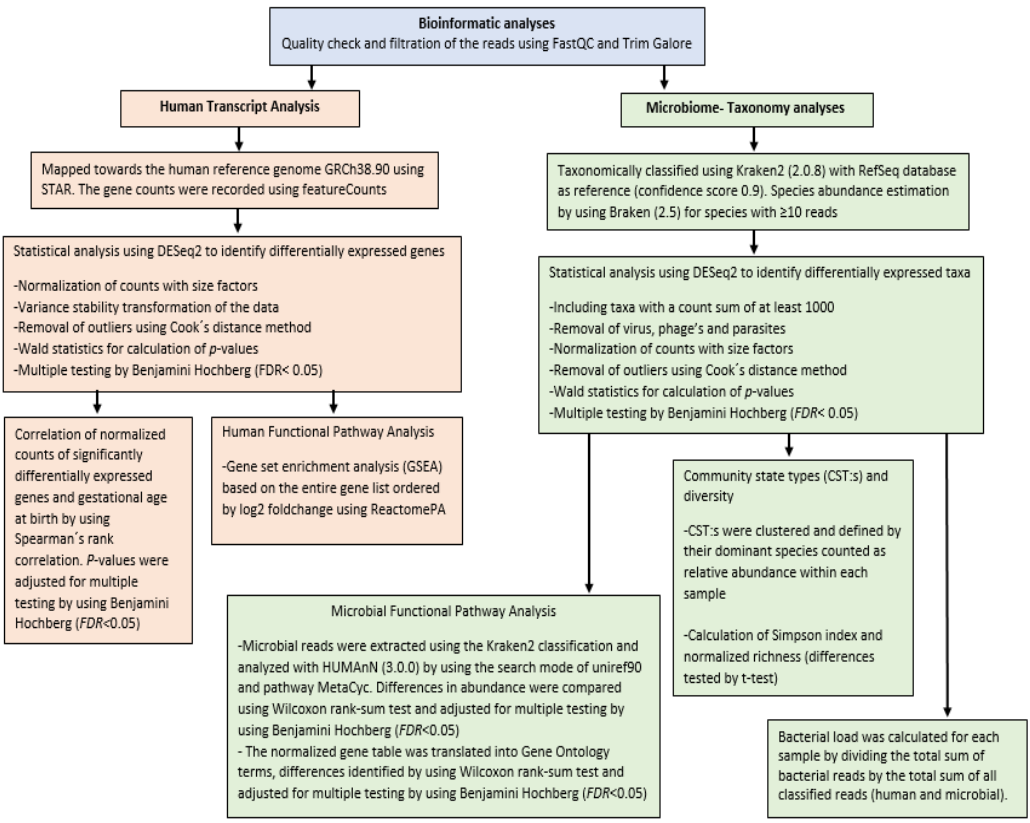


***Figure 22**. Flow chart summarizing the performed bioinformatic analyses in Paper III. All references to the referred software used are found under the heading "Method" in Paper III.*

Paper IV

The obtained miRNA-values from the PTD cases and term controls were subsequently analyzed with a non-parametric Mann-Whitney U-test (2-tailed) to compare the mean relative expressions between the groups. A *p*-value of <0.05 was considered to be statistically significant. The correlation between the normalized miRNA expression values and cervical length was calculated using Spearman's rank correlation[154].

The ability of a specific miRNA to discriminate between women who deliver preterm (<34+0, <32+0 weeks, <30+0 weeks or <28+0 weeks) *vs* at term is described as AUC. Youden´s index was used to determine the cut-off that yielded the highest proportion of correctly classified cases ("best cut-off")[155]. ROC curves were also created for models including both the specific miRNA and cervical length ≤30 mm and for models including both the specific miRNA and cervical length as a continuous variable.

Ethical permissions and considerations

All four studies in this thesis received ethical approval prior to study initiation. Ethical approval was given by the Regional Ethical Committee in Gothenburg: 825-13 (2013-11-11), T053-14 (2014-01-21), T691-14 (2014-09-19), T972-15 (2015-12-07), T122-16 (2016-02-25), T896-17 (2017-10-16), T645-18 (2018-07-09), T878-18 (2018-10-11), T970-18 (2018-11-01). All women signed written informed consent before entering the study, and all participation was voluntary.

All four studies are based on data and participants from the CERVIX-study[80]. The cervical length measurements were not disclosed to either the participants or the caregivers. One could argue that it was unethical to perform cervical length measurement without acting on a short cervix, even though all participating women were informed that the measurement results would not be disclosed or used for management of their pregnancy. At the time point of the study there was still no data on the prevalence of short cervix in Sweden, or on the best time point for measuring cervical length to identify pregnant women at high risk of delivering preterm. Neither were there any national guidelines on how to prevent spontaneous PTD in case of a short cervix, nor if cervical length should be measured to identify women at high risk for spontaneous PTD.

In all four papers data are derived from national health data registers and quality registers (described on page 42). National registers are important for many reasons, e.g., to enable health care professionals, policy makers, and researchers to follow up on quality measures of provided care, and to compare different health metrics between regions and patient groups, as well as for research. It could be argued that registers could be seen as "surveillance" of citizens and thereby being an integrity problem. It is important to know that the laws protecting the identity of a person in Sweden are highly regulated. Data used for research are in most cases pseudoanonymized, i.e., not traceable to a specific person[156]. From a philosophical perspective, with all the benefits of being part of a highly developed society, i.e., receiving modern, specialized and often expensive care, it is arguable and reasonable that citizens contribute with data on disease characteristics, interventions, and outcome, so that we can learn from it and further develop the care for those who need it.

In Paper III we sampled vaginal fluid in conjunction with the cervical length measurement. This is an additional procedure not part of given routine care. The sample collection was performed with a cotton pad from the posterior part of the vaginal fornix trying to avoid interfering with the cervix (which could potentially increase the risk of PTD particularly in case of a short cervix). The sampling of vaginal fluid could of course be uncomfortable. Therefore, all participants received careful information about the sampling procedure before inclusion. The benefit of obtaining the samples with the aim of finding new diagnostic markers for spontaneous PTD was considered to outweigh the discomfort of the sampling.

In Paper IV serological samples were taken and handled in a routine setting for infectious screening[147]. No extra samples were taken for the study, implying no harm to the participants of the study.

Results and comments

Paper I

The study population included 11 072 women with delivery data and cervical length measurement for Cx1, and 6288 women with delivery data and cervical length measurement for Cx2. An overview of the group composition as well as outcome is shown in **Figure 23**.

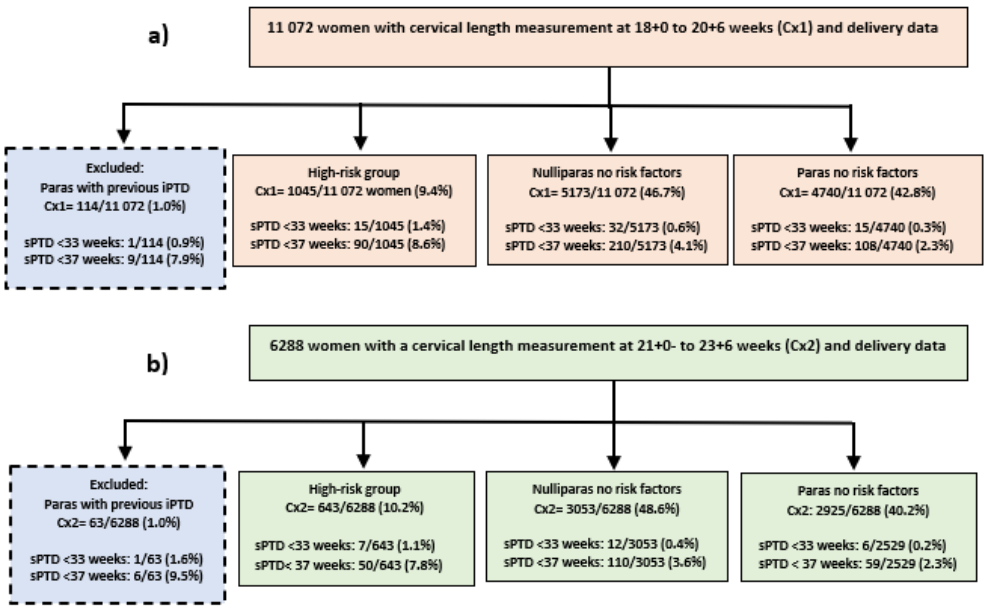


***Figure 23**. Overview of the outcome in the different risk groups. a) shows the study population with cervical length measurement at 18+0 to 20+6 weeks (Cx1), b) shows the study population with cervical length measurement at 21+0 to 23+6 weeks (Cx2). The high-risk group includes women with previous spontaneous PTD, previous late miscarriage and/or cervical conization. Nulliparas with no risk factors include nulliparous women with no cervical conization and no late miscarriages. Paras with no risk factors include parous women with only previous term deliveries, no cervical conization and no late miscarriages. Indicated PTDs were excluded because the association between previous indicated PTD and the risk of a subsequent spontaneous PTD is insufficiently known[128].*

Cervical lengths for the different risk-groups at Cx1 and Cx2 are shown in **Table 8**. It was shortest in high-risk women (mean cervical length 33.6 mm at Cx1, 33.5 mm at Cx2) and longest in parous women with no risk factors (36.5 mm at Cx1, 37.2 mm at Cx2). **Figures 24 and 25** are illustrations of the relationship between cervical length and spontaneous PTD in the CERVIX-study cohort. **Figure 24** shows how often spontaneous PTD occurred in the different risk groups in relation to cervical length at Cx1 and Cx2, and **Figure 25** illustrates the probability of a continued pregnancy at different gestational ages in relation to cervical length.

Table 8. Cervical length at 18+0 to 20+6 weeks (Cx1) and at 21+0 to 23+6 weeks (Cx2) by risk group.

		Cervical length* at 18+0 to 20+6 weeks			
Risk group	**Mean (SD) Median (range) (IQR)**	**0-20 mm**	**0-25 mm**	**0-30 mm**	**≥31 mm**
High-risk group† *n*= 1045	33.6 (6.6) 33.0 (9.0 to 58.0) (29.0; 38.0)	20 (1.9%)	99 (9.5%)	329 (31.5%)	716 (68.5%)
Nulliparas no risk factors‡ *n*= 5173	35.5 (5.8) 35.0 (3.0 to 60.0) (32.0; 39.0)	26 (0.5%)	173 (3.3%)	1017 (19.7%)	4156 (80.3%)
Paras no risk factors§ *n*= 4740	36.5 (6.4) 36.0 (9.0 to 60.0) (32.0; 40.0)	21 (0.4%)	163 (3.4%)	810 (17.1%)	3930 (82.9%)
		Cervical length* at 21+0 to 23+6 weeks			
Risk group	**Mean (SD) Median (range) (IQR)**	**0-20 mm**	**0-25 mm**	**0-30 mm**	**≥31 mm**
High-risk group† *n*= 643	33.5 (7.0) 33.0 (13.0 to 56.0) (29.0; 38.0)	24 (3.7%)	75 (11.7%)	216 (33.6%)	427 (66.4%)
Nulliparas no risk factors‡ *n*= 3053	35.5 (5.9) 35.0 (5.0 to 60.0) (32.0; 39.0)	23 (0.8%)	111 (3.6%)	576 (18.9%)	2477 (81.1%)
Paras no risk factors§ *n*= 2629	37.2 (6.7) 37.0 (4.0 to 60.0) (33.0; 41.0)	22 (0.9%)	84 (3.3%)	366 (14.5%)	2163 (85.5%)

SD, standard deviation; IQR, interquartile range; PTD, preterm delivery; sPTD, spontaneous preterm delivery
For categorical variables n (%) is presented
**Cervical length is the shortest of three measurements of the closed endocervical canal*
†Women with previous sPTD (singleton or multifetal) or late miscarriage or cervical conization
‡Nulliparous women with no cervical conization and no late miscarriage
§Parous women with only previous term deliveries, no cervical conization and no late miscarriage

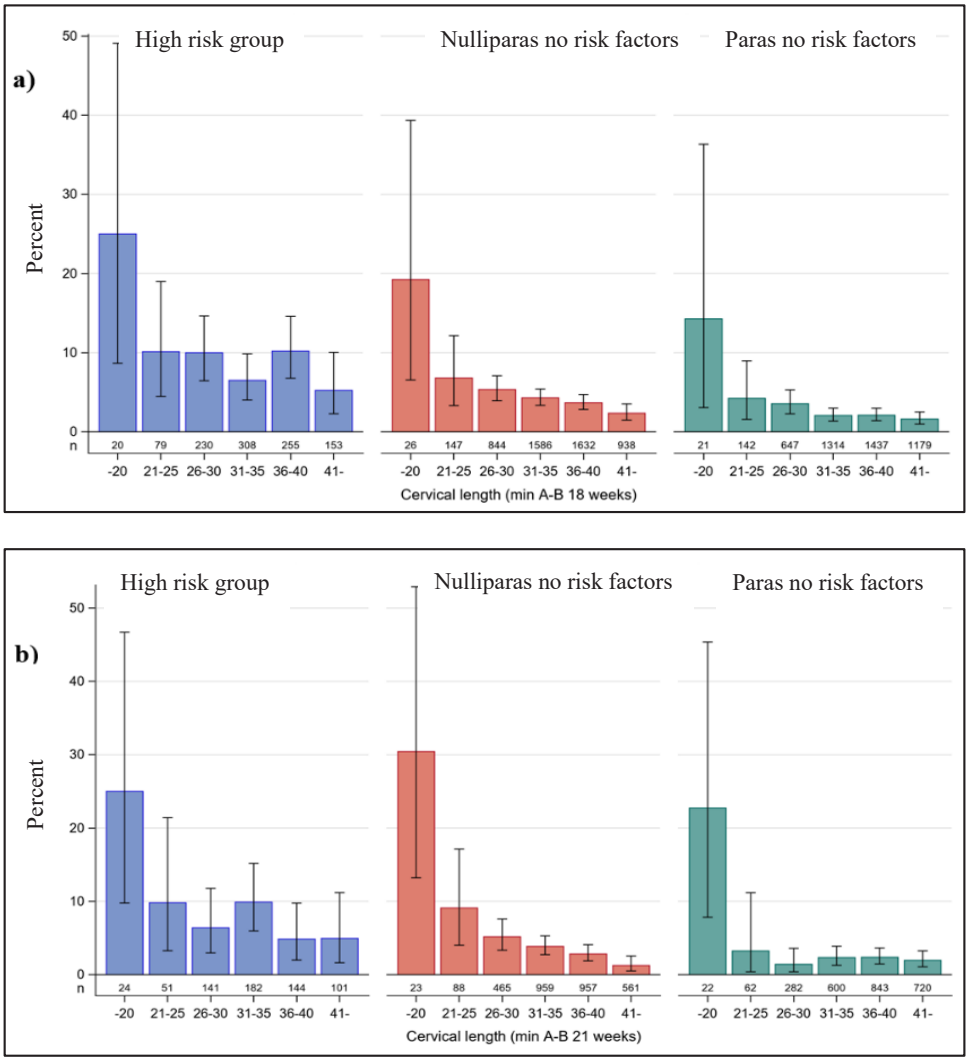


Figure 24. *Stock chart showing the event-rate (i.e., spontaneous PTD <37+0 weeks) in relation to cervical length in millimeters at 18+0 to 20+6 weeks (Cx1 (a)), and at 21+0 to 23+6 weeks (Cx2 (b)) in the three different risk groups. The percentage of spontaneous PTDs <37+0 weeks is shown on the y-axis. Cervical length (min A-B) means that measurements are taken from the internal os of the cervix to the external os of the cervix (see Figure 12). The high-risk group includes women with previous spontaneous PTD, late miscarriage or cervical conization. Nulliparas with no risk factors include nulliparous women with no cervical conization and no late miscarriages. Paras with no risk factors include parous women with only previous term deliveries, no late miscarriages, and no history of conization.*

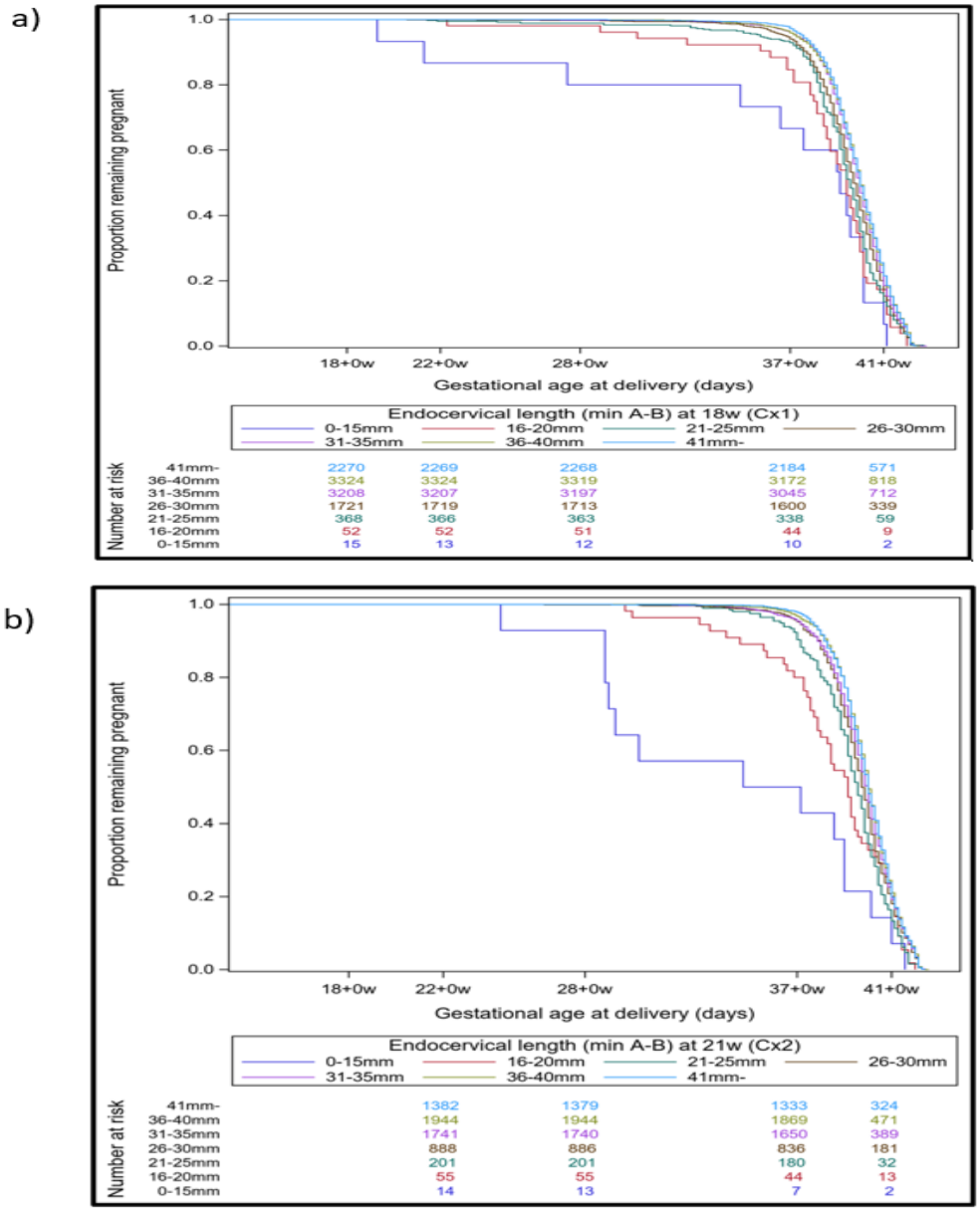


Figure 25. *Kaplan-Meier plots illustrating the probability of continued pregnancy at different gestational ages in relation to cervical length at 18+0 to 20+6 weeks (Cx1, (a) and at 21+0 to 23+6 weeks (Cx2, (b)) for the whole population, i.e., independent of risk group (Cx1= 10 958, Cx2= 6225). Women with indicated PTD (<37 weeks) are censored.*

The effect of cervical length on the risk of spontaneous PTD <33+0 weeks at Cx2 was similar in the three risk groups (OR 2.26 to 2.58) with no significant interaction effect (p=0.91). The ability to discriminate between those who delivered <33+0 weeks, compared to those who delivered at term, was better when cervical measurements were performed at Cx2 compared to at Cx1. The discriminative ability of spontaneous PTD <33+0 weeks at Cx2 was similar in the three risk groups (AUC 0.69 to 0.76) ***(Figure 26)***.

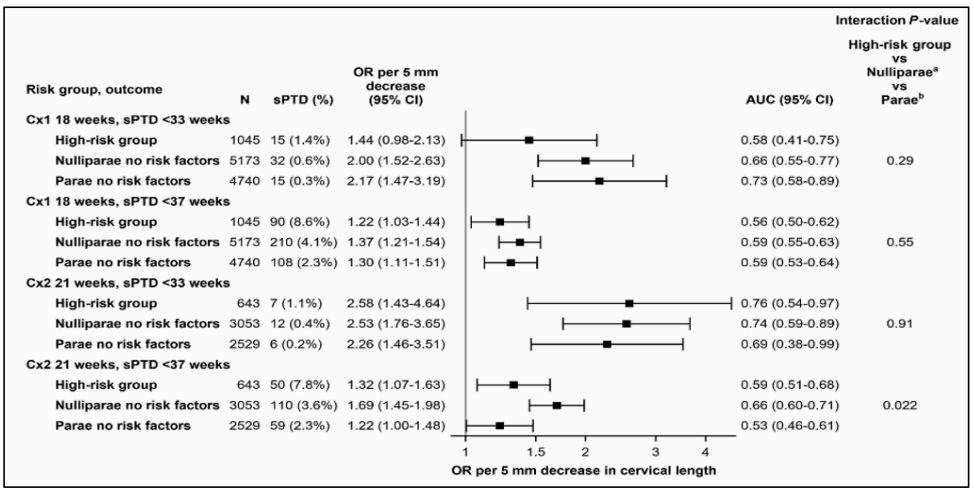


Figure 26. *The effect of cervical length (odds ratio per 5 mm decrease) on the risk of spontaneous preterm delivery and ability of cervical length to correctly discriminate between women who deliver spontaneously preterm vs at term (area under the receiver operating characteristic curve) in different risk groups. The high-risk group includes women with previous spontaneous preterm delivery, late miscarriage or cervical conization. Nulliparas with no risk factors include nulliparous women with no cervical conization and no late miscarriage. Paras with no risk factors include parous women with only previous term deliveries, no cervical conization and no late miscarriage. P-values for interaction analysis are shown.*

sPTD, spontaneous preterm delivery; OR, odds ratio; AUC, area under the receiver operating characteristic curve; CI, confidence interval, [a]Nulliparas with no risk factors; [b]Paras with no risk factors.

When using the commonly used 25 mm cervical length cut-off for defining a "short cervix", the number needed to screen (NNS) to detect one spontaneous PTD <33+0 weeks was five or six times higher in women with no risk factors compared to the high-risk group (843 vs 161 or 1018 *vs* 161). The number of false positive test results per one true positive test result (FP/TP) was also substantially higher in the former (27 *vs* 18 or 36 vs 18) ***(Table 9)***.

Table 9. The ability of transvaginal cervical length measurement at 21+0 to 23+6 weeks (Cx2, *n*= 6288) to discriminate between women who did and did not deliver spontaneously <33+0 weeks.

			Cervical length*			
			0-25 mm			
Risk group	**No. sPTD**	**AUC**	**n†**	**Sensitivity** [95% CI]	**Specificity** [95% CI]	**FP/TP NNS**
High-risk group‡ *n*= 643	7 (1.1%)	0.76	75 (11.7%)	4/7 (57.1%) [18.4%; 90.1%]	565/636 (88.8%) [86.1%; 91.2%]	18 161
Nulliparas with no risk factors§ n= 3053	12 (0.4%)	0.74	111 (3.6%)	3/12 (25.0%) [5.5%; 57.2%]	2933/3041 (96.4%) [95.7%; 97.1%]	36 1018
Paras with no risk factors¶ n= 2529	6 (0.2%)	0.69	84 (3.3%)	3/6 (50.0%) [11.8%; 88.2%]	2442/2523 (96.8%) [96.0%; 97.4%]	27 843

sPTD, spontaneous preterm delivery, No., number of; AUC, area under the receiver operating characteristic curve; CI, confidence interval; FP, false positive; TP, true positive; NNS, number of women needed to screen to detect one sPTD <33+0 weeks.

** Cervical length is the shortest of three measurements of the closed endocervical canal*
† Number of women with cervical length ≤25 mm
‡ Women with previous sPTD (singleton or multifetal), late miscarriage, or cervical conization
§ Nulliparous women with no cervical conization and no late miscarriage
¶ Parous women with only previous term deliveries, no cervical conization and no late miscarriage

Comments

In summary, second trimester transvaginal cervical length measurement is useful for identification of those at risk for spontaneous PTD in both high and low risk singleton pregnancies in a mainly Caucasian population with a low prevalence of spontaneous PTD. In all obstetric risk groups, the ability to predict spontaneous PTD was better when measurements were taken at 21+0 to 23+6 weeks (Cx2) compared to 18+0 to 20+6 weeks (Cx1) and was superior at predicting PTD <33+0 weeks compared to PTD <37+0 weeks. The study highlights the importance of considering both the difference in performance depending on the time point of cervical length measurement, as well as the difference in NNS between different obstetric risk groups before implementing a cervical length screening program.

Paper II

All suggested interventions gave better health outcomes in terms of less mortality and more QALYs in a lifetime perspective compared to No screening (no screening and no treatment with vaginal progesterone, i.e., current situation in Sweden). The best strategy in terms of improved health outcomes was Low-risk based screening (i.e., vaginal progesterone to those with previous spontaneous PTD or previous late miscarriage, and screening of all other women followed by treatment of those with a short cervix), irrespective of whether screening was performed at Cx1 (18+0 to 20+6 weeks) or at Cx2 (21+0 to 23+6 weeks) (***Table 10***).

Table 10. Cost and effects in terms of improved health outcome in different screening strategies. Base-case strategies are shown (cervical length ≤25 mm to indicate high risk of spontaneous PTD). Costs are presented per 100 000 women and are shown in US dollar.

	Difference in cost compared to No screening		Difference in health outcomes compared to No screening	
	Screening-year (USD)	Lifetime (USD)	Screening-year mortality *(n)*	Lifetime QALYs *(n)*
Cx1-strategies (Screening at 18+0 to 20+6 weeks)				
No screening, treat high-risk group	-1 201 000	-2 630 000	-2.1	+71
Nullipara screening	1 426 000	-1 454 000	-4.3	+148
Low-risk based screening	4 425 000	449 000	-6.0	+206
Universal screening	5 894 000	3 327 000	-4.0	+136
Cx2-strategies (Screening at 21+0 to 23+6 weeks)				
No screening, treat high-risk group	-832 000	-2 124 000	-1.8	+64
Nullipara screening	5 232 000	2 750 000	-3.6	+124
Risk-based screening	632 000	-491 000	-1.7	+58
Low-risk based screening	10 234 000	6 663 000	-5.3	+181
Universal screening	10 870 000	8 154 000	-4.1	+141

PTD, preterm delivery; USD, US dollar; QALY, quality adjusted life years; n, number

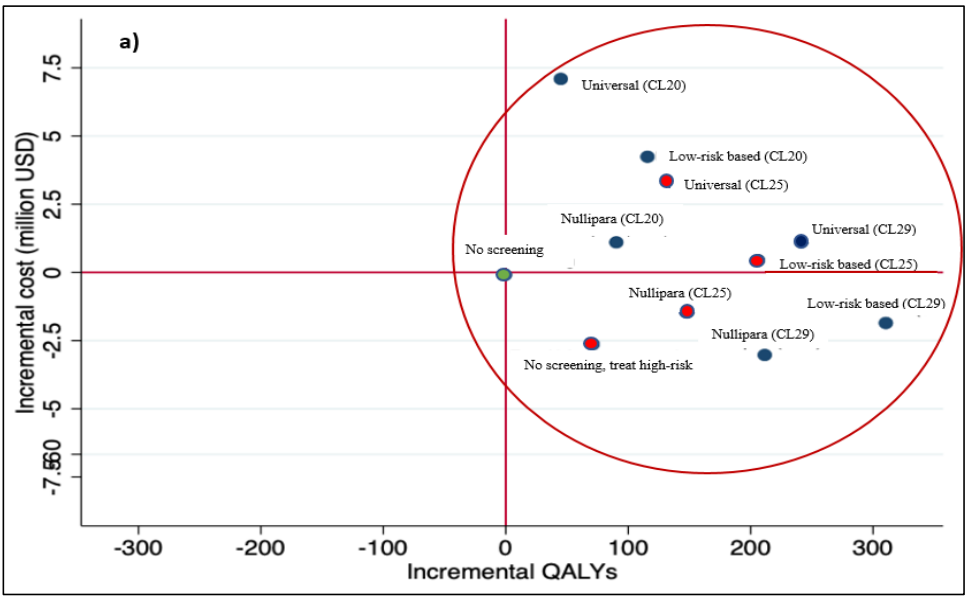
Cost-effectiveness results are presented both as ACER (added average costs per gained QALY compared to No screening) and as ICER (the incremental cost for each strategy compared to the less expensive strategy when all dominated strategies are excluded). Low-risk based screening (base-case) at Cx1 was shown to be cost-effective, while Low-risk based screening at Cx2 entailed high costs compared to

other alternatives **(Table 11, Figure 27 a and b).** The ACERs were 2200 USD for Low-risk based screening at Cx1 and 36 800 USD for Low-risk based screening at Cx2.

Table 11. Average cost per gained QALY (ACER) and Incremental cost per gained QALY (ICER) shown for base-case results (cervical length ≤25 mm to indicate high risk of spontaneous PTD). Costs in US dollar are presented per 100 000 women in a life-time perspective.

	Average cost per gained QALY *vs* No screening	**Incremental cost per gained QALY**
	USD/QALY	USD/QALY
Cx1-strategies (18+0 to 20+6 weeks)		
No screening, treat high-risk group	Dominant *	Reference
Nullipara screening	Dominant *	15 300 †
Low-risk based screening	2200	32 800 ‡
Universal screening	24 500	Dominated §
Cx2-strategies (21+0 to 23+6 weeks)		
No screening, treat high-risk group	Dominant *	Reference
Risk-based screening	Dominant *	Dominated ¶
Nullipara screening	22 200	Dominated **
Low-risk based screening	36 800	74 600 ††
Universal screening	58 000	Dominated ‡‡

USD, US Dollar; QALY, quality adjusted life year
** Lower costs and better health outcomes compared to No screening*
† Compared to No screening, treat high-risk group
‡ Compared to Nullipara screening
§ More expensive and worse health outcomes compared to Low-risk based screening
¶ More expensive and worse health outcomes compared to No screening, treat high-risk group
*** Dominated (by extension) by Low-risk based screening and No screening, treat high-risk group*
†† Compared to No screening, treat high-risk group
‡‡ More expensive and worse health outcomes compared to Low-risk based screening



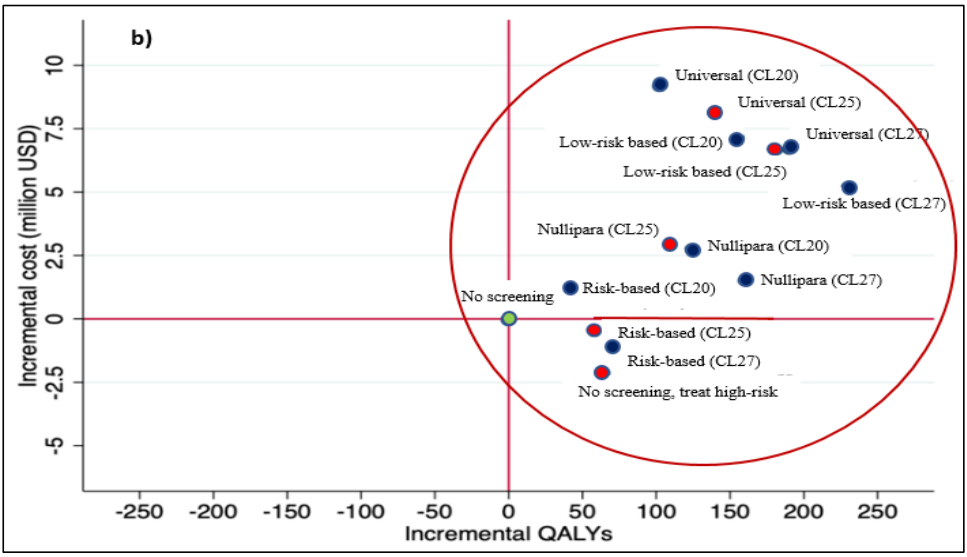
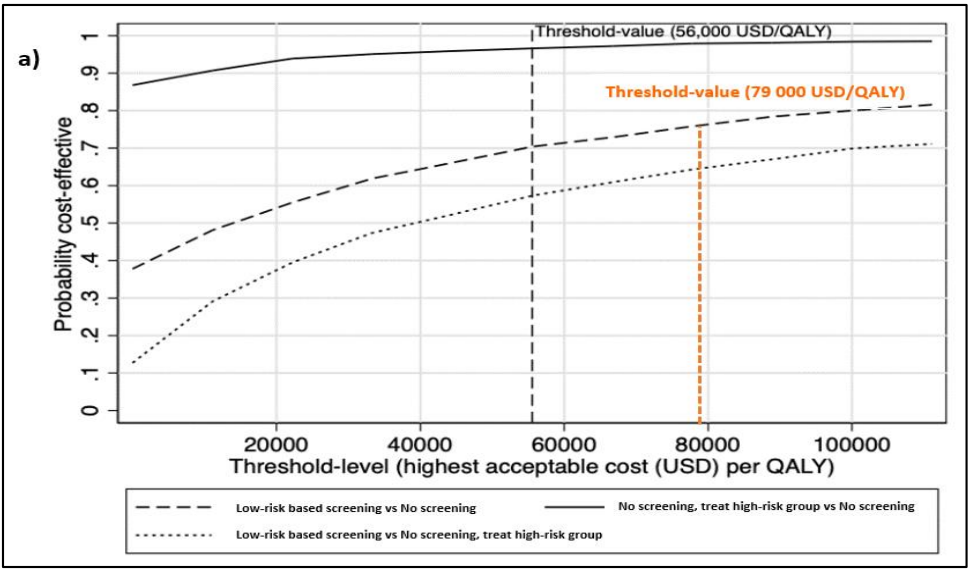


Figure 27a and b. *Cost-effectiveness plane showing the incremental cost-effectiveness ratio (ICER) per 100 000 women for all screening strategies considered at Cx1 (cervical length screening at 18+0 to 20+6 weeks) (a) and at Cx2 (cervical length screening at 21+0 to 23+6 weeks) (b). The No screening strategy is marked in green. Base-case results (CL25) are marked in red and other results in blue. The red ring illustrates that all strategies gave better health outcome compared to No screening. For comparative ICER-values se Figure 2, Paper II. USD, US dollar; CL20, cervical length ≤20 mm to indicate high-risk of preterm delivery; CL25, cervical length ≤25 mm to indicate high-risk of preterm delivery; CL27, cervical length ≤27 mm to indicate high-risk of preterm delivery; CL29, cervical length ≤29 mm to indicate high-risk of preterm delivery; QALY, quality adjusted life year.*

The deterministic sensitivity analyses showed that the cost-effectiveness of a strategy was particularly sensitive to, 1) the effectiveness of progesterone and 2) productivity-loss due to sick-leave during pregnancy. For the Cx1 strategies, Low-risk based screening remained the preferred strategy as long as progesterone reduced spontaneous PTD <33+0 weeks by at least 21% and spontaneous PTD 33+0 to 36+6 weeks by at least 7%. The corresponding numbers for Low-risk based screening at Cx2 were 36% and 12%, respectively. If assuming that either 50% or 100% of the women considered at high risk for spontaneous PTD (previous spontaneous PTD, previous late miscarriage or short cervix) would be held on sick leave during the pregnancy, none of the screening strategies would have an ICER below 56 000 USD (corresponding to the willingness to pay, WTP, per gained QALY recommended by the Swedish National Board of Health and Welfare[107]).

As previously described, probabilistic sensitivity analyses were performed for the most cost-effective base-case strategies. All prevalence probabilities, effectiveness of progesterone, costs as well as QALY-weights were varied in a Monte Carlo simulation to test the robustness of the model. These results are shown in **Figure 28 a and b.** At Cx1 the probability of Low-risk based screening to be cost-effective compared to No screening was 71%, using the WTP of 56 000 USD. The probability of Low-risk based screening being cost-effective at Cx2 was more uncertain (52%). If instead using a WTP of 79 000 USD[157] (~700 000 SEK) the corresponding numbers would be approximately 77% for Cx1, and 64% for Cx2 (**Figure 28 a and b)**.



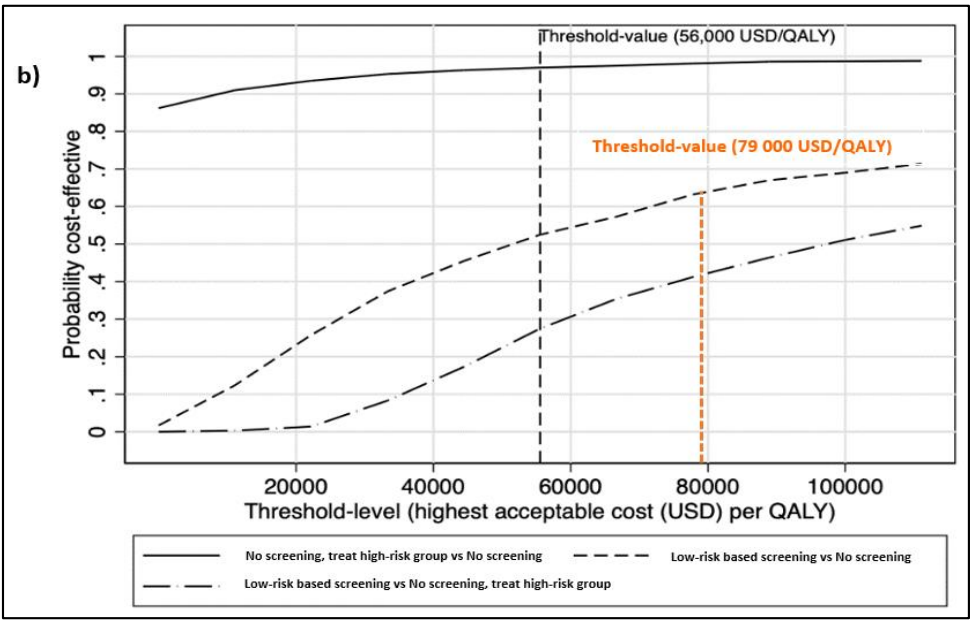


Figure 28a and b. *Cost-effectiveness acceptability curves for cervical length screening at 18+0 to 20+6 weeks (Cx1) (a) and at 21+0 to 23+6 weeks (Cx2) (b). Cervical length ≤25 mm is used to indicate increased risk of spontaneous PTD (base-case). The acceptability curves show the likelihood of the strategies "No screening, treat high-risk group" and "Low-risk based screening" being cost-effective when compared to each other, as well as when compared with "No screening". Willingness to pay (WTP) is shown in US-dollars (USD) on the x-axis, and the likelihood of the strategy being cost-effective is shown on the y-axis. The black dashed line on the y-axis corresponds to a WTP of 56 000 USD (approximately 500 000 SEK) and the orange line on the y-axis corresponds to a WTP of 79 000 USD (approximately 700 000 SEK).*

Comments

There is a high possibility that cervical length screening, followed by treatment with vaginal progesterone to those at an increased risk of spontaneous PTD, would be cost-effective in a Swedish setting. If we, hypothetically, would apply the higher proposed WTP of 79 000 USD[157] (~700 000 SEK) all alternatives would be considered cost-effective with good margins **(Table 11).**

However, for screening to be cost-effective, we need to strive for restraint in terms of sick leave in asymptomatic women classified to be at increased risk of spontaneous PTD. Performed sensitivity analyses assuming either 50% or 100% sick-leave for women at increased risk for spontaneous PTD (short cervix, previous spontaneous PTD or late miscarriage) resulted in screening no longer being cost-effective (WTP

of 56 000 USD). This highlights the importance of following current evidence-based recommendations of not prescribing sick-leave to asymptomatic women with the aim of reducing spontaneous PTD[158].

In the base-case scenarios, a cervical length cut-off of ≤25 mm was used to indicate an increased risk of spontaneous PTD. However, as seen in **Figure 25a and b** (including all investigated strategies and cervical length cut-offs), screening strategies with a cervical length cut-off ≤29 mm (Cx1) and ≤27 mm (Cx2) were the most cost-effective strategies. It would be advantageous to use these cut-offs, provided that vaginal progesterone has the same effect at a cervical length ≤29 mm and ≤27 mm as for a cervical length ≤25 mm. The scientific evidence for progesterone effect between >25 and ≤30 mm is scarce, though the EPPPIC-study found some evidence for progesterone treatment to be effective in women with a cervical length of ≤30 mm[82]. Further studies are needed before more liberal cut-offs (≤30 mm) can be used and implemented in a screening program.

Paper III

Vaginal samples were collected from 1219 women. Of these, 48 women delivered spontaneously <37+0 weeks (including two late spontaneous miscarriages <22+0 weeks) (**Figure 15**). In the dataset, a total of 4 907 632 710 reads (sequenced fragments) were annotated as either human or microbial with the proportion of human reads ranging from 3.1% to 98.9% **(Figure 29).** No statistically significant difference in bacterial load between the preterm (mean 0.45, 95% CI 0.39 to 0.52) and the term group (mean 0.39, 95% CI 0.36 to 0.43) was found (**Figure 30**). Nor was there an overall difference in either species diversity or richness between the groups.

Five major vaginal community state types (CSTs) were identified by the dominance of either one species of *Lactobacillus*, *Gardnerella vaginalis* or a mixture of bacterial species. Three CSTs were dominated by either *Lactobacillus crispatus* (CST I), *Lactobacillus iners* (CST II) or *Lactobacillus gasseri* (CST V), one by *Gardnerella vaginalis* (CST III), and one by a variety of different species e.g., *Streptococcus agalactica*, *Enterococcus faecalis* and *Bifidobacterium breve* (CST IV) (Figure 5, Paper 3). There was no statistically significant difference in the overall CST-distribution between the preterm and term group, apart from the absence of CST IV (mixture of different species) in the preterm group (**Figure 31**).

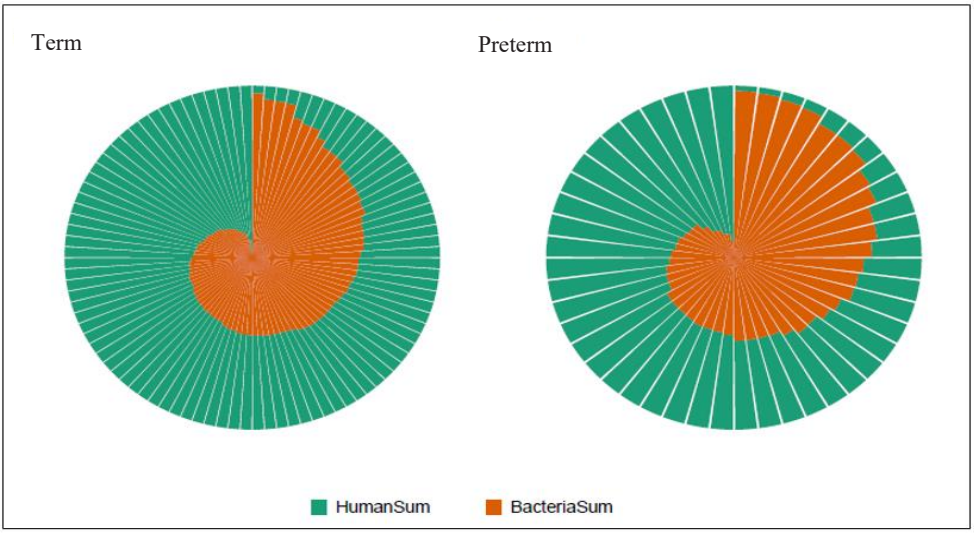


Figure 29. *Pie chart illustrating the proportion of human reads (green) and microbial reads (orange) in the term group (n= 96) and in the preterm group (n= 48).*

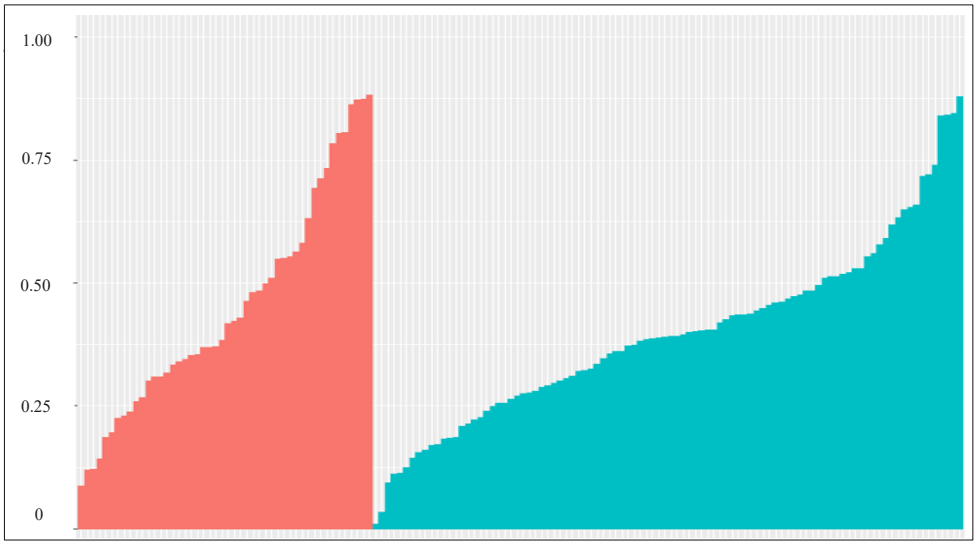


Figure 30. *Bacterial load in the preterm group (<37+0 weeks, n= 48) and in the term group (n= 96). The pink bars indicate the bacterial load in the preterm group (mean 0.45, 95% CI 0.39 to 0.52), and the blue bars indicate the bacterial load in the term group (mean 0.39, 95% CI 0.36 to 0.43).*

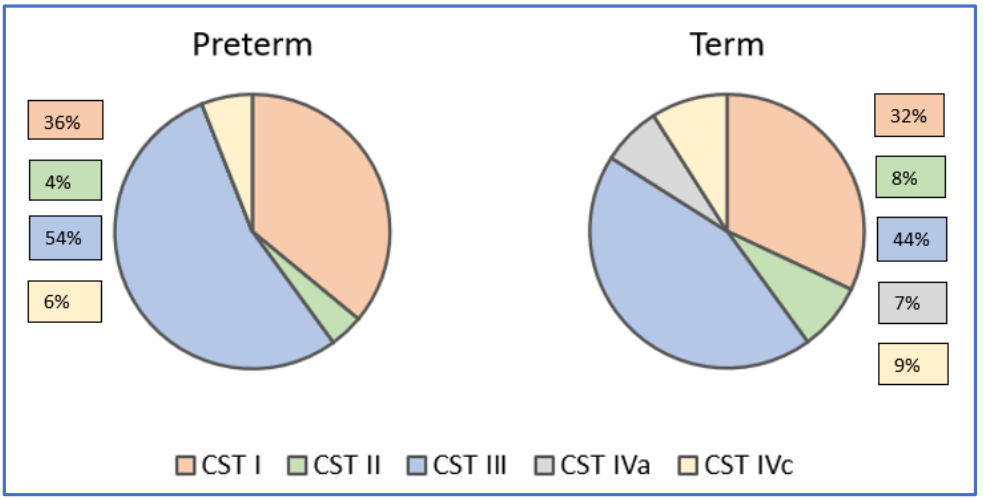


Figure 31. *Stacked pie chart showing the distribution of the identified vaginal community state types (CSTs) in the preterm group (<37 + 0 weeks, n= 48) and the term group (n= 96). No statistically significant difference was found apart from the absence of CST IV (mixture of different species) in the preterm group.*

A total of 17 human genes were significantly (*FDR <0.05*) differentially expressed in the preterm group compared to in the term group (**Table 12**). Of these 17 genes, 15 showed an overexpression in the preterm group compared to the term group. Human tissue kallikrein-2 (KLK2) was 10.6-fold higher; kallikrein-3 (KLK3) was 14.9-fold higher and the serine protease 30 (PRSS30P) was 22-fold higher in women who delivered preterm compared to those who delivered at term. Among the 17 differentially expressed genes there was a dominance of Metallothioneins (MTs) (4/17), all showing an overexpression in the preterm group compared to the term group (also shown in Figure 3, Paper III).

Table 12. Prevalence of significantly expressed human genes in women who delivered preterm and at term.

Human genes	Fold change*	FDR†	Preterm (<37+0 weeks *n*= 48)		Term (*n*= 96)		Prevalence ratio
			Reads‡	Prevalence (%)	Reads‡	Prevalence (%)	
AKR1C2	3.2	0.000	1723.27	37.50	1073.11	34.38	1.10
HOXB13	1.2	0.003	154.52	10.42	257.92	1.04	10.00
PRSS30P	22.2	0.003	3662.80	18.75	329.25	7.29	2.57
MT1L	2.8	0.009	5935.53	72.92	4226.49	75.00	-1.03
IGFL1	3.5	0.016	1504.35	37.50	870.67	29.17	1.29
KLK2	10.6	0.016	218.34	12.50	40.37	1.04	12.00
MT1M	3.1	0.016	9281.00	66.67	6022.29	72.92	-1.09
TGM4	15.5	0.016	613.33	10.42	78.09	1.04	10
TNIP3	-2.3	0.016	2645.89	66.67	12191.42	69.79	-1.05
AC107016.1	2.3	0.019	384.22	20.83	330.36	16.67	1.25
KLK3	14.9	0.019	193.07	14.58	25.05	0	14.58
TPM1	1.3	0.023	1616.58	31.25	2530.71	34.38	-1.10
MT1F	2.7	0.040	4560.34	60.42	3400.11	68.75	-1.14
AC008065.1	2.7	0.044	95.75	8.33	70.35	1.04	8
AC006262.3	3.8	0.048	383.08	18.75	200.80	7.29	2.57
HLA-DQB1	-2.2	0.048	2417.27	83.33	10685.90	91.67	-1.10
MT1A	2.3	0.048	5920.52	83.33	5082.26	80.21	1.04

FDR, false discovery rate
** Calculated by using the group sum of normalized values after group size correction. A gene is counted as present in a sample if the gene count is >9 (prevalence)*
† False discovery rate represents the corrected p-value for multiple comparisons between those who delivered preterm vs term (Benjamini Hochberg)159
‡ Normalized counts calculated with the ratio method160 within DESeq2. The sum of the normalized values is calculated for each gene within its group separately. Values are not corrected to group size

Comments

The study showed no difference in bacterial load, diversity, richness, or CST-distribution between women who delivered preterm compared to women who delivered at term. Nor did *Lactobacillus Crispatus* seem to be a protective factor against spontaneous PTD as proposed in three earlier studies with a mainly Caucasian population[53, 54, 59]. These results suggest that the answer to the enigma of spontaneous PTD is not to be found in the vaginal microbial transcriptome, at least not in our population.

Interestingly, 17 human genes were found to be differentially expressed in women who gave birth preterm compared to women who gave birth at term. Of these, the most interesting transcriptionally active genes were KLK2, KLK3, several isoforms

of MT1s and PRSS30P. KLK2 and KLK3 are part of the tissue kallikrein-related peptidase family consisting of 15 proteolytic enzymes, with diverse functions such as processing of growth factors, cleavage of extracellular matrix proteins and activation of other proteases[161]. The increase of PRSS30P could be of significance, since PRSS30P belongs to the serine protease-family, previously shown to exert KLK enzymatic activity in inflammatory processes[162]. It has been proposed that KLKs could be associated with mechanistically important pathways linked to PTD such as: 1) activation of antimicrobial peptides, 2) degradation of the cervical mucus plug by cleaving mucin proteins, and 3) breakdown of the fetal membranes by degradation of extracellular matrix components such as collagens, fibronectin and lamines[163, 164].

Another interesting finding was the overexpression of four different isoforms of MT1s and the increased activation of the Metallothioneins binding metal pathway in women delivering preterm compared to those delivering at term. MTs play an important role in regulating oxidative stress, inflammation, and hormone signaling[165, 166]. At present, there is very little information on the role of MTs in spontaneous PTD. In a small study by Lappas et al. it was demonstrated that MTs 1-4 were upregulated in intrauterine tissues in women with PTD complicated by bacterial infection (samples were taken at the time of birth)[167]. In another small study by Chaemsaithong et al[168], an increased expression of MT1A and MT2A in the myometrium was reported in women with arrest of cervix dilatation during labor. The authors attributed this to an increase in oxidative stress. This raises the question as to whether MT1 could be used as a marker of reactive oxygen species (ROS) production associated with PTD induced by infection or inflammation. A hypothetical illustration of the interaction between the different described genes and spontaneous PTD is seen in ***Figure 32***. Whether the KLK and MT genes, or their respective translated proteins are biomarker candidates for spontaneous PTD needs to be further explored.

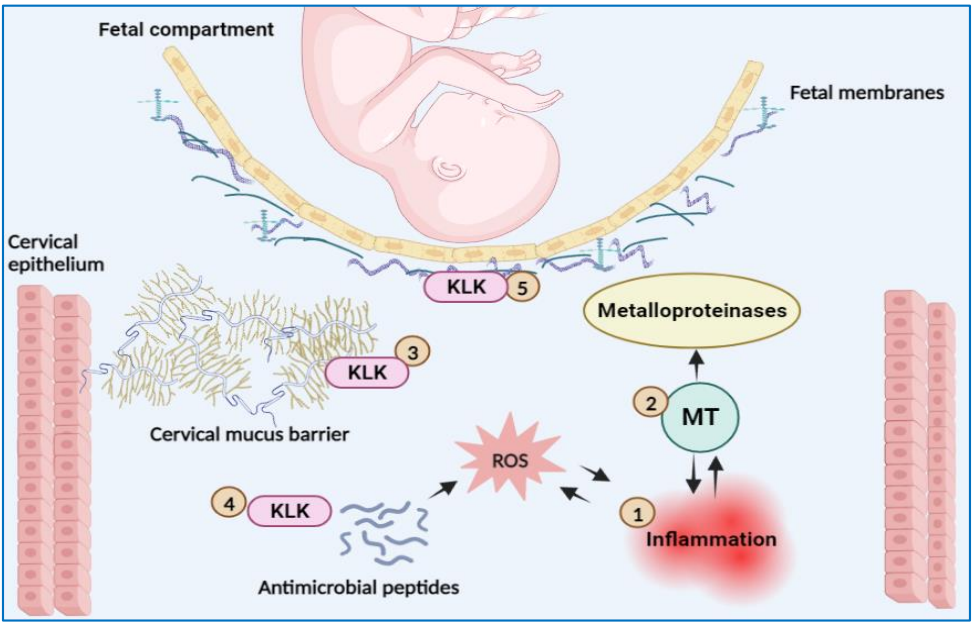


Figure 32. *Schematic illustration of the potential mechanisms of Kallikreins (KLKs) and Metallothioneins (MTs) in relation to spontaneous preterm delivery. KLK and MT mRNA are increased in vaginal fluid at gestational week 18+0 to 20+6 in women who subsequently deliver preterm. MTs are activated in response to cytokine-mediated inflammation and oxidative stress (1). MTs regulate metalloproteinases which degrades the extracellular matrix and have been associated with preterm prelabor rupture of fetal membranes (2). KLKs can be mechanistically involved in preterm delivery via degradation of the cervical mucus barrier (3), modulation of anti-microbial peptides (4) and preterm prelabor rupture of fetal membranes (5). ROS; reactive oxygen species.*
Illustration by Tove Wikström.

Paper IV

61 women with spontaneous start of delivery <34+0 weeks were included in the study. Of these, 27 women delivered <32+0 weeks, 17 women delivered <30+0 weeks and 8 women delivered <28+0 weeks (**Figure 18**). 205 women with term deliveries with spontaneous onset (39+0 to 40+6 weeks) were included as controls.

Cervical length at both Cx1 (18+0 to 20+6 weeks) and at Cx2 (21+0 to 23+6 weeks) was in general shorter in the preterm group (Cx1: 32.7mm, Cx2: 30.1 mm) compared to the term group (Cx1: 35.4 mm, Cx2: 35.8 mm). It was shortest in those who gave birth at <28+0 weeks in the Cx1-population (30.4 mm) (Supplementary Tables 1 and 2 in Paper IV).

There was no statistically significant difference in the mean relative expression level of any of the nine analyzed miRNAs between the preterm group (spontaneous PTD <34+0 weeks) and the control group. MiR-191-5p was significantly overexpressed in the group who delivered <32+0 weeks with an increasing difference in fold-change if the delivery occurred at <30+0 weeks or at <28+0 weeks (**Table 13**; Figure 2 in paper IV). Both miR-93-5p and miR-15b-5p were significantly overexpressed in the group who delivered <30+0 weeks compared to the control group, and for miR-15-5p this statistical association was also shown for delivery <32+0 weeks (**Table 13)**. No correlation was found between the expression level of the investigated miRNAs and cervical length measured at either Cx1 or Cx2.

The miRNA with the best discriminative ability was miR-191-5p with an AUC of 0.73 (95% CI 0.54 to 0.91) for spontaneous PTD <28+0 weeks at Cx1. When adding cervical length as a continuous variable AUC improved to 0.82 (95% CI 0.69 to 0.95) (**Figure 33**). Results for spontaneous PTD ≤28 weeks at Cx2 were not obtainable due to the insufficient number of cases.

Table 13. Mean relative expression of nine miRNAs in women who gave birth spontaneously <34+0 weeks, <32+0 weeks, <30+0 weeks or <28+0 weeks, presented as fold change compared to a control group of women who gave birth at term.

miRNA	Term 39+0 to 40+6 weeks (*n*= 205)	sPTD <34+0 weeks (*n*= 61)	sPTD <32+0 weeks (*n*= 27)	sPTD <30+0 weeks (*n*= 17)	sPTD <28+0 weeks (*n*= 8)
Let-7a-5p	1.00 (0.05)	0.96 (0.06)	1.10 (0.09)	1.12 (0.10)	1.09 (0.18)
miR-150-5p	1.00 (0.06)	0.92 (0.06)	0.96 (0.09)	0.91 (0.08)	0.95 (0.14)
miR-15b-5p	1.00 (0.05)	1.10 (0.10)	1.16 (0.12)	1.28 (0.18) *	1.35 (0.29)
miR-185-5p	1.00 (0.06)	1.14 (0.11)	1.29 (0.18)	1.24 (0.24)	1.38 (0.44)
miR-191-5p	1.00 (0.05)	1.09 (0.09)	1.34 (0.19) *	1.53 (0.27) **	1.85 (0.52) *
miR-19b-3p	1.00 (0.04)	1.09 (0.08)	1.24 (0.14)	1.27 (0.20)	1.32 (0.36)
miR-23a-3p	1.00 (0.05)	0.98 (0.09)	1.05 (0.12)	1.03 (0.17)	1.20 (0.34)
miR-374a-5p	1.00 (0.46)	0.65 (0.12)	0.61 (0.05)	0.59 (0.06)	0.52 (0.10)
miR-93-5p	1.00 (0.04)	1.11 (0.07)	1.27 (0.11) **	1.26 (0.14) *	1.13 (0.19)

sPTD, spontaneous preterm delivery
Results are presented as mean relative expression (fold change) and SEM (standard error of the mean). A 2-tailed p-value of <0.05 was considered statistically significant. A p-value of <0.01 is denoted with ***

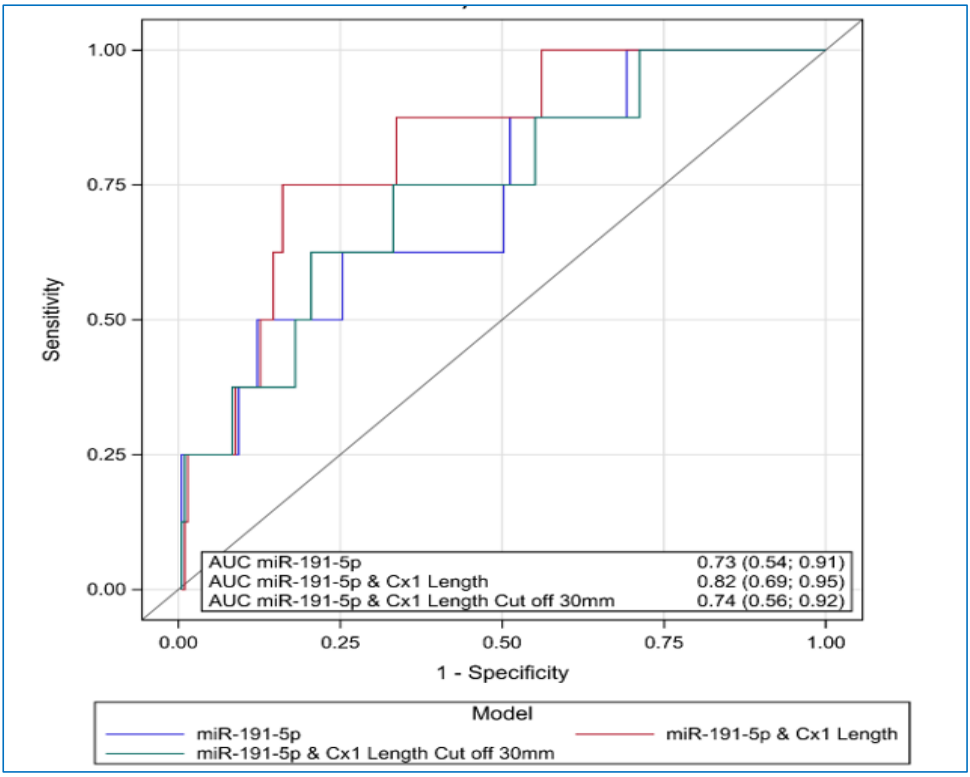


Figure 33. *The ability of miR-191-5p miRNA to discriminate between women who deliver preterm <28+0 weeks vs at term is described as area under the Receiver Operating Characteristic (ROC) curve (AUC). Youden´s index was used to determine the cut-off that yielded the highest proportion of correctly classified cases ("best cut-off")*[155].
Cx1, cervical length measurement at 18+0 to 20+6 weeks; miR, micro ribonucleic acid.

Comments

None of the nine investigated miRNAs shown by Cook et al. to be associated with spontaneous PTD[91] were significantly differentially expressed in those who gave birth <34+0 weeks in comparison to those who gave birth at term. However, one of the analyzed miRNAs (miR-191-5p) was significantly overexpressed in the group who gave birth <32+0 weeks, with an increasing difference in fold change with decreasing gestational age at delivery (Figure 2 in paper IV). Also, the level of two miRNAs (miR-93-5p and miR-15b-5p) were significantly overexpressed in those who gave birth at <30+0 weeks compared to those who gave birth at term. This is interesting since the overexpression of miR-191-5p, miR-93-5p and miR-15b-5p is in line with the findings of Cook et al., even though their study demonstrated a greater difference in relative expression between the preterm and term group[91]. Also, the

findings are consistent despite that 1) the analyses in paper IV were performed on routinely collected serum samples for infectious screening early in pregnancy instead of on prospectively collected plasma, and 2) that study IV was performed on a low-risk population instead of on a high-risk population.

MiRNAs are involved in multiple biological processes including cell proliferation, differentiation, apoptosis, migration, and cellular invasion[88, 169-171]. MiR-191-5p, mir-93-5p and miR-15-5p have been associated with different types of cancers, type-2-diabetes, inflammatory diseases, myocardial diseases as well as neurodegenerative diseases[170, 172], but very little is known about their potential pathophysiological role in spontaneous PTD. It would be of great interest to perform a prospective study, analyzing both serum and plasma, to further investigate the potential of using these nine miRNAs as future biomarkers for spontaneous PTD in a low-risk population. There is also a need to better understand the pathophysiological role of these nine miRNAs, as well as the upstream consequences of either downregulation or upregulation of these gene regulating miRNAs.

Summary, general discussion and future perspectives

The aim of this thesis was to clarify 1) if second trimester cervical length measurement has the same ability to discriminate between women who give birth spontaneously preterm *vs* at term irrespective of obstetric risk group classification, 2) if second trimester cervical length screening would potentially be cost-effective in a Swedish setting, and 3) to further explore the mechanisms of spontaneous PTD with the long-term objectives of identifying complementary biomarkers to improve the ability to predict spontaneous PTD.

In short, the above questions can be answered as follows: 1) Second trimester cervical length measurement can be used to discriminate between women who do and do not deliver spontaneously preterm in both high and low risk singleton pregnancies in a mainly Caucasian population with a low prevalence of spontaneous PTD. In all obstetric risk groups, the discriminatory ability was better when measurements were performed at 21+0 to 23+6 (Cx2) weeks compared to at 18+0 to 20+6 weeks (Cx1) and was also better in foreseeing early PTD (<33+0 weeks) compared to late PTD (<37+0 weeks) (Paper I). 2) There is a high possibility that cervical length screening, followed by treatment with vaginal progesterone of those at increased risk of spontaneous PTD, would be cost-effective in a Swedish setting. Question marks remain regarding whom and when to screen. This depends mainly on how much resources our society is willing to invest as well as on the logistical feasibility (Paper II). 3) No evidence was found that differences in the vaginal metatranscriptome can explain why some women deliver preterm while others do not. Seventeen human genes were found to be differentially expressed in women who gave birth preterm compared to at term. Whether these genes, or their respective translated proteins could serve as biomarker candidates in the future remains to be seen (Paper III). In Paper IV it was demonstrated that miR-191-5p, miR-93-5p and miR-15b-p were overexpressed in women delivering early preterm (either at <32+0 weeks or at <30+0 weeks) compared to those delivering at term. These exploratory results are consistent with the previous work by Cook et al.[91]. Whether these miRNAs could have roles as biomarkers in the future is still unclear.

Below follows a general discussion about the results, the strengths and limitations of the studies, considerations regarding the introduction of a screening program, as well as questions that need to be addressed in future research.

Strengths and limitations

General strengths that apply to all four studies

The major strengths that apply to all four studies are: 1) the blinding, i.e., the results of the cervical length measurements were not disclosed neither to the medical staff nor the participants, 2) the negligible rate of intervention to prevent PTD (0.2% received either progesterone or cerclage) between inclusion and outcome. Both blinding and a low intervention rate are crucial to reduce research bias, and in this case to estimate the true association between cervical length measurements and spontaneous PTD[173]. 3) The multicenter design since it increases the likelihood of the results being generalizable to a similar population 4) the minimal loss to follow-up (0.3%), 5) the detailed description and quality control of the cervical length measurements, and 6) the strict validation of risk factors for spontaneous PTD. The homogeneity of the study population can be seen both as a strength and a limitation. The strength is that one knows to what type of population the results are generalizable. The limitation is that the results might not be applicable to a population with other demographic characteristics, or to a population with a higher prevalence of spontaneous PTD.

Strengths and limitations Paper I

As far as I know, no other study has directly compared how well cervical length measurements distinguish between women who deliver spontaneously preterm and those who do not in different obstetric risk groups. A major strength is that all prevalences of cervical length, as well as rates of spontaneous PTD are from the blinded multicenter CERVIX-study[80].

Because spontaneous PTD is a relatively rare event, a subgroup analysis naturally reduces the number of women with spontaneous PTD in each risk group. This is a limitation since the reduced number of women experiencing spontaneous PTD in each risk group makes the test results less precise. Another limitation is that the Swedish National Patient Register, from which information about treatment for cervical dysplasia was obtained, does not provide full coverage. Before year 2001, specialized outpatient care and day surgery were not included in the National Patient Register, meaning that some participants with a conization prior to inclusion in the study might not have been included in the high-risk group in the analysis. Most probably the number of misclassified participants is low, but misclassification cannot be ruled out.

Strengths and limitations Paper II

This is to date the first study to estimate the cost-effectiveness of cervical length screening followed by treatment with vaginal progesterone of those at increased risk of spontaneous PTD in a Nordic health care context. Another strength is that spontaneous PTD and indicated PTD were identified and distinguished, which is not the case in previous cost-effectiveness studies[110-114]. This is important since it is unlikely that the identification of a short cervix and treatment with vaginal progesterone of those at risk for spontaneous PTD would result in a reduced number of indicated PTDs. Further, estimates of the cost of delivery, postpartum, and neonatal care were carefully estimated and based on up-to-date Swedish data, separating costs for babies born <33+0 weeks, at 33+0 to 36+0 weeks and ≥37+0 weeks. This stands in contrast to the cost-effectivness analyses identified in our systematic literature search, where costs regarding these variables were estimated from already published data (publication data ranging between year 2000 and 2007)[110-114]. Costs for long-term morbitidty (CP) were also carefully estimated, taking into account that the rate of CP depends on gestational age at birth[134]. Also a variety of health and societal costs were included in the analysis[136, 137].

The main limitation of the study is that the same progesterone effect was assumed for all applied cervical length cut-offs, despite that the progesterone effect is insufficiently known for cervical length between 26 to 30 mm[82]. Moreover, the progesterone effect was assumed to be the same for women with a previous spontaneous PTD or late misscarriage with unknown cervical length. This is most probably an overestimation based on a recently published meta-analysis (further discussed below[174]. Another limitation is that it was not possible to compare screening strategies at Cx1 (18+0 to 20+6 weeks) with those at Cx2 (21+0 to 23+6 weeks) because of the difference in prevalence of spontaneous PTD (late miscarriages <22+0 weeks were only included in the Cx1 population). The inability to compare the results of screening strategies at Cx1 with those at Cx2 makes it difficult to determine the optimal time point for screening. The reason to present results for both screening performed at Cx1 (i.e., at the time for the routine ultrasound scan) and at Cx2 (extra appointment) are several. In Sweden, the routine fetal ultrasound examination is scheduled at 18 weeks of gestation. Termination of a pregnancy because of e.g., malformation is, in most cases, not allowed after 21+6 weeks of gestation, which makes it impossible to postpone the routine scan[175]. There are advantages and disadvantages of screening at Cx1 and at Cx2. Screening at Cx1 would be less costly, as well as logistically and organizationally easier to implement compared to an extra appointment. Also, a cervical length screening program

combined with the well-accepted routine fetal screening program with high attendance would probably be more likely to reach general acceptance than a strategy involving an extra appointment. At the same time, we know that Cx2 cervical length measurements are better in discriminating between those who do and do not deliver preterm than measurements taken at Cx1[80, 133]. This has also been shown by others[68, 78]. Another limitation is that long-term morbitity was defined as CP, despite spontaneous PTD being associated with a multitude of short- and long-term morbidities, such as respiratory distress syndrome, bronchopulmonary dysplasia, necrotizing enterocolitis, neuropsychiatric problems, learning disabilities and hearing and visual impairment[13, 14, 176]. Moreover, lifetime costs for productivity loss for parents with a child with long term disabilities were not included. The reasons for the above limitations are that the full effect of impairment associated with spontaneous PTD, as well as its economic impact, is difficult to access[177]. The costs for spontaneous PTD are most probably underestimated in the study.

An inherent limitation of any decision analytic model is that a cost-effectiveness analysis is at best an approximation of real-life practice. To compensate for this and to mimic a real-world scenario as much as possible, all estimations were liberally modified in sensitivity analyses.

Strengths and limitations Paper III

As far as I know, the study is the first to compare both the human and the bacterial transcript profiles in vaginal fluid in women with a singleton pregnancy who delivered preterm *vs* at term, using high throughput sequencing. This was done in a comparatively large cohort of participants. Another strength is the homogeneity of the cohort regarding ethnicity. This is critical since previous studies have revealed that both the microbiome itself as well as the relationship between the microbiome and spontaneous PTD seem to differ depending on race and ethnicity[51, 52, 61, 178].

It is a limitation that vaginal sampling was only performed at one timepoint. This means that it is not possible to study possible longitudinal changes or interrelational changes between the human and microbial transcriptome activity. Also, the relationship between the total bacterial DNA and the activity (mRNA) of the bacteria was not studied. It would have been advantageous to study both, since the metatranscriptomic field is less explored than the metagenomic field. Finally, the study is underpowered for performing subgroup analyses, i.e., for performing separate analyses of early and late spontaneous PTD, or of different types of spontaneous PTD (PTL *vs* PPROM).

Strengths and limitations Paper IV

The major strength is that the study is performed on a comparatively large cohort of women. Other strengths are the blinded prospective two-center design of the study, the homogeneity regarding ethnicity and socioeconomic status of the study participants, as well as that no participant received preventive treatment for spontaneous PTD (progesterone or cerclage) between inclusion and outcome.

An important limitation is that archived serum samples were used for analysis, which limited the control of how each sample was handled from sampling to storage. This may have affected the quality of the miRNAs. Furthermore, some samples had a degree of hemolysis which potentially could have affected the levels of the analyzed miRNAs[179-181], even though interaction analyses did not reveal any association between hemolysis and the levels of the miRNAs analyzed.

Cervical length screening - advantages and disadvantages

Seen from a general point of view there are several aspects that need to be taken into account before implementing a screening program. In 1968 Wilson and Jungner wrote their report "Principles and practice of screening for disease" with the intention to standardize and define essential screening criteria[182]. The aim was to guide decision makers, as well as clinicians, to select conditions and diseases suitable for screening, weighing the risk of causing harm against the possibility of identifying a condition and finding targets for treatment. Since then, the established criteria by Wilson and Jungner have been reevaluated and modernized by Andermann et al. and presented in the Bulletin of the World Health Organization in 2008[183] (**Table 14**).

Table 14. Recommendations from the World Health Organization (WHO) of criteria that should be fulfilled before the setup of a screening program*.

- The screening program should respond to a recognized need
- The objectives of screening should be defined at the outset
- There should be a defined target population
- There should be scientific evidence of screening program effectiveness
- The program should integrate education, testing, clinical services, and program management
- There should be quality assurance, with mechanisms to minimize potential risks of screening
- The program should ensure informed choice, confidentiality, and respect for autonomy
- The program should promote equity and access to screening for the entire target population
- Program evaluation should be planned from the outset
- The overall benefits of screening should outweigh the harm

*Table modified from the publication: Andermann A, Blancquaert I, Beauchamp S, Déry V. Revisiting Wilson and Jungner in the genomic age: a review of screening criteria over the past 40 years. Bull World Health Organ. 2008;86(4):317-9.[183]

There is a need to reduce the number of spontaneous PTDs and most of the above screening recommendations by WHO are already fulfilled. This argues for the implementation of a cervical length screening program with the aim of reducing the number of spontaneous PTDs and thereby reducing neonatal mortality and morbidity. There is also a growing body of evidence that treatment with vaginal progesterone of asymptomatic women with a short cervix can prolong pregnancy[73, 82, 92]. The remaining question to answer is to whom a cervical length screening program should be directed. Should it include all women with a singleton pregnancy, or only women with a singleton pregnancy considered to be at increased risk of spontaneous PTD?

In Paper II, the most cost-effective screening strategy was "Low-risk based screening", i.e., treatment with vaginal progesterone of all high-risk women (previous spontaneous PTD or late miscarriage) without screening, complemented by screening of all other women and followed by treatment with vaginal progesterone of those with a short cervix. This strategy corresponds to the previously proposed strategy of "Universal screening of low-risk women" evaluated by several other authors in the US[66, 111]. Until recently, intramuscular 17-OHPC was recommended in the US to women with a singleton pregnancy with a history of a previous PTD, regardless of cervical length[184]. Makena® (17-OHPC) and its generics have recently been withdrawn since they have been shown non effective in reducing the risk of PTD[185]. In the latest update of recommendations from the American College of Obstetricians and Gynecologists (ACOG) it is concluded that treatment with vaginal progesterone can be considered in women with a singleton pregnancy with a history

of PTD and a shortened cervix, but that the body of evidence for treatment is insufficient in the absence of a shortened cervix[174, 184, 186]. This is in line with the recently published guidelines from the Swedish Society of Obstetrics and Gynecology (SFOG)[187]. This probably means that the "best strategy" in our health economic evaluation (i.e., treatment with vaginal progesterone of all high-risk women without cervical length measurement, complemented by screening of all other women followed by treatment of those with a short cervix) is not the strategy to choose when introducing a cervical length screening program in Sweden.

The implementation of a screening program of only high-risk women, i.e., women with a previous spontaneous PTD or a spontaneous late miscarriage, and possibly also women who have undergone cervical conization (in total 9.4% the CERVIX-study population[80, 133]), is tempting from a practical point of view since fewer women would have to be screened. However, approximately two-thirds of all PTDs occur in low-risk pregnancies, which means that this strategy would most probably only have a modest effect on the prevalence of PTD [188]. In our health economic evaluation, screening of only high-risk women at 21 to 23 weeks (Cx2, cervical length cut off ≤25 mm) was considered a "dominant" strategy, i.e., it would lead to lower costs and better health outcome compared to "No screening". For Universal screening, the cost per gained QALY compared to "No screening" was 24 500 USD when measurements were taken at Cx1, and 58 000 USD when taken at Cx2. However, it is important to consider the large differences in mortality and gained QALYs between the different strategies. Screening of high-risk women only reduces neonatal mortality by 1.7/100 000 deliveries, whereas universal screening reduces neonatal mortality by 4.0/100 000 deliveries at Cx1 and 4.1/100 000 deliveries at Cx2. At the same time, screening of high-risk women increased gained QALYs in a life-time perspective by 58/100 000 deliveries, while universal screening increased gained QALYs in a life-time perspective by 136/100 000 deliveries (Cx1) and 141/100 000 deliveries (Cx2), respectively. All this needs to be taken into consideration in the decision-making process.

An important aspect when considering the strategy of a screening program including all women (universal screening), is that the number needed to screen (NNS) to detect one spontaneous PTD <33+0 is relatively high, especially when using the proposed cut-off of ≤25 mm (at Cx1= 625, at Cx2= 629). If we hypothetically assume that vaginal progesterone would have the same effect at cervical length between 26 and 30 mm as for a cervical length ≤25 mm (assuming a 30% reduction of sPTB[73]), the situation might be different. If applying the best cervical cut-off´s from the CERVIX-

study[80] (Cx1 ≤29 mm, Cx2 ≤27 mm) to the CERVIX-study population, NNS would be 411 for Cx1 and 450 for Cx2. It is also important to recognize that the NNS to prevent one spontaneous PTD <33+0 weeks is much higher than to detect one (**Figure 34).**

NNS to detect one sPTD= numbers of screened women/numbers of sPTDs
Number of prevented sPTDs= assumed progesterone effect x numbers of sPTDs
NNS to prevent one sPTD= numbers of screened women/ numbers of prevented sPTDs

Figure 34. *Explanation of how "NNS to detect one sPTD" and "NNS to prevent one sPTD" was calculated. A full report of all probabilities used is found in supplementary Table 1 in Paper II.*
NNS, number needed to screen; sPTD, spontaneous preterm delivery; nr, number

If using the cut-off of ≤25 mm the NNS to prevent one sPTD <33+0 weeks would be 2171 for Cx1 and 2096 for Cx2. Correspondingly, if using the best cut-offs from the CERVIX-study[80] (Cx1 ≤29 mm, Cx2 ≤27 mm), NNS to prevent one sPTD would be reduced from 2171 to 1367 for Cx1 and from 2096 to 1498 for Cx2. The impact would most probably be even greater if we instead could use a cut-off of ≤30 mm as a definition of a short cervix. Using a higher cut-off would lead to a higher number of false positive test results per one true positive test result but would probably also lead to an improved detection rate[133]. Even though the positive effects of implementing a cervical length screening program would most probably outweigh any negative effects, it is still important to highlight the potential anxiety that a false positive test result could cause, the risk of unnecessary treatment and its subsequent costs, as well as the possible displacement effect in our resource limited health care system.

Future perspectives

Our health economic evaluation (Paper II), together with the HTA-analysis performed by Wennerholm et al. suggest that cervical length screening would most probably be cost-effective in a Swedish context[92]. However, further discussions on whom and when to screen is needed before the implementation of a Swedish cervical length screening program. It would be of great interest to further evaluate the effect of vaginal progesterone in women with a cervical length between 26 and 30 mm. In the EPPPIC study the authors concluded that there is a potential effect of vaginal progesterone in this interval, but the number of included women was small[82]. A well powered, multi-center RCT is needed to clarify this issue.

In the subgroup analysis in Paper I, the high-risk group (consisting of women with previous spontaneous PTD, late miscarriage or cervical conization) was divided into one group consisting of women with a previous spontaneous PTD or a late miscarriage as their only risk factor and one group of women with cervical conization as their only risk factor (Figure 2a and 2b in Paper I). Even though the number of women with the outcome of spontaneous PTD <33+0 weeks in each group was low (meaning imprecise estimates), the finding of a similar risk of early spontaneous PTD in the two groups was surprising (previous PTD or late miscarriage: 6/404 (1.5%), cervical conization as the only risk factor: 8/631 (1.3%)). There is a large knowledge gap in how to best prevent spontaneous PTD in women who have undergone treatment for cervical dysplasia. To my knowledge there is no RCT that has tested or compared prophylactic treatments to prevent spontaneous PTD in women with previous cervical conization[189]. There is an urgent need to further clarify this matter and a RCT is needed.

In the recently published meta-analysis by Conde-Agudelo et al. the authors found no effect of vaginal progesterone given to high-risk women (previous spontaneous PTD) with a singleton pregnancy and unknown cervical length[174]. Problematically, as in most reports on PTD, their outcome was any PTD (i.e., including both indicated PTD as well as spontaneous PTD) which could bias the results. Spontaneous PTD is a more clinically relevant outcome since it is not believed that indicated PTD can be prevented by treatment with vaginal progesterone. Moreover, even spontaneous PTD should be considered a surrogate outcome, as the key issue is neonatal mortality and short- and long-term sequelae of spontaneous PTD. Both neonatal mortality and severe neonatal morbidity are rare events, and the definitions of sequelae as well as its reporting differ between trials, which makes it difficult to interpret the results. Van't Hooft et al. have published a recommendation of a core outcome set for evaluations of intervention to prevent PTD which would harmonize the reporting of the outcome data, also including long term outcome of the infants[190]. Although the findings in the meta-analysis by Conde-Agudelo et al. indicate that high-risk women with unknown cervical length are not in benefit of routinely administered prophylactic treatment with vaginal progesterone, the question remains whether this would still be true if the outcome was set to spontaneous PTD instead of any PTD. Whether it would be feasible to conduct a RCT sufficiently large to answer this question, including neonatal mortality and morbidity as main outcomes (proposed by van´t Hooft et al.[190]), remains to be seen.

The full effects of impairments associated with PTD, and its economic effects are difficult to assess. This would require large cohort studies with the possibility of linkage between various national registers from different sectors and looking at variables such as long-term use of health and social care services, occupational status, income, receipt of social welfare benefits etc. Potentially a study like this could be carried out by using the existing Swedish health data registers in combination with data from SCB (Statistics Sweden). To fully understand the social and economic burden of PTD, large-scale follow-up studies of parent's health (physical and psychological), occupational situation and income status in a long-term perspective would also be of great value. As far as I know, no study has evaluated these long-term economic sequelae of PTD. Obviously, the heterogeneity of the outcome of PTD (ranging from severe impairment to a normal life) makes it difficult to study the long-term economic sequelae of spontaneous PTD. However, this kind of composite data has been explored in other areas of diseases and healthcare[177, 191], which means that it would probably be possible to do the same for PTD although it would require a multidisciplinary approach.

There is a continued need for research to find easily accessible biomarkers to predict spontaneous PTD, either to be used alone or as a complement to cervical length screening to improve the possibility of identifying those at risk for spontaneous PTD. Whether KLK and MT genes, or their respective translated proteins are biomarker candidates for spontaneous PTD needs to be further explored. This reasoning also applies to the miRNAs (miR-191-5p, mir-93-5p and miR-15-5p) shown to be associated with preterm birth in paper IV as well as in the study by Cook et al.[91]. It would be of great interest to perform a well powered prospective study, analyzing both serum and plasma, to further investigate the potential of using these miRNAs as future biomarkers for spontaneous PTD in a low-risk population. It is also important to highlight that the pathogenesis of spontaneous PTD is still insufficiently understood, limiting both its prediction and prevention. Research to further characterize the underlying molecular events preceding spontaneous PTD is crucial. This could in the future lead to better diagnostics and treatment.

To date the evidence for using ASA for prevention of spontaneous PTD is scarce and results are conflicting[92, 101-103]. Whether ASA could be a complementary drug with the aim of prolonging pregnancy and thereby improve neonatal outcome deserves further investigation. A well-powered RCT is needed.

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