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Preterm birth: aetiology and screening

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Preterm birth: aetiology and screening

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"Strength does not come from physical capacity. It comes from an indomitable will."

Mahatma Gandhi

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LIST OF ABBREVIATIONS

3D: Three-dimensional	HMGB1: High-mobility group box-1	RCTs: Randomized controlled trials
17-OHP-C: 17-alpha hydroxyprogesterone caproate	HPA: Hypothalamic-pituitary-adrenal	ROC: Receiver operating characteristic
AUC: Area under the curve	HPV: Human papillomavirus	ROIs: Regions of interest
ACOG: American College of Obstetricians and Gynaecologists	ICSI: Intra-cytoplasmic sperm injection	ROS: Reactive oxygen species
ADC: Apparent diffusion coefficient	IGFBP-1: Insulin growth factor binding protein-1	SDI: Socio-demographic index
AI: Artificial intelligence	IL: Interleukin	SNPs: Single nucleotide polymorphisms
AMPs: Antimicrobial peptides	ISUOG: International Society of Ultrasound in Obstetrics and Gynaecology	SMFM: Society for Maternal-Fetal Medicine
Antxr2: Anthrax toxin receptor 2	IVF: In vitro fertilization	SPINK5: Serine protease inhibitor Kazal type 5
APCs: Antigen-presenting cells	LMP: Last menstrual period	sPTB: Spontaneous preterm birth
aCAM: Acute chorioamnionitis	LPS: Lipopolysaccharide	SRD5A1: Steroid 5 alpha reductase type 1
BV: Bacterial vaginosis	MET: Mesenchymal-to-epithelial transition	SuPPoRT: Stitch, Pessary, or Progesterone Randomised Trial
CAPs: Contraction-associated proteins	MMPs: Matrix metalloproteinases	TAUS: Transabdominal ultrasound
cCAM: Chronic chorioamnionitis	MMC: Maternal fetal cells	TFF1: Trefoil factor 1
cfDNA: Cell-free Deoxyribonucleic Acid	MRI: Magnetic Resonance Imaging	TGF: Transforming growth factor
CI: Cervical incompetence	mRNA: Messenger ribonucleic acid	THBS2: Thrombospondin 2
CL: Cervical length	NICU: Neonatal Intensive Care Unit	TL: Term labour
CMP: Cervical mucus plug	NK: Natural Killer	TLRs: Toll-like receptors
COX : Cyclooxygenase	NPV: Negative predictive value	TNC: Tenascin-C
CR: Cervical remodelling	OR: Odds ratio	TNF-α: Tumor necrosis factor-alpha
CRH: Corticotrophin-Releasing Hormone	PAMPs: Pathogen-associated molecular patterns	TIMP: Tissue inhibitor of metalloproteinases
CVF: Cervical vaginal fluid	PAMG-1: Placental alpha microglobulin-1	Treg: Regulatory T cells
DAMPs: Damage-associated molecular patterns	PGDH: Prostaglandin dehydrogenase	TUCL: Transvaginal ultrasound cervical length
DCs: Dendritic cells	PGE2: Prostaglandin E2	TVUS: Transvaginal ultrasound
Dcn KO: Decorin	PGs: Prostaglandins	UCA: Uterocervical angle
DHEA: Dehydroepiandrosterone	PPT: Parto pré-termo	USA: United States of America
E3: Estrone-3-glucuronide	PPROM: Preterm premature rupture of membranes	VP : Vaginal progesterone
ECM: Extracellular matrix	PPV: Positive predictive value	WHO: World Health Organization
EMT: Epithelial-mesenchymal transition	PR: Progesterone receptors	
ER: Oestrogen receptor	Ptgs: Prostaglandin-cyclooxygenase-endoperoxide synthase	
fFN: Fetal fibronectin	PTB: Preterm birth	
FMF: Fetal Medicine Foundation	PTD: Preterm delivery	
GA: Gestational age	PTL: Preterm labour	
GAGs: Glycosaminoglycans	PTnotL: Preterm not in labour	
HA: Hyaluronic acid	QUS: Quantitative Ultrasound	
Has2: Hyaluronan synthase 2		
HLA: Human leukocyte antigen		

ABSTRACT

Preterm birth (PTB), defined as childbirth before 37 weeks of gestation, is a major global health challenge and the leading cause of neonatal morbidity and mortality. Beyond immediate perinatal outcomes, PTB is linked to long-term health complications and significant socio-economic burdens.

Our work offers a comprehensive review of the global epidemiology of PTB, highlighting regional disparities, classification systems and trends over time. We explore the multifactorial nature of PTB, considering demographic factors, medical conditions, obstetric histories and labour triggers, such as infections and genetic predispositions. A thorough review of the normal mechanisms that initiate labour is also included, as understanding these processes is crucial for advancing PTB research. Additionally, we evaluate current screening methods, such as ultrasound and biomarkers and examine prevention strategies that include pharmacological, non-pharmacological and surgical interventions.

While much of the research on PTB has historically focused on myometrial activity and membrane activation, there is a growing shift towards understanding the cervix as an active participant in pregnancy maintenance and labour initiation. This thesis emphasizes the cervix's critical role in PTB pathophysiology and that early cervical changes can serve as valuable screening markers to identify women at risk of PTB.

Objectives

This thesis ultimately, aims to bridge gaps in understanding the cervix's role in PTB and to contribute to the development of effective screening and prevention strategies. The primary objectives are:

1. To investigate the cellular mechanisms underlying cervical remodelling in PTB, with a focus on inflammatory processes and fibroblast changes during term and preterm labour.
2. To evaluate the efficacy of mid-trimester cervical length measurement via transvaginal ultrasound as a predictive tool for PTB, assessing its sensitivity, specificity,

cut-offs and clinical utility in identifying women at risk who may benefit from targeted interventions.

Methods

The research comprises two key studies:

1. **Cervical Remodelling Study:** A prospective observational study was conducted from October 2018 to March 2021 (with a 12-month hiatus due to the COVID-19 pandemic) across two Obstetrics and Gynaecology wards in Porto, Portugal. Participants included pregnant women divided into three groups: preterm spontaneous labour (PTL), term spontaneous labour (TL) and preterm not in labour (PTnotL). Gestational age was confirmed via first-trimester crown-rump length measurement. Preterm birth was defined as delivery between ≥ 24 and < 37 weeks. Cervical biopsies were performed transvaginally and samples were processed for histopathological analysis. Cellular analysis was done using QuPath® software to classify cells into categories such as neutrophils, mononuclear cells and fibroblasts. Statistical analyses (using SPSS®) included Kruskal-Wallis, Mann-Whitney and Wilcoxon tests to assess group differences and associations, with a p-value of < 0.05 considered statistically significant.
2. **Cervical Length Screening Study:** This retrospective, cross-sectional cohort study, was conducted at the Obstetrics and Gynaecology Department of Centro Hospitalar Universitário São João in Portugal. Data were collected from women who underwent second-trimester ultrasound between January 2013 and October 2017. Exclusion criteria included preterm labour, cervical cerclage, chorioamnionitis and deliveries before the 24th week. The primary outcome was the prevalence of a short cervix (≤ 25 mm) in the second trimester, with secondary outcomes including diagnostic performance of different cervical length cut-offs (e.g., 20mm, 25mm) for predicting PTB. Multivariable logistic regression and ROC analysis were performed, with statistical significance set at $p < 0.05$.

Key Findings

1. **Cellular Mechanisms in Cervical Remodelling:** The study involved 34 singleton pregnant women, categorized into three groups: PTL (n=14), TL (n=14) and PTnotL (n=6). In cervical samples, chronic inflammation was predominant, with a higher density of inflammatory cells in the endocervix (median 813 cells/mm²) compared to the exocervix (median 175 cells/mm²). Inflammation in the exocervix was more pronounced in PTL compared to TL, showing higher counts of neutrophils and mononuclear cells (p=0.027). Neutrophil inflammation was significantly increased in PTL compared to PTnotL (p=0.035), although mononuclear cell density did not differ significantly. No significant differences in fibroblast counts were found between groups.
2. **Efficacy of Cervical Length Screening:** From January 2013 to October 2017, 8,016 women underwent second-trimester ultrasounds, with 3,476 excluded due to factors such as delivery before 24 weeks (n=5), medical induction of PTB (n=241), cervical cerclage prior to ultrasound (n=19), chorioamnionitis diagnosis (n=3), absence of cervical length measurement (n=958) and non-compliance with Fetal Medicine Foundation measurement standards (n=2,426). The final cohort included 4,481 women, with a mean age of 30.7 years. The prevalence of spontaneous PTB was 6.9%, which decreased to 4.0% after exclusions. Most women had no significant medical (86.5%) or obstetric (96.7%) history. Multivariable analysis confirmed the negative correlation between cervical length and PTB, with factors such as maternal age ≥ 40 , prior PTB and cervical surgery being significant predictors. Short cervical length (≤ 25 mm) was strongly associated with PTB, with the optimal cut-off for predicting PTB identified at 20mm.

Limitations

The **cervical remodelling study** has several limitations that must be considered. First, the relatively small sample size (34 participants) reduces statistical power, limiting the ability to detect significant differences between groups. The study's observational design restricts the ability to establish causality between cervical remodelling and preterm birth. Moreover, cervical biopsies were taken from specific regions of the mucosa, which may not fully capture

the dynamic, three-dimensional changes occurring across the entire structure of the cervix. The use of histopathological techniques and QuPath® software for cell classification, while advanced, still introduces a degree of subjectivity, potentially leading to classification bias despite iterative refinement. Additionally, the reliance on non-parametric statistical tests may limit the detection of clinically significant differences. The study was also impacted by a 12-month hiatus due to the COVID-19 pandemic, which may have disrupted participant recruitment, retention and sample collection consistency. Lastly, the study primarily focused on morphological characteristics, not including specific markers or functional assays, such as immunohistochemistry, which may limit the depth of cellular analysis.

The **cervical length screening study** also has several limitations. First, the retrospective design depends on existing data, which may not capture all relevant variables or guarantee the accuracy of recorded information, potentially introducing information bias. The cross-sectional nature of the study limits the ability to establish causality or temporal relationships between variables. Furthermore, the exclusion of 3,476 participants from the original 8,016 due to incomplete data or measurement inconsistencies may introduce selection bias, especially if excluded cases differ from those included in the analysis. The study's single-centre design limits the generalizability of its findings to other populations. Variability in transvaginal ultrasound cervical length (TUCL) measurements, despite standardization efforts, could affect the accuracy of the results and while the 25mm cut-off for TUCL is the most widely accepted in the literature, it may not be universally applicable. Additionally, the study does not fully examine the impact of treatments like vaginal progesterone or Arabin Pessary on preterm birth rates. Lastly, the predominantly low-risk cohort may not represent other populations and the study's geographic limitations affect the external validity of the findings.

Conclusions

Preterm birth (PTB) is a significant challenge for global health, with relevant socio-economic impacts. This thesis highlights the importance of the cervix in the pathophysiology of PTB and the use of cervical length to screen women with early remodelling who are at risk of PTB. Future studies, particularly multi-centre and prospective ones with larger sample sizes, are needed to validate these findings and improve PTB prevention strategies.

RESUMO

O parto prematuro (PPT), definido como o nascimento antes das 37 semanas de gestação, representa um grande desafio para a saúde mundial e é a principal causa de morbidade e mortalidade neonatal. Para além dos efeitos imediatos na saúde perinatal, a prematuridade está associada a complicações a longo prazo e a elevados custos socioeconómicos.

O nosso trabalho oferece uma revisão detalhada da epidemiologia global do PPT, destacando as disparidades regionais, os sistemas de classificação existentes e as tendências ao longo dos últimos anos. Explora a natureza multifatorial do PPT, abordando fatores demográficos, condições médicas, antecedentes obstétricos e fatores que desencadeiam o trabalho de parto. Inclui ainda uma análise da fisiologia do trabalho de parto de termo, uma vez que a compreensão deste processo é essencial para o avanço da investigação sobre PPT. Acrescenta ainda a descrição de métodos de rastreio (como a ecografia e os biomarcadores) e as estratégias de prevenção atuais, incluindo intervenções farmacológicas, não farmacológicas e cirúrgicas.

Embora a pesquisa sobre a etiologia do parto prematuro se tenha historicamente centrado na atividade miometrial e na ativação das membranas, está a surgir uma mudança crescente para compreender o colo do útero como um participante ativo na manutenção da gravidez e na iniciação do parto. Esta tese sublinha a importância crítica do colo do útero na fisiopatologia do PPT e o papel das alterações cervicais precoces como marcador para rastreio de mulheres em risco de PPT.

Objetivos

Esta tese visa preencher as lacunas no conhecimento sobre o papel do colo do útero no PPT e contribuir para o desenvolvimento de estratégias eficazes de rastreio e prevenção. Os objetivos principais são:

1. Investigar os mecanismos celulares subjacentes à remodelação cervical no PPT, com foco nas alterações das células inflamatórias e fibroblastos durante o trabalho de parto a termo e o parto prematuro.

2. Avaliar a eficácia da medição do comprimento cervical no segundo trimestre por ecografia transvaginal como uma ferramenta preditiva do PPT. Avaliando a sua sensibilidade, especificidade, *cut-offs* e utilidade clínica na identificação de mulheres em risco, que possam beneficiar de intervenções direcionadas.

Métodos

A investigação é composta por dois estudos principais:

1. **Estudo de Remodelação Cervical:** Estudo observacional prospectivo realizado entre outubro de 2018 e março de 2021 (pausa de 12 meses devido à pandemia de COVID-19) em dois serviços de Obstetrícia e Ginecologia no Porto, Portugal. As participantes foram divididas em três grupos: trabalho de parto espontâneo prematuro (PTL), trabalho de parto espontâneo a termo (TL) e parto prematuro em ausência de trabalho de parto (PTnotL). A idade gestacional foi confirmada por medida do comprimento crânio-caudal no primeiro trimestre. O parto prematuro foi definido como o nascimento entre ≥ 24 e < 37 semanas. Foram realizadas biópsias cervicais transvaginais e as amostras foram processadas para análise histopatológica. A quantificação celular foi efetuada com o software QuPath®, classificando as células neutrófilos, mononucleares e fibroblastos. A análise estatística (usando SPSS®) incluiu testes de Kruskal-Wallis, Mann-Whitney e Wilcoxon para avaliar as diferenças entre grupos e suas associações, sendo o valor de $p < 0,05$ considerado estatisticamente significativo.
2. **Estudo de Rastreio do Comprimento Cervical:** Estudo retrospectivo, de coorte transversal, realizado no Serviço de Obstetrícia e Ginecologia do Centro Hospitalar Universitário São João, Portugal. Foram analisados dados de mulheres que realizaram ecografia no segundo trimestre entre janeiro de 2013 e outubro de 2017. Os critérios de exclusão incluíram trabalho de parto prematuro, ciclorrafia, corioamnionite e partos antes das 24 semanas. O principal *outcome* estudado foi a prevalência de colo do útero curto ($\leq 25\text{mm}$) no segundo trimestre, e os *outcomes* secundários relacionados com a performance diagnóstica de diferentes *cut-offs* (por exemplo, 20mm, 25mm) para prever o PPT. Foram realizadas análises de regressão logística multivariada e curvas ROC, com significância estatística definida em $p < 0,05$.

Principais Resultados

1. **Mecanismos Celulares na Remodelação Cervical:** O estudo envolveu 34 mulheres grávidas, divididas em três grupos: PTL (n=14), TL (n=14) e PTnotL (n=6). Nas amostras cervicais, predominou a inflamação crónica, com uma maior densidade de células inflamatórias no endocolo (mediana 813 células/mm²) em comparação com o exocolo (mediana 175 células/mm²). A inflamação no exocolo foi mais pronunciada no PTL em comparação com o TL, com maior número de neutrófilos e células mononucleadas (p=0,027). O infiltrado inflamatório neutrofílico estava significativamente aumentado no PTL em comparação com o PnotTL (p=0,035), enquanto que densidade de células mononucleadas não apresentou diferenças significativas. Não foram encontradas diferenças significativas nas contagens de fibroblastos entre os grupos.
2. **Eficácia do Rastreio do Comprimento Cervical:** Entre janeiro de 2013 e outubro de 2017, 8.016 mulheres realizaram ecografia no segundo trimestre, com 3.476 excluídas devido a fatores como parto antes das 24 semanas (n=5), indução médica de PPT (n=241), ciclorrafia antes da ecografia (n=19), diagnóstico de corioamnionite (n=3), ausência de medição do comprimento cervical (n=958) e medição do colo em não conformidade com as regras da Fetal Medicine Foundation (n=2.426). A coorte final incluiu 4.481 mulheres, com uma média de idade de 30,7 anos. A prevalência inicial de PPT espontâneo foi de 6,9% e diminuiu para 4,0% após aplicados os critérios de exclusão. A maioria das mulheres não tinha antecedentes médicos (86,5%) ou obstétricos (96,7%) de relevo. A análise multivariada confirmou a correlação negativa entre o comprimento cervical e o PPT, sendo a idade materna ≥ 40 , o PPT prévio e a cirurgia cervical alguns dos fatores preditores estatisticamente significativos. O comprimento cervical curto (≤ 25 mm) esteve fortemente associado ao PPT, sendo o ponto de cut-off ótimo para prever o PPT nesta população de 20mm.

Limitações

O **estudo de remodelação cervical** apresenta várias limitações. O tamanho relativamente pequeno da amostra (34 participantes) reduz o poder estatístico, limitando a capacidade de detectar diferenças significativas entre os grupos. O desenho observacional restringe a possibilidade de estabelecer causalidade entre as alterações cervicais e o parto prematuro.

Além disso, as biópsias cervicais foram realizadas em regiões específicas da mucosa, o que pode não refletir totalmente as alterações dinâmicas e tridimensionais do colo do útero. A utilização de técnicas histopatológicas e do software QuPath® para a classificação celular, embora avançadas, pode ainda assim conter algum grau de subjetividade, potencialmente levando a viés de classificação. A dependência de testes estatísticos não paramétricos pode também limitar a detecção de diferenças clinicamente significativas. O estudo foi impactado por uma pausa de 12 meses devido à pandemia de COVID-19, o que afetou o recrutamento de participantes, a retenção e a consistência na coleta de amostras. Além disso, o estudo focou-se apenas nas características morfológicas, não incluindo marcadores específicos ou estudo funcionais de ativação celular, por exemplo usando imuno-histoquímica, o que limitou o alcance da análise.

O **estudo do comprimento cervical** também tem várias limitações. O desenho retrospectivo, depende de dados existentes, pode introduzir um viés informativo. A natureza transversal do estudo limita a capacidade de estabelecer causalidade ou relações temporais entre as variáveis. A exclusão de 3.476 participantes dos 8.016 iniciais, devido a dados incompletos ou inconsistências nas medições, pode introduzir viés de seleção, especialmente se os casos excluídos diferirem dos incluídos na análise. O estudo foi realizado em um único centro, limitando a generalização dos resultados. A variabilidade nas medições do comprimento cervical via ecografia transvaginal (TUCL), apesar dos esforços de padronização, pode afetar a precisão dos resultados e embora o *cut-off* de 25mm para TUCL seja o mais consensual na literatura, pode não ser aplicável universalmente. O estudo também não avalia o impacto de tratamentos como progesterona vaginal ou Pessário de Arabin nas taxas de parto prematuro.

Conclusões

O PPT é um desafio a nível mundial, com impacto socioeconômicos relevante. Esta tese destaca a importância do colo do útero na fisiopatologia do PPT e o uso do comprimento cervical para rastrear mulheres com remodelação precoce que estão em risco de PPT.

São necessários futuros estudos, particularmente multicêntricos e prospectivos, com amostras maiores, para validar estes achados e melhorar as estratégias de prevenção do PPT.

1. INTRODUCTION

Pregnancy and childbirth are pivotal moments in a woman's life and ensuring the health and safety of both the mother and neonate is essential. Among the most significant obstetric complications, preterm birth (PTB) stands as a leading cause of neonatal morbidity and mortality worldwide. The consequences of PTB extend far beyond the immediate perinatal period, influencing long-term health outcomes for the child and imposing substantial socio-economic burdens on healthcare systems. Given these profound impacts, the early identification of risk factors for PTB and the implementation of effective preventive strategies have become crucial in contemporary obstetric practice.

Historically, research on PTB focused heavily on myometrial activity, particularly the role of uterine contractions as the primary mechanism for preterm labour. This perspective, rooted in the Danforth paradigm, positioned the cervix as a relatively passive structure, merely responding to uterine contractions. However, this view has undergone a significant transformation over the past few decades. Increasing evidence suggests that the cervix plays a far more active and pivotal role in PTB than previously recognized.

Research has increasingly emphasized the role of cervical remodelling, particularly as a precursor to PTB. In the 1990s, studies began to focus on cervical shortening as a key indicator of increased risk for PTB. The measurement of cervical length via transvaginal ultrasound in the second trimester has since become a cornerstone for identifying women at risk of preterm delivery. A shortened cervix is consistently associated with a significantly higher risk of PTB and interventions, such as vaginal progesterone therapy and cerclage pessaries, have shown promise in reducing this risk, establishing the cervix as a critical target for PTB prevention.

Despite advancements in clinical understanding of the cervix as PTB marker, substantial gaps remain in our comprehension of the pathophysiology and molecular mechanisms underlying cervical changes during labour. Much of the existing knowledge is based on animal models, with limited human cervical tissue evaluations from women in PTB. This lack of human tissue-based insights has hindered a deeper understanding of how cervical changes contribute to preterm delivery.

This thesis aims to address some of these gaps analysing the inflammatory remodelling of the cervix during labour, alongside a critical evaluation of the most consensual current cervical screening methods. Our research explores the cellular mechanisms involved in cervical remodelling, focusing on inflammatory cell densities and fibroblasts in cervical tissue samples from women experiencing spontaneous preterm labour and term labour. By investigating these cellular profiles, we seek to clarify the role of cervix cellular changes in the timing and onset of labour, offering a more comprehensive understanding of the processes leading to PTB.

Additionally, this study evaluates the utility of second-trimester transvaginal ultrasound cervical length measurements as a predictive tool for PTB. We assess the predictive value, sensitivity, specificity and role of transvaginal ultrasound cervical length (TUCL) in clinical decision-making, providing valuable insights into its practical application in clinical settings. Through this research, we aim to contribute to the existing body of knowledge on PTB prediction and prevention, enhancing our understanding of the cervix's role in premature delivery and refining screening strategies.

The ultimate goal of this work is to contribute to the development of more effective clinical guidelines and strategies aimed at reducing the incidence of preterm births by addressing critical gaps in our understanding of cervical function, remodelling and screening. By enhancing our knowledge in these areas, it may be possible to lower preterm birth rates, improving neonatal outcomes and shaping the future of preterm birth prevention.

2. BACKGROUND

2.1 DEFINITION OF PRETERM BIRTH

Preterm birth (PTB) is a complex phenomenon with evolving definitions. Initially, PTB was defined solely by birth weight < 2500 grams, a criterion that did not differentiate between infants born prematurely from those who were small for gestational age. A pivotal change by the World Health Organization (WHO) redefined PTB, shifting the focus from birth weight to gestational age. Today, the WHO defines preterm birth as any birth occurring before 37 completed weeks of gestation, or fewer than 259 days from the first day of a woman's last menstrual period (LMP). (1, 2)

I. GESTATIONAL AGE ASSESSMENT

The method of assessing gestational age (GA), including ultrasound, LMP, symphysis–fundal height or postnatal examination, affects population preterm birth rates. (3-5)

The use of the LMP to assess gestational age assumes that conception occurs on the 14th day of the menstrual cycle, coinciding with ovulation. While this method was once the gold standard for dating a pregnancy, it is now recognized as less accurate due to variations in menstrual cycle length and timing of ovulation. Currently, the most accurate method for assessing gestational age is the first trimester ultrasound fetal crown–rump. (6) However, the widespread use of ultrasound faces challenges, including the need for skilled technicians, specialized equipment and limited availability. (7) While second-trimester ultrasounds can also be used to determine gestational age, they have a greater margin of error compared to first-trimester ultrasounds. (2)

A combining ultrasound and LMP has been proposed as the "best obstetric estimate" and is gaining acceptance in many countries. (8)

The method chosen and the expertise of the user significantly impact reported preterm birth rates. The increased use of ultrasonography has led to more births being classified as preterm. (4, 5, 7) A Canadian study found a 9.1% preterm rate using ultrasound alone compared to 7.8% with "best obstetric estimate", emphasizing the need for standardized definitions. (9)

II. LOWER GESTATIONAL AGE CUT-OFF

The classic definition of PTB includes any delivery occurring between 20 and 37 completed weeks of gestation. However, the WHO defines preterm birth without specifying a lower gestational age boundary. (10) The International Classification of Diseases considers fetal viability as the lower limit for gestational age but does not assign a specific gestational age to define viability. Instead, it advises using 'signs of life' to determine a live birth. (6) The lack of an official lower gestational age cut-off can lead to misclassifications, as perceptions of viability vary widely across countries. In high-income countries, advances in neonatal care have enabled the survival of babies born as early as 22 to 24 weeks, while in many low-resource settings, babies born up to 32 weeks gestation are often perceived as nonviable. (6) Since the distinction between spontaneous abortion and spontaneous preterm birth (sPTB) affects the reported prevalence of prematurity, counting all preterm births, both live and stillborn, would enhance comparability, especially given the potential for stillbirth/livebirth misclassification. (6)

2.2 CLASSIFICATION OF PRETERM BIRTH

PTB is an unusual entity as it is defined by time rather than by a distinct clinical phenotype. Moreover, the 37 weeks cut-off is arbitrary since fetal development is a continuous. Even early term births (37 and 38 weeks) have higher rates of adverse outcomes when compared to full-term gestations (39-40 weeks). (2, 6)

To address these complexities, multiple classification systems have been developed, each with specific applications and purposes. These systems aim to guide research on the causes and determinants of PTB, identify at-risk populations, implement and monitor prevention strategies, facilitate PTB surveillance and enable international data comparisons. (2)

I. GESTATIONAL AGE

GA serves as a primary classification, dividing PTB into extremely preterm (<28 weeks), very preterm (28 - <32 weeks) and moderate preterm (32 - <37 completed weeks). This is a very important subdivision of preterm newborns because the mortality rates increase with decreasing gestational age. Analysing preterm data by gestational age subgroups is crucial for stratifying individual risk at a population level, aiding programme planning and assessing data quality. (1)

II. CLINICAL CONDITIONS

Conditions contributing to PTB are broadly categorized into three groups: sPTB (50%), preterm premature rupture of membranes (PPROM) (25%) and medically indicated preterm birth (25%). PPROM is defined as spontaneous rupture of the membranes at less than 37 weeks gestation at least 1 h before the onset of contractions. PPROM may lead to spontaneous labour or, in some cases, necessitate iatrogenic delivery after weeks or months. (11) Medical indications for provider-initiated PTB include maternal complications like severe hypertension or fetal well-being concerns.

III. PHYSIOPATHOLOGY

The presumed pathophysiological pathways (infectious/inflammatory, vasculopathic and stress-induced) are also utilised for sub-classification. The Global Alliance to Prevent Prematurity and Stillbirth proposed a comprehensive phenotype classification system in 2012, incorporating maternal conditions, fetal conditions, placental pathologic conditions, signs of parturition initiation and the pathway to delivery. (12) Similarly, the Eunice Kennedy Shriver National Institute of Child Health and Human Development identified potential PTB phenotype categories based on factors like infection/inflammation, decidual haemorrhage, maternal stress and others, further validated through a prospective cohort study. (13) These classification systems were applied in a prospective cohort study for testing; however, their use remains within an investigational context.

Given the multifactorial nature of PTB, the development of new classifications is imperative for a comprehensive understanding of this complex reproductive health issue.

2.3 EPIDEMIOLOGY OF PRETERM BIRTH

Over the past decade, extensive studies have illuminated the prevalence, trends and implications of PTB worldwide. However, the true global prevalence remains unclear due to inconsistent definitions and data gaps in many countries.

A 2012 systematic analysis estimated that in 2010, there were 14.9 million preterm births globally, accounting for 11.1% of all live births. The rates varied significantly among 184 countries, with South Asia and sub-Saharan Africa together contributing to over 60% of these preterm births. Even in high-income countries like the United States of America (USA), high rates persisted, placing it among the top ten countries with the highest PTB prevalence. The study noted an increase in estimated rates from 7.5% in 1990 to 8.6% in 2010 across 65 countries, with only three countries showing a decrease and 14 countries maintaining stable rates. (14)

In 2019, a systematic review and modelling analysis provided estimates for 2014, indicating a global preterm birth rate of 10.6%, equivalent to approximately 14.84 million preterm births. Rates varied by region, with North Africa showing a rate of 13.4% compared to 8.7% in Europe. North Africa, Asia and sub-Saharan Africa accounted for 78.9% of live births. This review highlighted an upward trend in global preterm birth rates from 9.8% in 2000 to 10.6% in 2014. Importantly, the study highlighted methodological advancements in data collection, rendering the 2014 estimates not directly comparable to those published in 2012 for the period 1990–2010. (15)

The year 2022 provided additional insights into neonatal preterm birth, with 15.22 million cases reported for 2019. Notably, the mortality rate for preterm births decreased by 47.71% compared to 1990. Significant regional variations were observed, with high Socio-demographic index (SDI) regions experiencing increased incidence but decreased mortality rates between 1990 and 2019. (16)

A more recent study in 2023, providing estimates for preterm birth in 2020, reported a global prevalence of 9.9%, accounting for 13.4 million preterm live births. (17) Southern Asia exhibited the highest preterm birth rate at 13.2%, almost double the rate in the Sustainable Development Goal region of Eastern Asia, Southeast Asia and Oceania (6.8%). Over 50% of all

preterm births in 2020 occurred in just eight countries and areas, with India having the highest number of preterm births (3.02 million), representing over 20% of all preterm births worldwide. The study observed minimal change in preterm birth rates globally from 2010 (9.8%) to 2020 (9.9%), with little regional variation over the past decade. (17)

Major variations in preterm birth rates exist based on geographic region and the income level of a country. When countries are grouped by World Bank income groups, it is evident that approximately 90% of all preterm births occur in low- and middle-income countries. The average preterm birth rate for low-income countries is close to 12%, compared to 9.4% and 9.3% for middle- and high-income countries, respectively. Notably, there are outliers, such as Ecuador, a middle-income country, which has a lower preterm birth rate of 5% than many high-income countries like Germany (9.2%), Canada (7.8%) and Israel (8%). (14)

These differences in preterm birth rates arise partly from variations in definitions, such as those for fetal viability and gestational age assessment. However, they also highlight genuine disparities linked to genetic factors, socioeconomic status and other social determinants. For instance, in the USA in 2016, the preterm birth rate was 14% among African-American women compared to 9% among white women. (18) Similarly, an analysis of preterm birth rates across 12 European countries showed higher rates among women with lower levels of education. (19) Understanding these epidemiological variations is essential for effectively advocating for and allocating resources towards surveillance, research, prevention and care related to preterm birth.

Regarding PTB sub-classifications, about 30–35% of preterm births are indicated, 40–45% result from spontaneous preterm labour and 25–30% follow preterm PPRM. (11) In terms of gestational age, approximately 5% of PTB occur at less than 28 weeks, 15% at 28–31 weeks, 20% at 32–33 weeks and 60–70% at 34–36 weeks. (11)

2.4 RISK FACTORS OF SPONTANEOUS PRETERM BIRTH

PTB is a complex and multifactorial phenomenon, making the identification and definition of risk factors crucial. Establishing these risk factors is valuable for several reasons. First, it allows for the early identification of at-risk women, enabling the implementation of targeted, risk-specific interventions. Second, understanding these risk factors helps define populations that are ideal for studying specific preventive measures or treatments. Finally, identifying risk factors can provide critical insights into the underlying mechanisms and pathways that contribute to preterm birth, enhancing our overall understanding and management of this condition.

2.4.1 DEMOGRAPHICAL FACTORS

A) MATERNAL AGE: plays a significant role in preterm birth risk, with the relationship showing a phasic pattern. Women older than 35 years have an elevated risk, with an odds ratio (OR) of 1.4 and increased risk further for those 40 years or older, especially at 45 years and older. (6, 20-22) Adolescent pregnancies also carry an increased risk of preterm birth. (6, 23)

B) RACE/ETHNICITY: influences preterm birth risk, with African/Black women facing a 1.5 times higher risk compared to Caucasians. Data from the USA shows the highest preterm birth rates among non-Hispanic Black women. (21, 24, 25) Importantly, the disparity is not only in prevalence but also in gestational age and recurrence. Black women are more likely to experience preterm birth at earlier gestations and multiparous black mothers face an increased repetition of preterm births. (26) Socioeconomic factors, such as disparities in prenatal treatments and medical technologies received by blacks compared to whites, contribute to these disparities, but even among high socioeconomic groups, non-Hispanic Black women experience higher preterm birth rates, suggesting potential genetic contributions to sPTB. (21, 25-27)

C) SOCIOECONOMIC FACTORS: play a pivotal role in the risk of preterm birth, shedding light on the intricate interplay between social conditions and maternal health. (20)

Socioeconomic status significantly impacts preterm birth risk. Women with lower income face higher risks, likely due to limited healthcare access and increased stress. (28, 29) The socioeconomic characteristics of a woman's neighbourhood also play a vital role. Communities with fewer resources, higher crime rates and limited access to healthcare facilities can contribute to elevated stress and hinder the adoption of healthy behaviours during pregnancy. (30)

Educational disparities significantly impact preterm birth rates. Women with lower educational attainment frequently experience higher rates of preterm birth due to factors such as restricted access to essential health information, delays in starting prenatal care and difficulties in adopting healthy behaviours. (20, 31)

D) BODY MASS INDEX (BMI): this measurement is calculated as weight in kilograms divided by the square of height in meters, is linked to the risk of prematurity. (20) Extremes in BMI are associated with preterm birth risk. (20, 21) Underweight women (BMI <18.5) face higher risks due to poor nutritional reserves, while overweight women (BMI ≥30) experience increased risks due to obesity-related complications such as insulin resistance and hypertension. (20, 32-36)

Additionally, insufficient weight gain during pregnancy has been associated with an elevated risk of preterm birth. (23)

E) SUBSTANCE ABUSE: The use of alcohol, tobacco and illicit drugs significantly increases the risk of preterm birth. For example, smoking during the first trimester is associated with a markedly higher risk of preterm delivery, including a 20% increased risk of extreme preterm birth before 28 weeks of gestation. (20, 36) Similarly, heavy alcohol consumption during pregnancy is consistently linked to a higher likelihood of PTB. (11, 21)

Illicit drug use, such as opioids, cocaine, cannabis, amphetamines and polysubstance use, represents a major risk factor for preterm birth. Among these, cocaine use is particularly concerning, with nearly one in four users experiencing preterm delivery. (21, 37-39)

F) MATERNAL STRESS: Stress is a significant factor influencing preterm birth, reflecting the complex relationship between psychological well-being and pregnancy outcomes. Elevated stress levels are consistently associated with an increased risk of preterm delivery. (40, 41) Chronic stress can induce hormonal changes and inflammatory responses that contribute to preterm birth. (42, 43) Acute stressors and traumatic events also increase this risk by activating the maternal hypothalamic-pituitary-adrenal (HPA) axis, leading to higher cortisol production and hormonal imbalances that affect birth timing. (44) Additionally, factors such as socioeconomic status, social support and racial disparities may exacerbate the impact of stress on preterm birth risk. (45)

G) OCCUPATIONAL FACTORS AND EXERCISE: Research on occupational factors and exercise shows mixed results, but certain physical stressors—such as shift work, prolonged standing and heavy lifting—are linked to a higher risk of PTB. (23) Lifting heavy objects (>100kg per day), standing for over 2.5 hours a day and engaging in physically demanding work are associated with increased preterm birth risk. (46) Shift work, especially with frequent night shifts and long hours, further elevates this risk. Working three or more consecutive nights and frequently having quick returns from night shifts during the first trimester show a 3–4 fold increased risk of preterm birth. Additionally, working frequent long shifts (≥ 10 hours) and any week exceeding 40 hours during the third trimester are associated with an increased risk of PTB. (47)

Regarding exercise, moderate and regular physical activity during pregnancy is generally safe and beneficial as it improves cardiovascular health and reduces risk of gestational diabetes. (48)

The historical recommendation of bed rest in high-risk pregnancy situations is now challenged by recent research suggesting limited efficacy and potential harms, such as deconditioning and psychological stress for the expectant mother. (36, 49)

H) MATERNAL DISEASES: While maternal diseases are commonly associated with iatrogenic prematurity, it's imperative to acknowledge that acute and chronic maternal illnesses also elevate the risk of sPTB. (23)

- **Maternal hypertensive disorders**, such as preeclampsia and gestational hypertension, consistently heighten the risk of sPTB. A prospective cohort study demonstrated that women experiencing spontaneous preterm delivery exhibit elevated blood pressure from 13 weeks until delivery, indicating a potential link between hypertensive conditions and preterm birth. (50) These disorders may compromise placental function and blood flow, leading to adverse pregnancy outcomes, including PTB. (11, 51)
- **Diabetes**, either pre-existing (Type 1 or Type 2) or gestational diabetes mellitus, contribute to an increased risk of preterm birth. Poorly controlled blood glucose levels can induce inflammation and vascular complications, potentially triggering preterm labour. (52, 53)
- **Autoimmune conditions**, such as systemic lupus erythematosus and rheumatoid arthritis, may heighten the risk of sPTB. These conditions can induce inflammation and immune system dysregulation, potentially impacting the maintenance of a healthy pregnancy. (54)
- **Chronic respiratory diseases**, such as asthma, have been associated with an increased risk of sPTB. The inflammatory nature of these conditions may play a role in triggering premature labour. (11)
- **Maternal abdominal surgery** in the second and third trimesters can stimulate contractions, potentially culminating in preterm delivery. (11)
- **Maternal psychiatric disorders**, including depression and anxiety, have been linked to a higher likelihood of sPTB. The intricate interplay between mental health and hormonal responses may contribute to this association. (41, 55)
- **Other chronic maternal illnesses**, like sleep-disordered breathing, haemorrhagic and hepatic disorders, chronic kidney disease, endometriosis, inflammatory bowel disease and cancer survivorship have been associated with an increased risk of PTB. (21, 56-61)

I) MATERNAL MICROBIOME: The regulation of the maternal-fetal interface involves a delicate balance between the maternal microbiome, trophoblasts and maternal immune cells. PTB can arise from disruptions in this equilibrium. (62-65) Research on the relationship between dysbiosis - disruption of normal vaginal microbiome - and PTB has yielded conflicting results. A North American Next-Generation Sequencing study by Romero et al. found no

significant difference in the abundance and structure of the vaginal microbiome across different types of birth. (66) However, other studies, such as those by Hyman et al. using chain-termination sequencing and Freitas et al. in Canada, reported a high diversity of vaginal microbiota in mothers who delivered prematurely. (67, 68) Additionally, emerging evidence suggests that women from different ethnic backgrounds harbour distinct vaginal microbial communities, which may contribute to understanding the aetiology of preterm labour and addressing racial disparities in PTB prevalence. (69, 70)

Recent studies have explored the potential link between the placental microbiome and preterm birth, likely through the activation of inflammatory processes in the chorioamniotic tissue. (71, 72) Additionally, the gut microbiome is proposed as a potential source of intrauterine infection, underscoring the complex interplay between microbial communities and pregnancy outcomes. (62)

J) GENETIC FACTORS: Substantial evidence emphasizes the significant impact of genetic factors on both gestational length and the risk of preterm birth. (73) Previous preterm birth remains the strongest predictor of subsequent prematurity, with high recurrence rates highlighting the genetic basis of sPTB. (20, 21, 74) Family history also plays a crucial role; the risk of PTB increases significantly if the mother, full sister, or maternal half-sister has a history of sPTB. Notably, this risk is higher when the mother herself was born preterm, emphasizing the maternal genome's pivotal role in transmitting this susceptibility. (21, 74, 75)

Supporting the role of genetic predisposition, heritability studies, particularly those involving twins, suggest that genetic factors account for approximately 30% of the risk of preterm birth. (76-78) Additionally, racial disparities in PTB rates persist regardless of socioeconomic status. (74) For example, women of African ancestry tend to have a stronger inflammatory response associated with PTB compared to those of European ancestry, pointing to the impact of genetic ancestry on PTB risk. (75) Overall, research underscores the primary contribution of the maternal genome to PTB, with minimal influence attributed to the fetal genome, further emphasizing the significance of maternal genetics in determining preterm birth susceptibility. (79)

2.4.2 OBSTETRICAL AND GYNAECOLOGICAL FACTORS

A) PRIOR PRETERM BIRTH is the strongest risk factor for repeated PTB. (20, 21) The risk is intricately linked to both the number and gestational age of previous deliveries. Women with a history of preterm births are more likely to face a recurrence in subsequent pregnancies. (11) According to Iams and colleagues, each previous preterm delivery raises the risk of recurrence by 1.5 to 2 times. (80)

Additionally, the risk inversely correlates with the gestational age of the prior preterm birth. (11) A prior preterm delivery at 28 weeks amplifies this risk to 35%, while experiencing two preterm deliveries before 32 weeks raises the risk dramatically to 70%. (20)

The recurrence risk is further influenced by the number of fetuses. For instance, if a woman had a previous preterm twin pregnancy and is currently carrying a singleton pregnancy, the risk is 10%, whereas it is 20% for women with a singleton pregnancy after a prior preterm singleton pregnancy. (81)

B) INTERGENESIC PERIOD defined as the time between the end of one pregnancy and the conception of the next, can influence various aspects of pregnancy. Short intergenesic periods, often characterized by pregnancies conceived within 18 months of a previous birth, pose notable challenges. This timeframe, associated with insufficient recovery time, can impact the mother's immune system, increase inflammation, impede normal placental development and lead to uterine over distension, all of which contribute to adverse outcomes.

The long-established link between short intergenesic periods and an elevated risk of adverse outcomes, particularly preterm birth, has been extensively documented. (6, 82, 83)

On the other end of the spectrum, recent meta-analyses propose that an interpregnancy interval exceeding 60 months does not elevate the risk of preterm delivery (PTD). (82)

C) CERVICAL FACTORS can significantly impact pregnancy outcomes. Research indicates that individuals who undergo cervical surgery face an increase in the likelihood of delivering their baby prematurely. (20, 21) Any cone depth of ≥ 10 –12 mm is associated with a risk of prematurity and this risk escalates with an increase in the cone size. (84) The consequences of a prior large loop excision of the transformation zone on the risk of sPTB in a subsequent

pregnancy are closely linked to the measurement of cervical length at mid-gestation. (85, 86) Several hypotheses exist regarding how cervical excision procedures may elevate the risk of preterm deliveries. These hypotheses encompass factors such as reduced mechanical support following stromal tissue removal, shortened cervical length, compromised antimicrobial defence post-cervical gland removal and alterations in cervicovaginal bacterial flora. (87) Beyond surgical interventions, various forms of cervical trauma or injury can contribute to an augmented risk of PTB. Examples include cervical lacerations during delivery, dilation and curettage procedures and malformations. (88-91)

D) UTERINE MALFORMATIONS, encompassing various structural abnormalities in the uterus, significantly influence pregnancy outcomes, particularly in relation to the risk of PTB. (92) A research indicates that women with uterine malformations, on average, face an overall risk of approximately 40% for PTB, but the risk of preterm delivery varies with the type of uterine malformation, being highest in women with uterus didelphys (56%), followed by those with a unicornuate uterus (43%) and a bicornuate uterus (39%). In contrast, malformations like a septate or arcuate uterus show a weaker association with preterm birth, with some studies presenting conflicting results. (21, 93)

E) OTHER GYNAECOLOGIC DISORDERS have been identified as potential contributors to premature birth:

- **Uterine fibroids**, benign tumours arising from the smooth muscle tissue of the uterus, have traditionally been associated with an increased risk of preterm birth. (94) However, recent studies involving large, general population cohorts have shown a minimal impact on preterm birth risk, contrasting with earlier findings predominantly from high-risk, academic tertiary populations. This discrepancy calls for a reassessment of the clinical assumption that fibroids are a significant risk factor for preterm birth. (95)
- **Endometriosis**, a condition characterized by the growth of endometrial cells outside the uterus, has also been implicated in preterm birth. The inflammatory response associated with endometriosis can contribute to an unfavourable uterine environment, thereby increasing the likelihood of preterm labour. (21, 96)

- **Pelvic inflammatory disease**, commonly caused by sexually transmitted infections, can induce inflammation in the reproductive organs. This chronic inflammation may compromise the integrity of the cervix, heightening the risk of preterm birth. (97)

2.4.3 COMPLICATIONS RELATED TO CURRENT GESTATION

A) ANTEPARTUM HAEMORRHAGE characterized by vaginal bleeding during pregnancy, is an independent risk factor for spontaneous preterm delivery. (20)

Studies have highlighted the importance of recognizing the timing of bleeding episodes. An episode of bleeding before 24 weeks are specifically associated with a relative risk of sPTB. (20, 98) While third-trimester bleeding is more linked to iatrogenic PTB resulting from placental complication. (99)

Moreover, the persistence of vaginal bleeding throughout more than one trimester even increase more the in the risk of premature rupture of membranes and eventual preterm birth. (99)

B) FETAL MALFORMATIONS are linked to an increased risk of preterm birth, underscoring the complex relationship between fetal development and the timing of delivery. (20) Abnormalities in the heart and central nervous system, such as neural tube defects and chromosomal disorders, notably heighten this risk. (100, 101) Additionally, specific malformations affecting the kidneys and gastrointestinal system can lead to polyhydramnios—an excess of amniotic fluid—which further elevates the risk of preterm birth. (20, 100)

C) MATERNAL INFECTIONS both overt and subclinical, are linked to 25 and 40% of PTB. (11) Infections in the reproductive and urinary tracts are significant risk factors for spontaneous PTB. (20, 102-104) Additionally, extra-genitourinary infections, such as periodontitis, pneumonia and appendicitis, as well as systemic infections caused by bacteria, fungi, or viruses, are also associated with an increased risk of PTB. (20, 60, 105)

- **Genital infections**, such as Chlamydia and Gonorrhoea, have been identified as significant contributors to the risk of preterm birth. For instance, a study revealed that C. trachomatis infection at 24 weeks gestation predisposed mothers to a risk of preterm

delivery two to three times higher than uninfected controls. These infections can induce inflammation in the reproductive tract, potentially triggering preterm labour. (21, 104, 106, 107)

- **Bacterial vaginosis (BV)**, characterized by an imbalance in the vaginal microbiome, is another maternal condition linked to preterm delivery. While the evidence is substantial, universal screening during pregnancy in low-risk women for BV remains a topic of ongoing research. (21, 108)

- **Non-genital infections** such as asymptomatic bacteriuria, pyelonephritis, pneumonia, appendicitis and viral infections have also been associated with an increased risk of spontaneous preterm. (20, 109) Periodontal disease, a chronic low-grade infection, has also been implicated in the causation of preterm birth. (21, 60, 103)

D) UTERINE OVER DISTENSION is a well-established risk factor for preterm birth. Overstretching of the uterine muscle triggers critical events preceding uterine contractions and cervical dilation. (23)

- **Multiple gestations** significantly increase the risk of both spontaneous and iatrogenic preterm deliveries. While twins often deliver between 32–36 weeks, the prevalence of preterm delivery before 32 weeks is 5% in dichorionic twins and 10% in monochorionic twins. (6, 20) Multiple gestations, accounting for 2–3% of infants but result in 15–20% of all preterm births. About 40% of twins experiencing spontaneous labour or PPRM before 37 weeks' gestation. (11)

- **Polyhydramnios**, characterized by an excess of amniotic fluid, can lead to overstretching of the uterine muscles. This overstretching triggers critical events that precede uterine contractions and cervical dilation, thereby increasing the risk of PTB. (23)

E) PLACENTA DISORDERS represent a significant category of complications during pregnancy that can contribute to the risk of preterm birth. (110)

- **Placenta Previa** is a disorder wherein the placenta partially or completely covers the cervix, potentially obstructing the birth canal. This condition increases the risk of spontaneous and iatrogenic preterm birth. (21)

- **Placental insufficiency** also increases the risk of PTB. An observational study concluded that “abnormal” accelerated villous maturation pattern of placental villi were associated with approximately 41% of cases of unexplained spontaneous preterm labour. (110) Lesions of maternal vascular malperfusion are also greater in the placentas of patients with preterm labour than in the control group. (110, 111)

F) ASSISTED REPRODUCTION, like in vitro fertilization (IVF) or intra-cytoplasmic sperm injection (ICSI), introduces a heightened risk of obstetrical and perinatal complications.

This increased risk is partly due to a higher incidence of multiple gestations, but even singleton pregnancies resulting from assisted reproduction have a greater likelihood of PTB compared to spontaneously conceived ones. The exact causes of this elevated risk remain unclear, as it is challenging to distinguish whether the increased risk is primarily related to underlying infertility issues (such as endometriosis), characteristics of the infertile couple (such as advanced maternal age), or potential complications arising from the assisted reproductive techniques themselves. (112, 113)

G) SHORT CERVIX LENGTH has been linked to a significantly heightened risk of spontaneous preterm delivery, making it a pivotal risk factor for prematurity. (See chapter 2.8.2 Cervix evaluation to predict preterm birth).

2.5 IMPACT OF PRETERM BIRTH

PTB remains a formidable global health challenge, representing not only a significant cause of neonatal mortality but also a profound contributor to long-term health complications, economic burdens and societal challenges. This intricate issue extends far beyond the immediate neonatal care, delving into the complex realms of economic, healthcare and enduring health ramifications. (114) PTB is one the largest single conditions in the Global Burden of Disease analysis given the high mortality and the considerable risk of lifelong impairment. (115)

2.5.1 GLOBAL IMPACT AND MORTALITY

Preterm birth is a leading cause of neonatal mortality, with approximately 3.1 million infants succumbing annually. Survival rates vary dramatically across the world, emphasizing stark global health care inequalities. In high-income countries, survival rates for infants born at 24 weeks can reach 50%, contrasting sharply with less than 10% survival in low-income settings at the same gestational age. (116)

The risks of mortality and morbidity escalate with the degree of prematurity. (114) While early PTB have higher risk of adverse outcomes, even late preterm births exhibit considerably increased morbidity and mortality compared to those born at term. (117)

Examining the trends over time reveals a notable shift, with a decrease of over 50% in neonatal preterm birth deaths in low-middle, middle, middle-high and high-SDI regions between 1990 and 2019. However, a concerning 4.62% increase occurred in low-SDI regions. Recent decades have witnessed a commendable increase in the survival of preterm infants, attributed to timely interventions, antenatal corticosteroid administration, improved Neonatal Intensive Care Unit (NICU) care and exogenous surfactant therapy. Yet, the persistence of unequal access to healthcare remains a significant factor contributing to these disparities. (16, 117)

Furthermore, preterm birth complications ranked as the most prevalent cause of under-5 mortality in various countries in 2019, underscoring the enduring impact on child health. (118)

2.5.2 ACUTE-TERM HEALTH COMPLICATIONS

Preterm birth ushers in a spectrum of acute complications that demand meticulous and specialized care, requiring a collaborative approach from a multidisciplinary team. This concerted effort involves neonatologists, paediatricians, respiratory therapists and various other specialized healthcare professionals. While virtually all organs and systems can be affected, the most frequent and severe complications manifest predominantly in the respiratory, gastrointestinal and neurological domains. (119)

One prevalent acute complication is Respiratory Distress Syndrome, notably common in infants born before 28 weeks. (15) Another frequent challenge is Bronchopulmonary Dysplasia, a chronic lung condition that can manifest due to prolonged mechanical ventilation and oxygen therapy, marked by inflammation and scarring of the lungs. (114)

The vulnerability of preterm infants extends to gastrointestinal emergencies, such as Necrotizing Enterocolitis, characterized by inflammation and necrosis of the intestinal tissue. (15, 114)

The intricate web of acute complications also involves the cerebrum, a vital organ susceptible to multiple challenges. Intraventricular haemorrhage, a serious neurological complication, entails bleeding within the brain's ventricles, with severity ranging from moderate to severe cases leading to long-term neurodevelopmental issues. (15) Periventricular leukomalacia, another neurological complication, involves damage to the white matter surrounding the brain's ventricles and is often associated with enduring cognitive and motor impairments. (120) Hypoxic-ischemic encephalopathy, stemming from insufficient oxygen and blood flow to the brain, results in neurological damage and is a severe complication of prematurity. Additionally, cerebral palsy, linked to deafness, blindness, cognitive delay and behavioural difficulties, poses a significant challenge. (119)

Vision-related concerns, such as Retinopathy of Prematurity, arising from abnormal blood vessel development in the retina, may potentially lead to vision impairment. (121) Children born before 28 weeks face a six-fold risk of myopia and hypermetropia, resulting in 25% of this group requiring glasses by the age of six. (114, 122)

Furthermore, very preterm infants face an elevated risk of hearing impairment, approximately 25 times higher than that of the general paediatric population. (114, 123)

In addition to these complexities, newborns born prematurely encounter heightened risks of hypothermia, hypoglycaemia, jaundice, anaemia, sepsis and many other complications. (114)

2.5.3 LONG-TERM HEALTH COMPLICATIONS

Despite enhanced early survival rates, adults who entered the world prematurely encounter a heightened susceptibility to chronic disorders, including cardiovascular, respiratory and neuropsychiatric conditions. Studies spanning the last 50 years reveal a modest increase in mortality among adults born preterm. (124, 125)

The long-term ramifications encompass various health domains. Elevated blood pressure in adulthood, coupled with an increased prescription of antihypertensive medications, accentuates the risk of ischemic heart disease, which emerges in early adulthood. Additionally, preterm birth heightens the risk of stroke, both type 1 and type 2 diabetes later in life, lipid disorders and cardiometabolic syndrome in adulthood. (125)

Reduced pulmonary function, especially pronounced in those with a history of bronchopulmonary dysplasia, persists into early adulthood. This is reflected in substantially reduced expiratory airflow, with higher risks of chronic obstructive pulmonary disease in later years. Early respiratory symptoms as asthma and increased bronchodilator use, continue to echo into adulthood. (125)

The kidney is also affected in these adults and there is a heightened susceptibility to chronic kidney disease. (125)

Cerebral palsy emerges as the most common long-term neurodevelopmental disability associated with preterm birth. (114) The impact on neurodevelopmental outcomes is profound, with the association extending into adulthood, manifesting in various disorders. (119) Epilepsy, observed either concurrently with cerebral palsy or in isolation, is more prevalent among survivors of preterm birth. (114)

Psychiatric disorders, including depression, anxiety, bipolar disorder and psychosis, also exhibit an increased incidence in adults born prematurely. (125)

Despite these challenges, the vulnerability of this population often goes unrecognized. A comprehensive approach involving screening and management guidelines in primary care settings is imperative to address the nuanced health complications stemming from prematurity. (126)

2.5.4 SOCIO-ECONOMIC BURDEN

The socio-economic burden of preterm birth far beyond immediate healthcare costs, intricately weaving a complex tapestry of financial challenges for families and society at large. In the United States alone, the burden amounted to a staggering \$25.2 billion (\$64,815 per preterm birth) in 2016. (127)

Medical care costs constitute a substantial portion of societal expenses and they represent two-third of all societal costs of prematurity. NICU expenses, medications, respiratory support and treatments for acute complications contribute significantly to this financial strain, emphasizing the critical role of healthcare expenditure in addressing preterm birth challenges. (127)

The economic landscape is further shaped by disability-specific lifetime medical expenses, special education, rehabilitation treatments and lost productivity costs linked to developmental disabilities associated with preterm birth. Families navigating the intricate intersection of emotional and economic challenges find that caring for a child with lasting health issues brings not only financial strains but also profound psychological hardship, necessitating medical treatments, therapies and special education. (15, 52, 127)

Preterm birth's repercussions extend also into the workforce, hindering individuals' full participation and resulting in lost productivity and economic contributions. (127)

Hospitals and healthcare providers must allocate resources strategically to meet the specialized needs of preterm infants, further influencing the socio-economic landscape. (127)

The impact of preterm birth also reaches into the educational sphere, with an elevated risk of cognitive impairments and attention deficit and hyperactivity disorders. This heightened risk translates into a greater demand for special education services, as survivors of preterm birth are 1.3 to 2.8 times more likely to require these provisions. This aspect adds an extra layer to the socio-economic considerations associated with preterm birth. (114)

2.5.5 INTERCONNECTED GENERATIONAL CYCLE

PTB initiates an interconnected generational cycle, where those born prematurely, especially females, face increased risks of delivering preterm or low birthweight infants. Breaking this cycle necessitates comprehensive interventions targeting the root causes and mitigating the generational impact of preterm birth. (128)

2.6 LABOUR MECHANISM

Labour, or childbirth, is a complex process involving a series of precise physiological changes that culminate in the delivery of a newborn. Studying the human labour process is challenging due to limited sample availability. Consequently, much of our understanding derives from animal studies. However, because of anatomical, gravitational and hormonal differences between animal and humans, these findings must be interpreted with caution.

2.6.1 NORMAL LABOUR MECHANISM

A) TRIGGER OF LABOUR

The onset of normal labour is orchestrated by a complex interplay of hormonal, genetic and inflammatory factors. Since 1995, it has been widely accepted that labour results from a multifaceted interaction of various stimuli, rather than a single-stimulus theory. (129, 130) Studies have shown that rodent models with genetic mutations affecting molecules involved in parturition can still deliver at term, suggesting the presence of redundant pathways that ensure labour onset. (131, 132)

Both maternal and fetal factors play active roles in initiating labour, whether at term or preterm, although their precise contributions are still under investigation and debate. (129, 130)

I. HORMONAL REGULATION:

Hormones play a pivotal role in labour, with progesterone withdrawal being a key mechanism. The hormonal shift from progesterone to oestrogen is thought to be crucial in initiating the preparatory phase of labour. (129, 133, 134)

○ Progesterone withdrawal:

Progesterone is essential for a healthy pregnancy, playing key roles in maintaining uterine quiescence, preserving cervical integrity and modulating the immune response at the maternal-fetal interface. (135) As the most extensively studied immunomodulator in this context, progesterone inhibits lymphocyte proliferation, prolongs allograft survival,

modulates antibody production and reduces proinflammatory cytokine production by macrophages. (136)

Given its critical role in sustaining pregnancy, progesterone withdrawal has been proposed as a trigger for the initiation of labour. In rodent and ruminant pregnancy models, a reduction in peripheral progesterone levels, signalling a pivotal transition in the reproductive process and precedes the onset of labour. (62, 137)

Supporting this concept, experiments using progesterone receptor blockers like RU486 have provided valuable insights into the mechanisms of labour induction in animals. By inhibiting progesterone receptors (PR), these interventions mimic the conditions associated with progesterone withdrawal, underscoring the hormone's essential role in maintaining uterine quiescence during gestation and the subsequent inflammatory cascade triggered by its decline. (137, 138)

In humans, there is no significant decrease in peripartum progesterone levels. However, labour can be induced with progesterone antagonists and its prevention is possible with progesterone supplementation. This has led to the concept of "functional progesterone withdrawal," where labour is initiated despite sustained or even elevated circulating progesterone levels. (11, 37, 136, 139, 140) This functional decline is thought to involve several contributing factors.

- Change in receptor isoform: The myometrium exhibits two primary isoforms of PRs, PR-A and PR-B. PR-B is the dominant isoform, mediating the key effects of progesterone by inhibiting the production of critical components like oxytocin receptors, prostaglandins (PG) and gap junction proteins. In contrast, PR-A primarily acts as a suppressor of PR-B's activity. As parturition approaches, a functional progesterone withdrawal occurs, characterized by an increase in the myometrial PR-A/PR-B ratio, leading to a reduced overall effectiveness of progesterone. (10, 130, 139, 141)
- Inflammatory activation: Inflammatory signals also play a role in the functional decline of progesterone. As labour approaches, there is an upregulation of inflammatory pathways, leading to increased production of proinflammatory cytokines. This inflammatory milieu contributes to the altered responsiveness of gestational tissues to progesterone. (130)

- **Metabolic inactivation:** Progesterone levels decline in the nucleus of labouring myometrial cells due to metabolic inactivation by enzymes, like 20 α -hydroxysteroid dehydrogenase. This process, responsive to inflammatory signals, may be responsible for progesterone modulation in inflammation-driven preterm deliveries. (130)

- **Genetic and epigenetic changes:** The altered expression of genes related to PR, along with epigenetic modifications such as changes in histone deacetylase methylation, contribute to the modified responsiveness of gestational tissues to progesterone. (130) For example, as term approaches, the increased expression of miR-200 family members in the myometrium antagonizes progesterone's effects, promoting its breakdown and the upregulation of proinflammatory mediators. (62, 135)

○ **Enhance oestrogen responsiveness:**

Oestrogen plays a vital role in preparing the uterus for labour by enhancing uterine contractility and excitability, thereby increasing the sensitivity of myometrial cells to uterotonic agents like oxytocin. Additionally, oestrogen stimulates the secretion of PG from the decidual cells of fetal membranes, which are crucial for progressing labour. (142, 143)

Evidence suggests that the oestrogen-to-progesterone ratio at term, rather than the absolute levels of these hormones, is the key factor driving "functional oestrogen activation" and "functional progesterone withdrawal," processes essential for initiating labour. (11, 37) "Functional oestrogen activation" is characterized by increased expression of oestrogen receptors (ER), particularly through the upregulation of ER α in the cervix during the later stages of pregnancy. This receptor enhancement continues up to the onset of labour and then declines in the postpartum phase. Elevated ER α levels are also linked to spontaneous preterm labour and delivery, underscoring the pivotal role of oestrogen in orchestrating these critical reproductive events. (144, 145)

Furthermore, the interplay between steroid hormones plays a crucial role in managing cellular processes like the epithelial-mesenchymal transition (EMT) and inflammation

important in cervical remodelling (CR). (144) (See chapter 2.7.2 Function of the cervix - b) cervical ripening)

- **Oxytocin:**

Contrary to common misconception, oxytocin does not act as the primary initiator of labour; instead, it plays a crucial role as an enhancer once the process has already commenced. Indeed, oxytocin concentrations do not rise before the onset of labour and its clearance remains constant. This characteristic suggests that oxytocin is not the trigger but rather a crucial participant in the later stages of labour. (11, 37)

II. PLACENTAL CLOCK

The placenta is a critical organ in pregnancy, acting as a vital connection between the mother and foetus. It not only facilitates the essential exchange of nutrients, oxygen and waste products, but also functions as an endocrine organ, producing a range of hormones and cytokines crucial for labour. (139, 146)

Among these placental products, Corticotrophin-Releasing Hormone (CRH) is particularly notable for its role as a temporal regulator of labour. CRH orchestrates neuroendocrine and inflammatory responses, influencing both the duration of pregnancy and the timing of labour. Maternal plasma CRH levels rise progressively throughout pregnancy, peaking at delivery. CRH engages in a feedback loop with proinflammatory cytokines, intensifying the inflammatory response that facilitates labour initiation. This phenomenon is referred to as the 'placental clock,' highlighting CRH's critical role in determining when labour begins. (62, 139, 146)

In response to both physiological and pathological stress, the placenta activates stress-related pathways that affect the levels of key hormones such as ACTH, cortisol and PG. These hormones play a significant role in triggering labour by preparing the uterus for contractions. (139)

Additionally, oxidative stress and cellular senescence in the fetal amnion and chorion can trigger labour. In senescent cells, damage-associated molecular patterns (DAMPs) migrate from the nucleus to the cytosol, acting as alarmins that initiate an inflammatory cascade and cellular damage leading to labour onset. (130, 146-149)

This intricate network of interactions underscores the placenta's central role in timing labour and highlights the complex mechanisms involved in both normal and preterm labour. (146)

III. FETAL CONTRIBUTION

Fetal contributions to the initiation of labour at term encompass various key mechanisms, including the maturation of the fetal HPA axis, production of placental steroid hormones and mechanical distention of the uterus and membranes. (10)

Research in sheep has clearly established the vital role of the fetal HPA axis in initiating labour. Studies involving the ablation of the fetal hypophysis or adrenal glands have shown that such interventions prevent the onset of labour, underscoring the HPA axis's critical role in labour initiation. This evidence is likely relevant to humans as well, as prolonged pregnancies have been observed in cases involving anencephalic foetuses, which lack a functional fetal HPA axis. (37, 150)

The foetus signals the onset of labour primarily through the secretion of hormones such as cortisol, oestrogen, placental CRH and surfactant protein A, all of which influence the timing of labour. As the fetal HPA axis matures, increased production of corticotrophin leads to the release of cortisol from the fetal adrenal glands. Cortisol facilitates the maturation of fetal tissues, including the production of surfactant and phospholipids critical for respiratory development. Elevated levels of surfactant protein A in the amniotic fluid play a key role in activating macrophages and enhancing inflammatory processes within the fetal membranes, cervix and myometrium. This connection between fetal maturity and labour onset underscores the importance of the foetus's developmental readiness in initiating parturition. (37, 74)

Furthermore, fetal cortisol stimulates placental 17 α -hydroxylase activity, resulting in decreased progesterone secretion and increased oestrogen production. This shift in the oestrogen-to-progesterone ratio promotes the formation of PG, initiating a cascade of events that lead to labour. Additionally, the fetal adrenal production of dehydroepiandrosterone (DHEA), which is converted to oestrogens by the placenta, further influences the hormonal environment and impacts myometrial activation. (10, 11, 37, 74, 139)

Recent research has identified cell-free Deoxyribonucleic Acid (cfDNA) as a potential marker for labour onset. Derived from placental cell degradation, fetal cfDNA acts as a DAMP that

can trigger labour through interactions with Toll-like receptor 9. This interaction stimulates an inflammatory responses, potentially initiating the labour process. cfDNA levels, typically present in maternal plasma and increasing throughout gestation, peak just before labour begins. Elevated cfDNA levels in mid-trimester samples have been associated with a higher risk of spontaneous preterm delivery, suggesting its role as a potential common trigger for labour. (62, 135, 146, 147)

Additionally, oxidative stress and cell senescence in fetal tissues prompt the release of sterile inflammatory mediators, such as damage-associated molecular patterns (DAMPs), contributing to the inflammatory upregulation that determines the timing of birth. (133, 146)

IV. LOSS OF IMMUNE TOLERANCE

The foetus is the most successful semi-allograft and the progression of pregnancy hinges upon the delicate balance of maternal immune tolerance towards the foetus. Failures in this tolerance can lead to adverse outcomes. (151)

Throughout pregnancy, the maternal immune system engages in a complex interplay with inflammation, starting from implantation and continuing through to labour. Initially, the immune system responds to fetal or paternal antigens by initiating inflammatory processes crucial for successful implantation. Subsequently, an immunomodulatory environment is established, characterized by anti-inflammatory cytokines, regulatory T cells (Treg) and placental cells that release immunomodulatory cytokines. Progesterone, a critical immunomodulator, supports fetal allograft survival by balancing immune activation and defending against microbial infections during pregnancy. (151-153)

Several mechanisms work together to create a tolerogenic environment during pregnancy. Key players include anti-inflammatory molecules like transforming growth factor (TGF) and specialized Treg cells, particularly CD4+FOXP3+ T cells, which suppress anti-fetal immune responses. Tolerogenic DCs in the decidua present fetal antigens to maternal CD4+ T cells, promoting Treg cell generation. This interaction between Treg cells and DCs/macrophages induces a tolerogenic phenotype in both cell types. Treg cells suppress effector T cell responses and maintain anergy in conventional T cells, preventing anti-fetal reactions. Placental cells further support this environment by upregulating Treg cells and limiting the

activation of inflammatory effector T cells, promoting immune tolerance within the placental microenvironment. (146, 152)

Allograft rejection, driven by both innate and adaptive immune responses, is associated with adverse obstetric outcomes. Key alloantigens, particularly those within the human leukocyte antigen (HLA) system play a crucial role in transplant rejection and recent research suggests that immune activation and molecular signatures indicative of graft rejection often occur before the onset of labour. (153)

Additionally, fetal maternal cells and maternal-fetal cells (MMCs) have been investigated for their roles in labour. MMCs, which cross the placenta to the foetus, contribute to tolerance of maternal antigens in fetal immune cells and may influence the timing of birth. (152) Furthermore, microvesicles, exosomes and cell-free proteins and nucleic acids, such as cfDNA, increase in maternal circulation during the pregnancy, peaking at term and has been implied in labour starting. While the exact role of these molecules throughout pregnancy remains unclear, it is believed that they facilitate communication between the foetus and the mother during critical events such as labour. (146, 152)

While the exact role of these molecules throughout pregnancy remains unclear, they are believed to facilitate communication between the foetus and the mother during critical events such triggering labour.

V. GENETIC FACTORS

Numerous studies underscore the genetic aspect of delivery timing, as investigations into twins and familial patterns have unveiled a hereditary impact on gestational duration. Genetic research places significant emphasis on the hereditary factors influencing gestational duration, pinpointing specific single nucleotide polymorphisms (SNPs) linked to spontaneous preterm labour. (154, 155) Polymorphisms identified in cytokine genes, Toll-like receptors (TLRs) and other immune-related genes play a pivotal role in shaping individual predispositions to the timing and progression of labour. (76-79, 156, 157)

VI. ENVIRONMENTAL FACTORS

Environmental factors play a pivotal role in influencing the timing of labour initiation. Various external elements and conditions contribute to this process, including stressful life events, socioeconomic status, infections, obesity and exposure to toxins. (10, 139)

For example, mechanical distension and uterine stretching, resulting from fetal growth, excess amniotic fluid, or multifetal pregnancy, is a recognized trigger for labour. The stretching of human myometrium leads to the release of PG, expression of gap junction protein and increased oxytocin receptor expression. These changes promote uterine activity and the degradation of the extracellular matrix (ECM), facilitating cervical ripening and rupture of the fetal membranes. This pro-inflammatory stretch response establishes a connection between the onset of labour and the finite capacity of the uterus. (10, 130, 139, 158, 159)

Considering the intricate nature of the labour process, it becomes clear that various other factors may play a role in its initiation. (135)

B) THE COMMON PATHWAY OF LABOUR:

The initiation of labour at term is a multifaceted and not yet fully understood phenomenon, but it is recognized as an inflammatory event affecting various gestational tissues. (10, 147)

The process of parturition involves three key stages: activation of the amnion and chorion, ripening of the cervix and activation of the myometrium. These stages can occur in different sequences and combinations, with changes in gestational tissues often preceding observable symptoms in patients by several weeks. (10, 139)

I. UTERINE TRANSITION

The uterine transition refers to the complex process of modifying the uterus from a quiescent to an active phase. The myometrium's activation involves four physiological phases. Initially, during **Phase 0**, the uterus is maintained in a state of active quiescence by inhibitors such as progesterone, prostacyclin and relaxin. As pregnancy nears term, **Phase I** begins, characterized by an increase in the expression of contraction-associated proteins (CAPs) in response to uterotonins like oestrogen, signalling the preparation for labour. (10, 133, 147)

The onset of labour marks **Phase II**, where contractions transform from irregular to regular, coordinated and powerful, driven by PGs and oxytocin. This phase involves a crucial shift from anti-inflammatory to proinflammatory signalling pathways. Uterine activation proteins, such as cyclooxygenase (COX) 2, are regulated by proinflammatory mediators and steroid hormones, facilitating the transition to active labour. Finally, **Phase III** occurs post-delivery and involves uterine involution. This phase is primarily mediated by oxytocin, which aids in returning the uterus to its pre-pregnancy state. (10, 133, 147)

II. ACTIVATION OF THE CHORION/AMNION

The activation of the amnion and chorion during parturition plays a crucial role in the inflammatory processes that drive labour. The amniotic membrane, for instance, influences the composition of the amniotic fluid and is significantly involved in this inflammatory response through the production of COX-2 and Prostaglandin E2 (PGE2). Elevated levels of CRH and cortisol further stimulate COX-2 and PGE2 production in the amnion, exacerbating inflammation as pregnancy progresses towards term. Conversely, the chorion initially acts as an inhibitor of parturition by producing Prostaglandin Dehydrogenase (PGDH), which suppresses PGE2 activity and mitigates inflammation. However, with the approach of labour, chorionic activation leads to a reduction in PGDH production, a change regulated by glucocorticoids and progesterone. This reduction in PGDH increases inflammation, weakens the membranes and can contribute to their rupture. (10)

Both the amnion and chorion play crucial roles in labour by producing PGs and cytokines, which elevate inflammation and drive the progression of labour.

III. CERVICAL REMODELLING

Structural changes in the cervix are crucial for the passage of the foetus. Cervical activation involves continuous remodelling throughout pregnancy, including softening, ripening, dilation and repair. (See chapter 2.7 The role of the cervix in labour)

C) THE ROLE OF INFLAMMATION IN THE COMMON PATHWAY OF LABOUR

In reproductive physiology, inflammation plays a crucial role throughout various stages, including ovulation, implantation, decidualization, placentation and parturition.

Inflammation is a complex tissue response to harmful stimuli, characterized by plasma protein exudation, leukocyte recruitment and the activation of inflammatory mediators. During childbirth, this traditional definition of inflammation—marked by pain, heat, redness, swelling and loss of function—evolves into a process known as "inflammatory activation." This term refers to the initiation and coordination of inflammatory cells, pro-inflammatory mediators and signalling pathways essential for the intricate processes of childbirth. Inflammatory activation is key to the mechanism of parturition and occurs in various tissues, including the myometrium, fetal membranes and cervix. (75, 130)

The inflammatory process begins weeks before the onset of labour and becomes evident with physiological changes leading up to delivery. Trigger signals recruit and activate inflammatory cells, which release proteases, chemokines, cytokines and uterotonins like oxytocin and PGs. Granulocytes, monocytes and lymphocytes dynamically respond to chemoattractant released by fetal membranes, playing a crucial role in the inflammatory response. This cascade of inflammation results in the local expression of additional mediators, including chemokines, cytokines, proteases and prostaglandin synthases, initiating structural changes, apoptosis and alterations in cellular contents. The infiltration of these leukocytes into the uterus, including the myometrium and cervix, amplifies the inflammatory cascade through heightened secretion of pro-inflammatory mediators. This positive feedback loop of leukocyte activation triggers the release of further cytokines, transforming inflammatory activation into a self-sustaining process. Additionally, decidual Natural Killer (NK) cells, maternal macrophages and fetal antigen-specific cells are significant contributors to labour. (75, 130, 147, 160)

The transition from a quiescent to a proinflammatory environment marks the initiation of labour, involving a three-step process characterized by uterine contractility, cervical ripening and membrane rupture. (75, 130)

I. MYOMETRIUM

In the myometrium, there is a critical shift in signalling pathways, including cytokines, prostaglandin receptors and CAPs like oxytocin. The expression of CAPs increases before term labour (TL) as they are released from progesterone-mediated transcriptional repression. Elevated levels of cytokines and ligands for pro-contractile receptors facilitate the

transformation of the uterus from a quiescent state to an active, contractile muscle, promoting regular contractions. (130)

II. CERVIX

The traditional inflammatory model of labour posits that immune cell infiltration during cervical ripening leads to ECM degradation and remodelling. However, recent studies suggest that inflammation contributes more to active dilation and postpartum tissue repair rather than initiating cervical ripening itself. (141, 161-164) (See chapter 2.7 The role of the cervix in labour)

III. AMNIO

In the amnion, increased expression of inflammatory cytokines and heightened protease activity are crucial for processes such as ECM dissolution and apoptosis. These events contribute to the separation of chorioamniotic membranes from the decidua, leading to structural weaknesses that reduce the tensile strength of the membranes and increase their susceptibility to rupture. (135, 136, 139)

IV. INFLAMMATORY MOLECULES

Cytokines, small signalling proteins secreted by immune cells, emerge as crucial architects in labour. The intricate balance between pro-inflammatory and anti-inflammatory cytokines creates a finely tuned microenvironment essential for labour. Key cytokines such as Interleukin (IL)-6, Tumour Necrosis Factor-alpha (TNF- α) and IL-1 β act as critical regulators, influencing cellular processes, promoting uterine contractions and facilitating CR. (146, 152, 159, 165)

The onset of labour is marked by a surge in the expression of IL genes, driven by the infiltration of leukocytes into the uterus. Pro-inflammatory cytokines, notably IL-1 β and TNF- α , play a direct role in stimulating myometrial contractions by enhancing calcium influx into smooth muscle cells. These cytokines also promote the production of other inflammatory mediators, including matrix metalloproteinases (MMPs), which contribute to ECM degradation, CR and membrane rupture. (75, 135, 139)

V. RECEPTOR-MEDIATED SIGNALLING AND IMMUNOLOGICAL INTERFACES

Inflammatory responses during labour are significantly influenced by TLRs, which are specialized pattern recognition receptors. TLRs detect pathogen-associated molecular patterns (PAMPs) from microorganisms and DAMPs, also known as alarmins, which are released from damaged or stressed cells. These receptors are critical for initiating and amplifying inflammation during labour. (130, 146-149) TLRs are found in both immune and non-immune cells and their expression increases as labour approaches and their activation starts a chain reaction in the inflammatory process, leading to both normal and preterm labour. (146) Activation of TLR4 in the uterus is crucial for timely parturition, controlling uterine activation and labour onset. Studies with mice lacking TLR4 have shown delayed delivery viability, emphasizing its significance in labour initiation. Interestingly, TLR4 expression in decidual endothelial cells, rather than immune cells, is crucial for initiating the inflammatory response. Maternal genetic variations in TLR4, such as the Asp299Gly polymorphism, are linked to an increased risk of early preterm delivery, highlighting the clinical importance of TLR-mediated inflammation in PTB. (146, 166)

When alarmins interact with TLRs on antigen-presenting cells (APCs) like macrophages and DCs, it sets off a series of inflammatory events. These APCs then stimulate other immune cells, such as T cells, neutrophils and NK cells, which release more pro-inflammatory cytokines and chemokines. This sequence of events is crucial for initiating labour and can contribute to various pregnancy complications. (75, 147, 148, 160)

Furthermore, activated APCs can stimulate invariant NK T cells, leading them to produce inflammatory cytokines and cytotoxic substances. This intensifies inflammation at the fetomaternal interface. The activity of iNKT cells can also cause tissue damage, which releases additional DAMPs, further fuelling the inflammatory response in a continuous cycle. (148, 167)

2.6.2 SPONTANEOUS PRETERM LABOUR: NOT SIMPLY AN ACCELERATED VERSION OF TERM LABOUR

Both term and preterm birth involve overlapping yet distinct processes. While term birth and sPTB share a common physiological pathway described before, the triggers initiating these processes differ. It's important to recognize that preterm birth is not simply an early onset of

labour but involves complex and multifaceted mechanisms that go beyond just the timing of delivery. Understanding these differences is essential for comprehending the full spectrum of parturition and addressing the challenges associated with preterm birth. (10, 37, 139, 168)

The 'common pathway' outlined earlier comprises three essential elements within the uterine environment: (i) stimulation of myometrial contractility, (ii) cervical ripening characterized by dilation and effacement and (iii) activation of the decidual/membrane interface. While TL is typically initiated by natural physiological processes, spontaneous preterm labour (PTL) often involves abnormal activation of one or more components of the common pathway, wherein the normal cascade of events is prematurely stimulated or disrupted. (37, 135, 139)

The activation of uterine components within the common pathway can occur synchronously or asynchronously. Synchronous activation typically presents as clinical PTL, while asynchronous activation gives rise to different clinical manifestations, referred to as phenotypes. For example, predominant activation of the membranes may precipitate PPROM, activation of the cervix may lead to cervical insufficiency and activation of the myometrium may provoke heightened preterm uterine contractions. The activation of each component carries distinct risks for impending preterm delivery. (168)

What triggers PTB? Premature labour is a multifaceted phenomenon without a single initiating factor. Instead, it arises from a confluence of multiple biological mechanisms and is influenced by various contributing factors. (10, 149) As described by Romero, PTB can be understood as described by Romero, it can be viewed as "one syndrome, many causes." (135) These factors include infection, inflammation, haemorrhage, cervical pathology, mechanical conditions, progesterone deficiency, maternal and fetal biological or psychosocial stress, allograft reactions, allergic phenomena and potentially other unidentified contributors. (10, 37, 52, 62, 74, 135)

Our understanding of the intricate etiopathogenic processes underlying PTB remains incomplete. Researchers are increasingly using mouse models to explore these complexities. These models simulate clinical scenarios associated with infection or inflammation using various agents, including immune stimulants and hormones. Additionally, genetically modified mouse strains have provided valuable insights into proteins and signalling pathways involved in both normal and preterm labour. However, findings from these models must be interpreted with caution due to their inherent limitations. (146, 149)

Below is a detailed overview of various potential triggers of preterm birth, each illustrating the intricate and multifaceted nature of prematurity.

A) INFLAMMATION

In various pathologies like cancer, autoimmune diseases and metabolic disorders, aberrant, excessive, or suppressed inflammation is often observed. Excessive maternal inflammatory responses devoid of microbial involvement have been associated with negative obstetric outcomes like Preeclampsia. In the context of PTB, inflammation plays a pivotal role, underpinning a complex cascade of events that can lead to early labour and delivery. (146, 148)

The balance between pro-inflammatory and anti-inflammatory mediators is vital in regulating placental function, uterine contractions, membrane rupture and cervical dilation—all of which are hallmarks of PTB. (62, 152) Inflammation is central to PTB for several reasons. Firstly, infections are significant contributors to premature birth. Secondly, maternal conditions associated with systemic inflammation, such as autoimmune and inflammatory diseases, are linked to an increased risk of PTB. Finally, genes involved in inflammatory responses are closely associated with intra-amniotic inflammation and PTB, highlighting inflammation as a fundamental factor regardless of its specific triggers. (11, 62, 75, 139) Gilman-Sachs and colleagues have demonstrated the role of inflammation in PTB, with intra-amniotic inflammation found in 30-50% of women experiencing PTB. (169)

Inflammatory activation is observed in gestational tissues during labour, regardless of gestational age. The differences between term and preterm labour may stem from an early disruption in inflammatory signalling. Such disruptions can trigger the common inflammatory pathway prematurely, leading to preterm birth. (130) PTB is marked by an increase in proinflammatory mediators such as IL-1, IL-6, IL-8, TNF- α and Interferon- γ , coupled with a decrease in anti-inflammatory mediators such as IL-10 and IL-4. (147, 152) Additionally, PTB is associated with heightened choriodecidual inflammation, characterized by the presence of M1 macrophages and NK cells. These findings underscore the central role of inflammation in PTB, regardless of its initiating factors. (147)

The discovery of TLRs has been instrumental in advancing our understanding of the immune response to pathogens and they have been implicated in pregnancy complications such as

PTB and PPRM (136) TLRs, acting as sentinels, initiate critical steps in the inflammatory cascade leading to labour. Research has largely focused on TLR4, with genetic variations such as the Asp299Gly polymorphism linked to an increased risk of early preterm delivery. (146, 166) However, other TLRs also play roles in modulating the timing of birth. (136, 146)

The confirmation of inflammation in PTB can be achieved through the detection of either intra-amniotic inflammation or postpartum chorioamnionitis.

- **Intra-amniotic inflammation** manifests in two distinct clinical phenotypes: intra-amniotic infection, characterized by the presence of microorganisms in the amniotic fluid and sterile intra-amniotic inflammation, occurring in the absence of microorganisms. (149) The concept of triple "I" - intrauterine inflammation, infection, or both - has been proposed to encompass scenarios where inflammation plays a role, even without clear evidence of infection. Romero et al. study on sterile intra-amniotic inflammation in PTB revealed sterile intra-amniotic inflammation and intra-amniotic infection in 26% and 11% of women, respectively. (170) Subsequent studies have corroborated these findings. (149, 171) Both microbial-associated and sterile intra-amniotic inflammations trigger similar cytokine mediators, with IL-6 and IL-1 playing pivotal roles in uterine mediation during labour transition. (147, 152)
- **Chorioamnionitis**, which involves inflammation of the fetal membranes (chorion and amnion) and placenta, is a key factor in PTL. It is now classified into two types: acute and chronic chorioamnionitis (cCAM).

Acute chorioamnionitis (aCAM) is marked by neutrophil infiltration in the amnion and chorionic villi, commonly detected in postpartum placentas. It affects 20–60% of preterm births, especially in very preterm cases and is typically linked to ascending or haematogenous bacterial infections. However, recent studies indicate that excessive cytokine levels alone can trigger aCAM, even without clear microbial infection, which may explain the inconsistent efficacy of antibacterial treatments for preterm birth. (148, 167) This emphasizes the influence of non-microbial factors, shedding light on why the use of antibacterials for PB treatment may not consistently lead to positive outcomes. (167)

In contrast, chronic cCAM features lymphocyte, plasma cell and monocyte infiltration, leading to prolonged inflammation and is more commonly observed in preterm births occurring later in gestation. While aCAM is typically associated with infections, cCAM can

arise from non-infectious and immunological causes that resemble maternal-fetal allograft rejection. (130, 148, 167)

I. MICROBIAL-INDUCED INFLAMMATION

The link between infection and PTB is well-documented, with infections during late pregnancy often triggering PTB. (130, 160) From an evolutionary standpoint, PTL triggered by infection may serve as a mechanism to eliminate infected tissue, safeguarding maternal reproductive health. This adaptive response, while protective, results in the compromise of delivering prematurely. Interestingly, inflammation near term may offer benefits, enhancing fetal defence against infections and promoting lung maturation, which is crucial for newborn survival. (135)

Despite intrauterine infection being a leading cause of PTB, its true prevalence remains uncertain. (169) Current microbiological studies estimate that intrauterine infections account for 25–40% of preterm births, though this might be an underestimate due to difficulties in detection. (11, 136, 139) Variations in laboratory techniques impact prevalence estimates, with molecular methods often identifying higher rates of infection compared to standard cultures. (130, 160, 172)

Elevated inflammatory markers in amniotic fluid often indicate chorioamnionitis, but confirmation usually occurs post-delivery through histological analysis. The incidence of infection-related preterm birth is inversely related to gestational age, with microbial invasion found in up to 70% of very early preterm births, decreasing to 11% at later stages. (130, 160, 173, 174)

- **Microorganisms** can enter the amniotic cavity through several pathways: ascending from the lower genital tract, haematogenous spread via the placenta, accidental introduction during medical procedures, or retrograde movement through the fallopian tubes. Ascending infection from the vagina and cervix is the most common cause. (11, 135, 175, 176) pathogens frequently associated with PTB include *Ureaplasma Urealyticum*, *Mycoplasma Hominis*, *Streptococcus Agalactiae*, *Escherichia Coli*, *Fusobacterium*, *Trichomonas Vaginalis* and *Gardnerella Vaginalis*. (107, 130, 136, 167) For instance, *Chlamydia Trachomatis* at 24 weeks' gestation doubles the risk of PTB, but clinical trials

have not consistently shown reduced PTB rates with its treatment. (177) BV also increases the risk of PTB, particularly when diagnosed before 16 weeks' gestation. (108)

Infections from distant sites can also induce PTB via haematogenous spread of bacteria or cytokines. For example, severe periodontitis, untreated pyelonephritis, malaria, pneumonia and other extrauterine infections have been linked to increased PTB risk. (60, 109, 135, 160, 178, 179)

Although less frequently than bacteria, various microorganisms, including virus and fungi can trigger a premature labour. (130, 180, 181)

- **Mechanism** by which infection leads to PTB has been elucidated through animal studies involving Lipopolysaccharide (LPS) inoculation, a molecule found in the outer membrane of Gram-negative bacteria like E. COLI. (143, 182) At the maternal-fetal interface, TLRs detect microbial invaders: TLR4 recognizes lipopolysaccharides from Gram-negative bacteria, while TLR2, TLR3 and TLR9 also play roles in initiating labour. Activation of these TLRs triggers the release of inflammatory molecules, including chemokines, cytokines, PGs and proteases, which drive the inflammatory cascade leading to labour. The variability in maternal immune responses accounts for why some women develop ascending intra-amniotic infections leading to PTB, whereas others remain unaffected. (10, 11, 62, 135, 139, 148, 160, 167) The variability in maternal immune responses accounts for why some women develop ascending intra-amniotic infections leading to PTB, whereas others remain unaffected. (130, 135)

II. STERILE INFLAMMATION

Sterile inflammation, characterized by intrauterine or intra-amniotic inflammation in the absence of apparent detectable microbial triggers, has emerged as a significant contributor to various diseases, including cardiovascular diseases, diabetes mellitus, lung disorders and cancers. (167) This form of inflammation has also been implicated in various pregnancy-related complications, such as infertility, recurrent pregnancy loss, premature delivery and preeclampsia. Notably, studies have shown that a considerable proportion of patients with PTL exhibit microbe-negative amniotic fluid, indicating that sterile inflammation is a more common cause of PTB than previously thought. (130, 148) However, it is crucial to consider that culture tests may yield false negatives and that bacteria localized to the fetal membranes,

without entering the amniotic fluid, might be misclassified as sterile inflammation due to the lack of apparent microbial invasion. (130)

- **Mechanism** - the pathogenesis of sterile inflammation is not yet fully understood. Potential triggers include both endogenous factors, such as high-mobility group box-1 (HMGB1), IL-33 and IL-1 α and non-pathogenic exogenous agents, like asbestos, silica particles, nanoparticles and smoking. These molecules are categorized as DAMPs, also known as alarmins. (130, 167) The exact mechanisms that initiate the release of these alarmins are not completely clarified, but placental dysfunction, hypoxia, trauma and maternal metabolic abnormalities are believed to play contributing roles. (148, 167) DAMPs implicated in PTB include HMGB1, fetal cfDNA, platelet-activating factor, IL-1 α , IL-33, heat shock proteins and S100 proteins. (130, 148, 167) For instance, HMGB1, released from stressed cells, activates immune responses via receptors such as TLR2 and TLR4, which can drive intrauterine inflammation and the activation of inflammatory pathways. Its presence in the amniotic fluid has also been associated with the onset of labour and elevated HMGB1 levels have been linked to early delivery, suggesting its potential role as a preterm birth trigger. (146, 148, 167) Similarly, cfDNA resulting from placental cell death can enter maternal circulation and act as an alarmin by binding to TLR9, triggering NF- κ B-dependent inflammatory responses. Elevated levels of cfDNA are associated with a higher risk of spontaneous preterm delivery, indicating its role in mediating communication between fetal, placental and maternal compartments, which can signal the onset of labour in both normal and preterm deliveries. (62, 135, 146, 183)

B) DECIDUAL DISORDERS

Among the spectrum of placental pathologies implicated in sPTB, acute inflammation, notably aCAM, stands out as the most prevalent and extensively documented and has been described above. Following this, vascular lesions emerge as the second most prevalent pathological feature, with decidual haemorrhage also identified as a pertinent factor associated with PTB. (139, 147) Moreover, there is a growing recognition of disorders related to deep placentation as substantial contributors to PTB. (139)

I. VASCULAR DISEASE

Approximately 30% of patients experiencing PTB present with placental lesions indicative of maternal vascular underperfusion. Similarly, a comparable proportion demonstrates a failure in the physiologic transformation of the myometrial segment of the spiral arteries. (62, 135, 152)

These vascular lesions encompass both maternal and fetal components of PTB placentas. Maternal lesions may include failed physiological transformation of myometrial spiral arteries, atherosclerosis, spiral artery thrombosis, or a combination thereof, while fetal lesions involve reductions in the number of arterioles in the villi and fetal arterial thrombosis.

These lesions may contribute to sPTB by inducing uteroplacental ischemia, although the precise mechanisms remain uncertain. A proposed mechanisms linking uteroplacental ischemia to sPTB involve the activation of the renin–angiotensin system, which may lead to myometrial contractility directly or via the release of PGs. Furthermore, thrombin released following decidual necrosis and haemorrhage, initiated by severe uteroplacental ischemia, is suggested to activate the common pathway of parturition, ultimately culminating in sPTB. (139)

Placentas from women delivering preterm also, often exhibit a greater degree of failed transformation of spiral arteries compared to those delivering at term. (139) Notably, an abnormal maternal plasma antiangiogenic profile during the midtrimester has been observed in some individuals delivering preterm with placental vascular lesions. (135) Despite these observations, the specific reasons why some women with failed arterial transformation develop PTB or other obstetric complications remain elusive. (139)

II. DECIDUAL HAEMORRHAGE

Decidual haemorrhage is a significant factor in preterm birth, with persistent vaginal bleeding beyond the first trimester notably increasing the risk. This condition correlates with a seven-fold higher likelihood of premature rupture of membranes and preterm birth. The mechanism connecting decidual haemorrhage to preterm birth involves thrombin, which triggers the coagulation cascade. Thrombin binds to myometrial receptors, directly stimulating uterine contractions and potentially leading to PTL. (10) Additionally, thrombin contributes to the degradation of the ECM in the chorioamniotic membranes, heightening the risk of membrane

rupture. Evidence indicates that increased thrombin generation in mothers is associated with a higher risk of spontaneous PTL. (62)

Moreover, uterine bleeding may also result from vascular lesions in the placenta or inadequate transformation of spiral arteries. (135)

III. DECIDUAL SENESENCE

Premature decidual senescence is emerging as a key mechanism in sPTB, especially when acute inflammation is not present. In murine models, conditional deletion of uterine Trp53 leads to sPTB in 50% of cases, characterized by decidual senescence and elevated mTORC1 signalling, even without progesterone withdrawal. Interventions like rapamycin, an mTOR inhibitor and progesterone have shown potential in counteracting premature decidual senescence and preventing preterm birth. (135, 184, 185)

In human studies, decidual senescence near the basal plate of the placenta has been observed in cases of PTL, contrasting with its absence in term deliveries. Recent research also suggests that senescence in fetal membranes and the presence of cfDNA might act as triggers for sterile inflammation. (135, 147)

C) DISRUPTION OF MATERNAL-FETAL TOLERANCE

Maternal immune adaptation is essential for maintaining pregnancy, given the semiallogeneic nature of the foetus and placenta, which express both maternal and paternal antigens. Disruption of maternal-fetal tolerance is linked to adverse pregnancy outcomes such as recurrent pregnancy loss, fetal death, PTL and PPRM. (135, 146, 186)

Maternal sensitization to fetal HLA is commonly seen in patients with cCAM, a condition frequently associated with late sPTB. This sensitization is marked by increased maternal anti-HLA class I seropositivity compared to normal term births. Additionally, C4d immunoreactivity on umbilical vein endothelium, indicative of classical complement system activation, is a distinctive feature of PTL, serving as a marker of antibody-mediated rejection. (135, 151)

In turn, cCAM resembles maternal allograft rejection and fetal graft-versus-host disease, marked by maternal T-cell infiltration into the chorioamnion, leading to CD8+ T cell-mediated trophoblast apoptosis. (135, 151, 186) CXCR3 and their ligands CXCL9 and CXCL10 - involved in the chemotaxis of activated T cells, dendritic cells and NK play a key role in this process,

guiding the migration of immune cells and influencing angiogenesis. CXCR3 is expressed in the placenta and fetal membranes and upregulated in cases of premature delivery and maternal anti-fetal rejection. (151, 186) Higher levels of CXCR3, CXCL9 and CXCL10 in the amniotic fluid are linked to placental lesions from maternal rejection, though spontaneous labour at term does not significantly alter these chemokine levels. (186).

In the developing foetus, maternal antibody-mediated rejection, potentially facilitated by placental transfer of antibodies, can trigger a systemic fetal alloimmune response, similar to conditions like Rh incompatibility and alloimmune thrombocytopenia. This process is marked by a distinct form of fetal systemic inflammation with elevated T cell chemokines, as seen in cCAM. This reflects the complex interaction between maternal and fetal immune systems. Additionally, intact maternal antibodies persist in fetal tissues, potentially contributing to conditions like neonatal alloimmune thrombocytopenia. (135, 151)

The breakdown of maternal-fetal tolerance may also be relevant to PTL following fetal surgery or stem cell transplantation, which increase maternal T cell presence in fetal circulation. Additionally, maternal stress, infection, diet, or alterations in vaginal microbiota can disrupt immune tolerance and lead to excessive inflammation, complicating the pathophysiology of preterm birth. (146, 147)

Given the similarity between cCAM and maternal anti-fetal cellular rejection, it is hypothesized that maternal antibody-mediated rejection against the foetus may also contribute to PTB and PPROM.

D) FETAL FACTORS

As previously discussed, research in sheep highlights the crucial role of the fetal HPA axis in regulating delivery timing, a mechanism likely relevant to human parturition, as suggested by cases of prolonged pregnancies with anencephalic foetuses. (37, 150) Dysregulation or premature activation of these processes may contribute to the pathogenesis of PTB.

Premature activation of the fetal HPA axis, triggered by factors such as fetal infection and ischemia, is implicated in PTB. CRH, produced in the central nervous system, placenta, chorion, amnion and decidua, is thought to mediate stress-induced PTB by stimulating PGE2 production. (10) The activation of the fetal adrenal gland produces DHEA, which the placenta converts into oestrogens that enhance contraction-related proteins in the myometrium.

Additionally, fetal stress increases placental CRH production, further stimulating COX-2 and PGE2 activity. (10, 135)

Furthermore, the fetal immune system plays a role in PTB, with evidence suggesting that fetal immune cells, particularly macrophages, are crucial in initiating the inflammatory cascade leading to parturition. Comparative studies of cord blood from term and preterm infants have revealed differences in immune parameters, such as cytokine levels and T cell phenotypes, which may reflect alterations in immune development and contribute to the pathogenesis of PTB. (146)

E) ENVIRONMENTAL FACTORS

Numerous risk factors such as stress, cervical trauma, genital tract infections, ethnicity and toxic exposure may contribute to the multifaceted nature of PTB (See chapter 2.4 Risk factors of spontaneous preterm birth).

I. UTERINE OVERDISTENTION

Uterine distension plays a crucial role in the onset of PTL and delivery. As pregnancy progresses, the expanding uterus accommodates the growing foetus, creating increased tension on the myometrial walls. Similar to other smooth muscle tissues, the myometrium responds to this tension with enhanced contractions. Abnormal myometrial stretch, caused by conditions such as polyhydramnios or multiple gestations, triggers the expression of various proteins, including gap junction proteins, oxytocin receptors and PGs, in the amnion, myometrium and cervical cells. These changes stimulate uterine activity and contribute to ECM degradation, facilitating cervical ripening and membrane rupture. (10, 135, 139)

Studies in nonhuman primates have demonstrated that inflating intra-amniotic balloons can induce uterine contractility, leading to PTL by initiating an "inflammatory pulse." (135) Furthermore, research using human amniotic epithelial cell lines and fetal membrane explants to simulate fetal membrane distention in vitro has shown increased expression of proinflammatory genes, such as IL-8 and pre-B cell enhancing factor. (187)

II. MATERNAL STRESS

Beyond inflammation, stress plays a crucial role in triggering the premature onset of labour, with various stressors such as heavy workloads, anxiety and depression potentially affecting pregnancy outcomes. Maternal stress activates placental stress-related pathways, leading to increased secretion of CRH, which stimulates both maternal and fetal HPA axes. Elevated CRH levels are observed in plasma and amniotic fluid and there is increased placental messenger ribonucleic acid (mRNA) expression in women delivering preterm, especially in cases linked to vaginal infections or microbial invasion of the amniotic cavity. A complex interaction exists between CRH and proinflammatory cytokines, creating a feedback loop: placental CRH enhances IL-1 β secretion, while IL-1 β stimulates CRH production. This interplay is supported by studies showing correlations between stressful life events, poor social circumstances, psychological factors and environmental conditions with sPTB. (135, 139) These findings suggest that external stressors can modulate placental functions, contributing to the risk of preterm birth. (139)

III. GENETIC ROLE

As previously described, PTB is influenced by a combination of genetic predispositions and gene-environment interactions helps explain why not all pregnancies exposed to certain environmental factors result in PTB. For example, maternal smoking combined with specific genetic polymorphisms significantly increases the risk of sPTB. Similarly, certain genetic variations are linked to maternal BV and PTB. (10)

Gene polymorphisms affecting the maternal immune response during pregnancy are particularly noteworthy. Variants that lead to an inadequate immune response to infectious stimuli may increase the risk of sPTB. For instance, the TNF allele has been associated with an exaggerated inflammatory response to infections, heightening the risk of complications. (139) Conversely, polymorphisms associated with reduced immune responses, such as those leading to decreased IL-6 production, have been linked to a lower risk of sPTB and PPROM. (159)

A comprehensive study by Romero et al., which analysed 775 SNPs across 190 candidate genes, identified a significant connection between PTD and a specific SNP within the tissue inhibitor of metalloproteinase 2. This research also found distinct haplotypes associated with

maternal PTB in genes such as IL6 receptor, Collagen Type IV Alpha 3 Chain, Lactoferrin and Fibroblast Growth Factor 1 and with fetal PTB in genes including Insulin-like Growth Factor 2, IL2 and Collagen Type IV Alpha 1 Chain, suggesting both maternal and fetal genetic contributions to PTB risk. (188) In parallel, research focusing on maternal data revealed 210 differentially expressed genes in spontaneous PTB, characterized by the upregulation of genes and cell types tied to innate immunity and the downregulation of those associated with adaptive immunity. (147, 152) Moreover, a systematic review spotlighted 189 SNPs within 84 genes that have been linked to PTB, highlighting the complexity and breadth of genetic factors at play in this condition. (156) Genome-wide association studies have become essential for exploring the intricate genetic variants associated with multifactorial diseases like PTB. (157) Additionally, single-gene disorders such as myotonic dystrophy, Ehlers–Danlos syndrome and neurofibromatosis have also been linked to an increased risk of early delivery. (163)

2.7 THE ROLE OF THE CERVIX IN LABOUR

During pregnancy, the cervix plays a critical role as a mechanical barrier, requiring closure until term and undergoing significant remodelling to ensure successful delivery. Studies utilizing sequential assessments of cervical length have demonstrated that cervical ripening begins several weeks before the myometrial contractions of labour, underscoring that parturition in women is a prolonged process, with uterine contractions marking a relatively late stage. (141) Despite this, most research on PTB has focused on the myometrium and fetal membrane, leaving the cervix's pivotal role in delivery underexplored.

Progress in understanding cervical function has been hindered by the challenges of studying cervical tissue during pregnancy, a period often regarded as a "protected state." However, advancements in animal models, imaging technologies and molecular biology have provided valuable insights into CR processes and their impact on pregnancy outcomes.

A comprehensive review of the current understanding of normal cervical tissue remodelling is crucial for elucidating the cervix's role in both full-term and preterm delivery. This approach will uncover key knowledge gaps, guiding future research and enhancing pregnancy care.

2.7.1 STRUCTURE OF THE CERVIX

Cervical tissue architecture includes the endocervical canal, lined with mucin-secreting columnar epithelium and the ectocervix covered by nonkeratinized stratified squamous epithelium. The junction between these cells forms a barrier against ascending infections from the vagina. (158)

Vascularization and blood flow, supplied by branches of the uterine artery, provide oxygenation and communication with the rest of the body. The cervix exhibits a complex vascular network with distinct zones of vessels. Innervation plays a crucial role, with the cervix being one of the most densely innervated reproductive organs. Autonomic innervation involves sympathetic and parasympathetic components, contributing to the complex sensory and pain transmission system. While the innervation of the uterus decreases significantly during pregnancy, the cervix maintains a consistent degree of innervation. Despite being an

emerging area of research, understanding cervical innervation is essential for comprehending its role in reproductive processes. (158)

The classical view of cervical tissue architecture considered the cervix a relatively homogeneous, primarily composed of ECM/collagen (85-90%) with minimal cellular content (10-15%), encompassing fibroblasts, smooth muscle cells, glandular cells, vascular cells and immune cells. However, this viewpoint has been challenged by recent research (see section III - shift the paradigm). (189-191)

I. CERVICAL EXTRACELLULAR MATRIX

The cervical ECM, secreted by epithelial cells and fibroblasts, comprises five major components: fibrillar collagens, elastin fibres, proteoglycans, glycosaminoglycans (GAGs) and matricellular proteins. This ECM plays a pivotal role in maintaining the structural integrity of the cervix, with collagen being the primary contributor to its passive biomechanical strength. However, the cervix's mechanical resilience extends beyond collagen, as it relies on the presence of proteoglycans, GAGs and matricellular proteins within the ECM. Proteoglycans facilitate the organization of collagen fibrils, GAGs regulate tissue hydration levels and matricellular proteins govern matrix assembly and cell-matrix interactions. Together, these components ensure the robustness and functionality of the cervical tissue architecture. (192, 193)

Collagen is a key component of the ECM in the cervix, constituting approximately 90% of its structure and imparting strength and resilience to this tissue. (194, 195) type 1 and type 3 collagen are particularly vital for this strength, with type 1 collagen alone accounting for about 70% of the fibrous ECM in the cervix. Additionally, a small amount of type 4 collagen is situated near blood vessels and smooth muscle cells. (161, 196) The synthesis of collagen is a sophisticated process, involving intricate posttranslational modifications, fibril assembly and enzymatic cross-linking mechanisms. (193, 195)

Water content is another critical aspect of cervical tissue composition. In nonpregnant and first-trimester cervical tissue, water makes up 75%-80% of its composition. This percentage increases by approximately 5% in the third trimester of pregnancy. The hydration level significantly influences cervical mechanical properties, with alterations in hydration levels profoundly affecting the mechanical response of the collagen network. (193)

Glycosaminoglycans are integral components of the cervical ECM, encompassing hyaluronan (HA), chondroitin sulphate, dermatan sulphate, heparan sulphate and keratan sulphate. These hydrophilic macromolecules are pivotal for maintaining tissue hydration and providing compressive strength. The diversity of GAGs is finely regulated by factors including type, size, composition, degree of sulphation and their ability to bind to collagen and other proteins. With the exception of HA, GAG chains are sulphated and covalently linked to a core protein, forming proteoglycans. HA exists as a free GAG within the ECM, exerting a significant influence on cervical hydration and inflammation. Elevated HA concentration has been linked to cervical ripening and contributes to the disorganization of collagen fibres. (191, 193)

Proteoglycans, such as decorin, constitute a significant portion of cervical ECM, influencing mechanical properties, cell proliferation, adhesion, growth factor signalling and immune cell function. During pregnancy, changes occur in proteoglycan concentration, with a decrease in decorin concentration observed near term pregnancy, potentially impacting collagen network stability. Interestingly, despite the decrease in decorin, there is a simultaneous increase in proteoglycan synthesis, indicating heightened matrix turnover processes. This suggests a dynamic remodelling of the ECM during pregnancy, potentially to accommodate the changing mechanical demands and physiological needs of the cervix as it prepares for labour and delivery. (141, 193)

Elastin, found in elastic fibres within the cervix, contributes to the tissue's elasticity. While elastic fibres may not significantly contribute to initial matrix strength, they play a crucial role in restoring the cervix's shape following deformation during delivery. (193)

II. CELLULAR CONTENT (STROMA)

The cervix comprises two primary cellular compartments: the epithelial and the stromal layer. Within the epithelial layer, which lines the cervical canal, three distinct regions can be identified: the ectocervix, the transformation zone and the endocervix. Conversely, the stromal layer primarily consists of the ECM, which encompasses fibroblasts, immune cells and smooth muscle cells. (197)

Epithelial cells are pivotal guardians of cervical integrity throughout pregnancy. Initially sparse within the nonpregnant cervix, endocervical epithelial cells undergo significant proliferation during gestation, eventually constituting approximately 50% of the cervical mass by term.

(141) These cells serve vital functions as a protective barrier along the cervical lumen, shielding the underlying stroma from pathogens originating in the lower reproductive tract. They play an active role in secreting essential cytokines, chemokines and antimicrobial factors, orchestrating a defence response against invading pathogens and facilitating the recruitment and activation of inflammatory cells. (141, 197)

The cervical stroma, a key component of the cervix, is primarily composed of fibroblasts. These cells play a vital role in producing the ECM, which gives the cervix its structure. Additionally, the stroma contains smooth muscle cells, glandular cells, vascular cells and immune cells. (163)

Smooth muscle cells, although constituting a smaller proportion of cervical tissue compared to fibroblasts, are indispensable for cervical function. Their capacity to contract and relax in response to hormonal and mechanical signals regulates cervical tone, influencing processes such as cervical ripening, dilation during labour and the maintenance of pregnancy. Recent research has challenged the notion of a homogenous distribution of smooth muscle cells within the cervix. Instead, studies have revealed varying distributions and abundances of smooth muscle cells along its length, with a notable concentration at the internal os. (158, 191)

The cervix also harbours various immune cells, including neutrophils, eosinophils, macrophages, dendritic cells and lymphocytes. These immune cells dynamically change in composition and distribution in response to hormonal fluctuations, microbial exposure and reproductive events such as menstruation, pregnancy and childbirth. For instance, during pregnancy, the cervix undergoes immunological adaptations to accommodate the semi-allogeneic foetus while maintaining protection against infections. Positioned at the lower end of the uterus, the cervix acts as a barrier between the uterus and the vagina, making it vulnerable to microbial invasion. Immune cells are strategically dispersed throughout the cervical tissue to actively monitor and combat pathogens. (163) Neutrophils serve as the first line of defence against infections in the cervix, armed with antimicrobial molecules and enzymes to neutralize pathogens and initiate inflammatory responses. Eosinophils contribute to defence against parasites and allergic reactions. Macrophages, versatile immune cells, engulf and digest pathogens, debris and dying cells, playing essential roles in tissue repair and remodelling, particularly during pregnancy and childbirth. Dendritic cells capture and present

antigens to activate adaptive immune responses, shaping the immune response in the cervix. Lymphocytes, including T cells, B cells and NK cells, orchestrate adaptive immunity. T cells recognize and target specific pathogens, B cells produce antibodies for long-term immunity and NK cells directly kill infected or abnormal cells in the cervix. (136)

III. SHIFTING THE PARADIGM

Advancements in our understanding of the cervix are challenging traditional perspectives, revealing a complex and heterogeneous tissue composition. Contrary to the previous notion of homogeneity, recent studies highlight distinct regional differences between the internal and external openings of the cervix. (189, 190, 195)

Recent investigations into collagen dynamics have uncovered significant heterogeneity within the cervix. The strength of the collagen network is closely linked to collagen crosslinks, with notable variations observed between the internal and external openings. This emerging understanding emphasizes the importance of considering location-specific differences in cervical architecture, which directly influence functional and physiological distinctions. (191) Pioneering studies from the 1970s delineated the intricate architecture of the cervix's collagen network, identifying three crucial zones pivotal for resisting cervical dilation. The first zone comprises collagen fibres located in the middle of the stroma, oriented circumferentially around the endocervical canal, thereby providing essential resistance to cervical dilation. Additionally, there are inner and outer zones of collagen fibres, running parallel to the endocervical canal, likely serving to anchor the cervix to the uterus. (158)Recent advancements in optical coherence tomography further validate the heterogeneous nature of collagen fibre orientation, with circumferentially oriented fibres in the middle stroma playing a pivotal role in maintaining cervical integrity. (158)Furthermore, the strength of the collagen network is intricately tied to collagen fibre alignment and the degree and type of cross-linking present between collagen fibrils. Recent research underscores the greater heterogeneity in collagen crosslinking within the internal opening compared to the external opening, highlighting the complexity of cervical tissue composition and its implications for cervical function. (192, 195, 198)

Moreover, contrary to the prevailing notion of the human cervix as a predominantly homogeneous, collagen-based structure with minimal cellular components, our

investigations revealed a significant presence of smooth muscle. Remarkably, approximately 50–60% of the tissue at the internal os comprises abundant smooth muscle, with additional bundles oriented circumferentially around the cervix's periphery. Notably, this muscular arrangement mirrors the reported collagen network "zone" identified by Yao et al. This muscular orientation persists through the mid-cervix, gradually diminishing in the lower half, where smooth muscle content decreases. At the external os, smooth muscle is sparsely scattered, constituting around 10% of tissue content. (158, 199) Functional assessments demonstrate greater contractility of cervical tissue from the internal os compared to the external os, as demonstrated in functional organ baths, aligning with recent rodent studies highlighting a significant smooth muscle component responsive to contractile agonists. (190) (158) These findings, coupled with heightened innervation during pregnancy, prompt speculation about the intriguing possibility of smooth muscle at the internal os forming a specialized sphincter, as proposed in recent literature. (200)

The concept of a cervical sphincter is not new; historical studies from the 1950s highlighted the pregnant cervix's remarkable ability to contract independently in response to various stimuli. This phenomenon was substantiated by electromyography studies, which correlated cervical contractile activity with the duration of the latent phase of labour. (158) Renewed attention has been sparked by recent observations that delve deeper into the functionality of cervical smooth muscle, alongside the implementation of ultrasound cervical length measurement. These advancements have unveiled notable regional disparities in cervical tissue dynamics during pregnancy. As cervixes undergo a process of shortening, often accompanied by funnelling or dilation/failure of the internal os, while the external os remains tightly closed. (10, 201)

Heterogeneity in cervical morphology across different subregions may also extend to the distribution or density of immune cell phenotypes. This heterogeneity was suggested in a flow cytometry study of macrophages in the cervix of pregnant mice. Analysing cells from a dispersed cervix may obscure the diverse range of phenotypes coexisting within each subregion or the vascular compartment. (161)

2.7.2 FUNCTION OF THE CERVIX

The cervix plays dual roles during pregnancy and childbirth. Firstly, it acts as a protective barrier, guarding against invading microorganisms and enduring the gravitational forces exerted by the expanding uterus. Secondly, it undergoes a gradual softening and eventual dilation to facilitate the successful passage of the foetus through the cervix and vagina. This delicate balance between maintaining structural integrity and accommodating the demands of childbirth is essential for ensuring a healthy pregnancy and safe delivery. (141, 161, 202, 203)

During pregnancy, the cervix orchestrates a series of endogenous defence mechanisms to combat infections, serving as the first line of defence and safeguarding its barrier function. These mechanisms include physical barriers, antimicrobial peptides and immune cell activation. (152, 201, 204) The cervical epithelial layers play a crucial role in forming a formidable barrier, achieved through junction proteins that seal intercellular spaces, effectively thwarting bacterial invasion. In studies conducted on mice, temporal changes in tight junction proteins such as claudin 1 and 2 and desmoglein have been observed during pregnancy. These proteins are upregulated during cervical ripening to strengthen barrier formation, thereby enhancing protection of the cervix and the reproductive tract against infections and other insults. (162, 205)

The mucus produced by cervical epithelial cells forms an additional defensive layer against infection, chemical and mechanical insults. The cervical mucus plug (CMP) assumes a crucial role in deterring bacterial infiltration from the vaginal canal into the uterine cavity. Abundant in antimicrobial peptides (AMPs) such as secretory leukoprotease inhibitor, lysozyme, lactoferrin, calprotectin, α -defensins and human β -defensin, the CMP acts as an additional barrier against microbial intrusion. Studies have demonstrated the robust antibacterial properties of cervical mucus, inhibiting various pathogenic bacteria effectively. (204, 206)

Furthermore, AMPs secreted by cervical cells play a pivotal role in recruiting and activating inflammatory cells and antimicrobial factors to combat invading pathogens. These AMPs exhibit potent bactericidal action. Studies investigating cervical fluid proteins and their correlation with PTB offer intriguing insights. For example, while levels of elafin or human β -defensin-1 in cervical fluid were not associated with PTB in a United Kingdom study, another

investigation suggested that reduced levels of β -defensin-2 protein slightly elevated the risk of sPTB. (204)

In summary, the cervix employs a diverse array of physical, chemical and immunological defence mechanisms to combat bacterial and viral infections, ensuring the protection of the developing foetus within the uterine cavity. (201, 204)

2.7.3 CERVICAL REMODELLING

The cervix plays a crucial role in both initiating and completing the birthing process, challenging past assumptions of its passive role in response to uterine contractions. Recent research emphasizes the dynamic nature of cervical changes, which commence well before the onset of contractions. Clinical observations such as the Hegar sign and increased cyanosis indicate early signs of cervical softening as early as one month after conception, highlighting the proactive nature of cervical changes. Ultrasound examinations further confirm this, revealing shortened cervical length weeks before labour onset. (10, 133, 147, 189)

CR throughout pregnancy encompasses a series of dynamic changes occurring in four overlapping phases. The first phase, softening, represents a prolonged and progressive transformation where the cervix gradually becomes more pliable. Following this, the ripening phase emerges, characterized by a notable acceleration in softening and an increase in compliance as the term approaches. As delivery draws near, the dilation phase commences, marking the active process of cervical opening just before childbirth. Finally, postpartum repair denotes the period after birth during which the cervix undergoes restoration and healing. Together, these phases of CR highlight the intricate and sequential adjustments the cervix undergoes to facilitate the birthing process and ensure maternal and fetal well-being. (10, 158, 203, 207)

Forceful uterine contractions alone cannot bring about delivery if the cervix retains its rigidity. Conversely, cervical incompetence may lead to preterm birth even in the absence of active labour contractions. Therefore, timely and appropriate alterations in cervical function during gestation are vital for achieving a successful pregnancy outcome. The meticulous coordination of cervical rigidity regulation and ripening is essential for the successful delivery of a full-term infant(141)

The cervix's innervation in both women and rodents is dense, with nerve fibre density either sustained or increased toward term. (208, 209) Disruption of parasympathetic nerves projecting to the cervix delays birth and disrupts the typical increase in macrophage presence at term. These pathways include sensory neuropeptidergic fibres that induce local vasodilation and inflammation. (210) Notably, the cervix demonstrates an augmented density of neuropeptidergic fibres preceding birth. (211) Furthermore, central connections from the paraventricular nucleus to the cervix may contribute to the circadian control of birth timing in mammals, suggesting neural involvement in regulating cervical processes leading to parturition. (202, 209) The evidence indicates a likely neural involvement in overseeing cervical processes preceding parturition, implying a potential role for the nervous system in instigating a "sterile" neurogenic inflammatory state during CR. (158, 202, 211, 212)

Comprehensive biochemical studies in humans remain somewhat limited, thus necessitating our reliance on findings from studies conducted in animal models. (158, 162, 163)

A) CERVICAL SOFTENING

Cervical softening refers to the progressive transformation of the cervical tissue's properties and composition during pregnancy. This essential physiological process involves a notable decrease in tissue stiffness and a concurrent increase in compliance, while ensuring the maintenance of tissue competence. (193, 195) Early signs of this phenomenon emerge as early as one month after conception in women, marked by the proliferation of endocervical epithelial cells and significant local matrix remodelling facilitated by native cervical cells like fibroblasts, myofibroblasts and epithelial cells. (141, 192, 207)

Throughout pregnancy, the overall collagen content in cervical tissue remains stable, with no discernible fluctuations in the ratio of type I to type III fibrillar collagens. Consequently, changes in collagen type ratios are unlikely to significantly affect the progressive decline in tissue stiffness. (163, 192) However, despite this consistent ratio, there is an upregulation in collagen turnover, leading to continuous breakdown and replacement of collagen. This dynamic turnover indicates an ongoing remodelling process within cervical tissue, contributing to its softening while preserving the overall integrity and structure of the tissue. This aligns with the notion that alterations in collagen organization during normal term pregnancy represent rearrangement rather than degradation of collagen. (163, 213)

While the content of collagen remains constant, its morphology undergoes significant changes throughout pregnancy. Specifically, collagen type I fibres transition from long, thin fibres to thicker, curved fibres as term approaches. These structural alterations likely initiate with the reduction in cross-linked collagen and matricellular proteins during cervical softening and continue during ripening. This progressive transformation contributes to increased tissue compliance without compromising competence, effectively preparing the cervix for the subsequent phase. (163)

In rodents, cervical softening is marked by a decrease in collagen solubility and a decline in enzymes responsible for collagen crosslink formation, leading to a shift towards more immature and weaker collagen cross-links. During normal CR, older and more cross-linked collagen undergo breakdown, replaced by new and less cross-linked collagen, enhancing tissue compliance. (161, 191, 192, 195, 198, 202) Quantitative analysis of pyridinoline crosslinks underscores their crucial role in maintaining collagen stability across various connective tissues. The reduction in lysyl oxidase activity during early pregnancy, a key enzyme promoting robust pyridinoline cross-links, is thought to significantly contribute to cervical softening and relaxation necessary for CR processes. (192, 195, 198)

Changes in matricellular proteins, including thrombospondin 2 (THBS2) and tenascin-C (TNC), also exert a significant influence on cervical softening and its effects on cervical mechanical strength. The downregulation of these proteins during pregnancy correlates with alterations in collagen architecture and protease activity, ultimately resulting in reduced mechanical strength of the cervix. As the expression of THBS2 and TNC decreases, there is a gradual substitution of mature, cross-linked collagen with forms that are less cross-linked, facilitating collagen remodelling and promoting cervical softening while simultaneously preserving tissue integrity. (191, 192, 198)

In addition to the extensive changes in the ECM, cervical epithelial cells are believed to play a crucial role in cervical softening. These cells perform essential functions including immunoprotection and barrier maintenance, which likely serve to safeguard the stromal compartment as the ECM undergoes remodelling. Immune cells localize to the epithelial/subepithelial regions of the cervix during softening and proteins involved in epithelial repair and maintenance, such as trefoil factor 1 (TFF1) and serine protease inhibitor Kazal type 5 (SPINK5), are induced. TFF1 aids in protecting and repairing mucosal epithelia,

while SPINK5 prevents the degradation of proteins crucial for barrier formation. Although further research is needed to delineate the specific roles of TFF1 and SPINK5 in the cervix, their upregulation during cervical softening suggests that epithelial functions are crucial for maintaining cervical competency. (163)

Overall, across various species, the cervix undergoes functional adaptations during pregnancy, including softening and preservation of cervical integrity until late pregnancy when an accelerated phase of cervical ripening begins. It has been suggested that simultaneous changes in cervical volume, hypertrophy, hyperplasia and geometric alterations counterbalance the softening effects until term. Subsequently, cervical ripening allows for full cervical dilation in the presence of uterine contractions. (141)

B) CERVICAL RIPENING

The process of cervical ripening, following the initial softening phase, is pivotal for preparing the cervix for dilation during uterine contractions, facilitating the passage of a term foetus. Although the term "cervical ripening" is often used interchangeably to describe changes in the cervix before and during labour, it seems that the dilation of the cervix in active labour involves distinct processes from those occurring during cervical ripening before labour. (141) Due to the brief duration of both ripening and dilation phases, distinguishing between these two overlapping stages of remodelling becomes challenging. (162, 163, 193, 207)

Cervical ripening reflects structural changes in the cervix, clinically indicated by an elevated Bishop score and characterized by a decreased ability to maintain closure as a load-bearing organ. This phase is characterized by a rapid loss of tissue compliance and integrity, typically observed hours before birth in mice and days or weeks before birth in women. (162, 193, 214, 215)

Throughout the ripening process, the ECM undergoes critical alterations essential for cervical preparation leading up to labour. These modifications, which begin during the softening phase, are notably intensified as ripening progresses. They encompass shifts in cross-linking patterns, augmentation of collagen fibre diameter, increased spacing between fibrils and a distinct transition in fibre morphology from straight to wavy fibres. These dynamic changes collectively contribute to the structural readiness of the cervix for dilation during childbirth. (163, 191)

The modulation of the GAG chain is crucial for maintaining cervical integrity and shaping its mechanical properties by influencing collagen fibril packing and tissue viscoelasticity. (162, 207) Increases in hydrophilic GAGs and aquaporin water channels lead to tissue hydration, causing dispersion and disorganization of collagen fibres. Consequently, collagen solubility rises, rendering the tissue more susceptible to endogenous proteases. (141)

HA, the primary GAG, exhibits heightened synthesis during cervical ripening, contributing to collagen fibre spacing and tissue hydration, subsequently disrupting or weakening the collagen network. The increased synthesis of HA during term cervical ripening coincides with the peak of tissue compliance, likely orchestrated by the oestrogen-driven upregulation of hyaluronan synthase 2 (Has2) expression, indicating a probable hormonal control over cervical GAG levels throughout pregnancy and parturition. (141, 216) The functions of HA depends on its size and interactions with other molecules. Large molecular weight HA enhances viscoelasticity in tissues, while low-molecular-weight HA exhibits proinflammatory functions. (163) The functional properties of HA vary depending on its size and interactions with other molecules. Larger molecular weight HA enhances tissue viscoelasticity, whereas low-molecular-weight HA exhibits proinflammatory functions by binding to cell surface receptors and stimulating macrophages recruited to sites of inflammation. This activation prompts the production of chemokines that sustain the inflammatory response by recruiting additional inflammatory cells. Consequently, once collagen is solubilized by hydrophilic HA, an inflammatory cascade is initiated, propagated and amplified by the activation of macrophages within cervical tissue, highlighting the diverse and cell-specific roles of HA during cervical ripening. (141, 163, 217)

Elevated levels of proteoglycans, observed in individuals with a ripe cervix, exert significant influence within the ECM. They contribute to collagen organization and affect crucial cellular processes such as adhesion, migration and proliferation. (141, 218) Studies have highlighted the importance of proteoglycans, with findings showing that mice lacking the proteoglycan decorin (Dcn KO) exhibit reduced mechanical properties compared to wild-type mice. Specifically, assessments using a load-to-break ring test revealed decreased strength and stiffness in Dcn KO mice. Proteoglycans, elevated in patients with a ripe cervix, play pivotal roles within the ECM, impacting collagen organization, as well as influencing cell adhesion, migration and proliferation.

In the 1980s, Mont Liggins proposed an inflammatory model of labour, suggesting that immune cell infiltration during cervical ripening leads to ECM degradation and remodelling, a concept supported by numerous immunohistochemically studies implicating macrophages or neutrophils in cervical ripening. However, this theory encountered a setback as most of these studies obtained samples postpartum, capturing alterations related to parturition rather than events preparing the cervix for labour onset. (161-163, 219, 220)

Recent evidence from human and animal studies prompts a reconsideration of the role of inflammatory cells in cervical ripening, suggesting that they primarily contribute to active dilation and postpartum tissue repair rather than initiating cervical ripening. (141, 161-164, 218, 219, 221, 222)

Research conducted on cervical tissues from women before and after ripening (but prior to labour) failed to reveal significant increases in granulocyte numbers in the ripened cervix before labour onset. It appears that a surge in neutrophil census in the cervix occurs only near labour or after evident collagen degradation in both women and rodents. (219) Additionally, depletion of neutrophils has no effect on the timing of parturition and myeloperoxidase activity, a marker of functional neutrophils, is not detectable until postpartum. Despite the presence of monocytes, resident macrophages and neutrophils in the cervix during ripening, mRNA expression of proinflammatory genes and MMPs is not induced. (163) This contradicts the long-held assumption that MMPs play a major role in cervical ripening. The role of MMPs and other collagenases in the ripening process remains unclear, but concentrations of MMPs and increased numbers of leukocytes correlate more strongly with the extent of cervical dilation during labour rather than the ripening phase. (141, 223)

In mice, an increased residency of macrophages occurs in the prepartum cervix stroma a day before labour, but their precise role in ripening remains unclear. (161, 224) Macrophage gene expression profile increases postpartum, suggesting a role in cervix recovery as a protector against microbial invasion and promoter of tissue repair. (162) Furthermore, supporting the theory that immune cell activation is not essential for physiological ripening at term, Sakamoto et al. reported no correlation between the degree of clinical cervical ripening and IL-8 concentrations in cervical tissue. (221) Moreover, in a transgenic mouse model, cervical ripening failed to occur despite the infiltration of neutrophils and macrophages into the

tissue, suggesting that the presence of inflammatory cells alone may not be sufficient to initiate ripening. (225)

The specific roles of monocytes, eosinophils and other cell types in the process of cervical ripening remain unclear. (141, 164) In rodents, there's an observed increase in transcripts encoding the chemotactic factor eotaxin1, followed by a significant rise in eosinophil presence on the day of birth. (161) While the function of eosinophils in the cervix is not fully understood, recent studies suggest they may promote the differentiation of monocytes into alternatively activated macrophages during postpartum repair. (162, 163) Additionally, mast cells show increased numbers and activity in ectocervix biopsies from term-pregnant women compared to those in the first trimester or non-pregnant individuals, though their exact role remains undetermined. (161)

B-lymphocytes and plasma cells do not contribute to cervical ripening or dilatation, while the decrease in T-lymphocyte count in the subepithelial cervix during late pregnancy and vaginal delivery suggests a potential down-regulation of local immunity, possibly influencing the late pregnancy period. (219)

In summary, existing evidence does not strongly endorse a direct involvement of neutrophils, eosinophils, or mast cells in the softening or ripening phases of CR. However, these cells might contribute to prepartum dilatation or peripartum processes aimed at cervix repair to restore barrier functions postpartum. (161) The prevailing evidence indicates that cervical ripening, mediated by collagen dispersal through increased synthesis of GAGs like HA, may not entirely be regulated by an inflammatory response. (141)

Evidence suggests that PGs play a critical role in late-term cervical function. Studies have shown that the administration of exogenous PGs increases collagen solubility and alters ECM components, mimicking the effects of cervical ripening and dilation. Despite these findings, the levels of PGs such as PGE2 and PGF2 do not increase. Moreover, inducing ripening with a progesterone inhibitor does not lead to a rise in PGs, creating a gap in our understanding of their role in this process. (182, 226, 227)

The coordination of CR events may also involve epithelial cells. During the latter half of rat pregnancy, mucosal epithelial cells undergo significant proliferation, driven by hormones like relaxin and oestrogen. (228) By gestation day 16 and 17, as the softening phase transitions into ripening, the epithelium develops mucin-secreting vacuoles essential for immune

surveillance and lubrication. Simultaneously, these cells express steroid 5 alpha reductase type 1 (SRD5A1), influencing local progesterone metabolism. The decline in ovarian progesterone synthesis, coupled with increased progesterone metabolism by SRD5A1, facilitates the shift to the cervical ripening phase. (229) Ripening involves controlled adjustments in barrier properties, hydration levels and intercellular communication, as indicated by alterations in the expression of tight junction proteins (e.g., claudin 1 and 2), aquaporin's, gap junction proteins (e.g., Connexin 26 and 43) and desmogleins. (230-232) Further research is necessary to fully elucidate the critical roles played by epithelial cells in CR. (163)

I. EPITHELIAL-MESENCHYMAL TRANSITION

EMT is a complex biological process involving the transformation of epithelial cells into mesenchymal cells. This transition encompasses various molecular events, including the activation of transcription factors, altered expression of cell-surface proteins, cytoskeletal reorganization and localized production of ECM-degrading enzymes, leading to phenotypic changes in the cell. Studies have highlighted the potential role of EMT in the intricate process of CR during pregnancy. (203, 218, 233, 234)

Human and animal studies show that EMT markers in the cervix change during pregnancy. (234) EMT is associated with the downregulation of E-cadherin, an epithelial marker, particularly evident in individuals with a ripe cervix compared to those with an unripe cervix. (218). Additionally, changes in the expression levels of vimentin and cytokeratin have been observed in the cervix of pregnant mice undergoing remodelling. Vimentin mRNA expression increases significantly throughout pregnancy, whereas cytokeratin 8 mRNA expression decreases markedly.

During pregnancy, EMT occurring in endocervical epithelial cells and cervical stromal cells poses potential risks to the cervix by compromising cervical epithelial barrier integrity and fostering inflammation and ECM degradation within the cervical stroma. (233) Reversible EMT, alongside its counterpart, mesenchymal-to-epithelial transition (MET), orchestrates cellular remodelling in both the amnion and cervix, contributing to the dynamic changes observed during gestation. (233, 234) This cyclic remodelling process is intricately regulated by steroid hormones. Evidence suggests that oestrogen enhances EMT and cellular migratory

potential, particularly evident in pathological conditions like endometriosis and gynaecologic malignancies. (235) Confirming oestrogen's role in EMT, studies demonstrate that blocking ER inhibits EMT, thereby suppressing the invasiveness and migratory capabilities of human breast and endometrial cancer cells. (236, 237) In pregnancy, third trimester levels of oestrogen permit an EMT-mediated impairment of the remodelling process in the cervix in vitro, suggesting a potential contribution of oestrogen in cervical ripening. (233) Conversely, progesterone promotes MET in both amnion epithelial and cervical epithelial cells. (238, 239) While a non-reversible state of EMT induced in response to infection or other risk exposures may lead to persistent inflammation and degradation of the cervical matrix. (197, 218, 234) These insights underscore the significance of EMT in CR during pregnancy, emphasizing the need for further investigation. (234)

C) DILATION

Distinguishing between the ripening and dilation phases is often challenging, as the two stages overlap. (141, 162, 163, 193, 207)

The structural changes in the cervix during the ripening phase result in a loss of integrity and tensile strength, preparing it for the subsequent phase of dilation. (161)

Unlike ripening, dilation is characterized by increased inflammation, infiltration of leukocytes, PGs, proteases and collagenases into the ECM, with concentrations of MMPs and leukocytes correlating with the extent of cervical dilation during labour. (141, 161, 197, 207)

Studies by the Norman group have extensively explored the inflammatory processes in the cervix at term, revealing higher densities of macrophages and neutrophils, along with elevated expression of proinflammatory cytokines like IL-1b, IL-6, IL-8 and TNF α in labouring women. Moreover, augmented concentrations of granulocyte-macrophage colony-stimulating factor during labour may stimulate resident myeloid cell growth and activity. Structural alterations, including collagen degradation and reduced cell nuclei density, are observed in cervix biopsies from later stages of pregnancy, likely mediated by proinflammatory cytokines promoting ECM digestion. The dilation phase is marked by characteristic signs of inflammation, supported by heightened presence of inducible nitric oxide synthetase in the cervix stroma of term women. (161)

D) POSTPARTUM REPAIR

Recovery during phase 4 of remodelling is essential for restoration of the barrier function of the cervix. (161, 240)

Postpartum recovery involves a rapid modulation of gene expression associated with the synthesis, processing and repair of the ECM. Unlike the processes observed during ripening, myeloid-derived immune cells play a crucial role postpartum, contributing to heightened neutrophil levels and increased myeloperoxidase activity. Diverse macrophage phenotypes, including M1 and M2 subsets, actively participate in clearing matrix debris such as HA and inadequately assembled collagen. (162, 163) Additionally, eosinophils may promote the differentiation of monocytes into alternatively activated macrophages during postpartum repair, suggesting a complex interplay between immune cells and CR processes. (162, 163)

Furthermore, postpartum upregulation of genes involved in collagen synthesis, cross-linking, proteoglycan metabolism and wound healing facilitates tissue repair, suppresses angiogenesis and expedites the cervix's swift recovery. Such recovery is crucial for safeguarding the reproductive tract and ensuring the success of subsequent pregnancies. (161, 163, 240)

2.7.4 PREMATURE CERVICAL REMODELLING:

Romero et al. have characterized cervical ripening as pivotal in the “premature parturition syndrome”. PTB often exhibits a prolonged preclinical phase characterized by changes in gestational tissues that precede symptomatic labour by several weeks. (10, 139) Early signs of cervical ripening, such as cervical shortening and patency of the internal os, can be detected through routine vaginal examinations weeks before the onset of uterine contractions and sPTB. (141, 241) Ultrasound imaging has further validated these clinical observations, revealing early cervical alterations, such as funnelling and reduced cervical length, weeks before the onset of sPTB. (241-252) This evidence underscores that cervical activation often occurs as an initial event in spontaneous PTB, rather than a consequence of myometrial activation. (10, 253)

The concept of "Precocious Cervical Ripening," first described by Papiernik et al. in 1986, encompasses multiple cervical changes such as softening, shortening, funnelling and internal os dilation. (241, 254) Subsequent research has demonstrated an inverse correlation between

cervical length (CL) and gestational age at birth, challenging the binary notion of cervical incompetence (CI) and supporting a model of “continuum of cervical competence”, with CI representing an extreme end. (241-248, 255-257) As evidence of the role of premature cervical changes has accumulated, the term “Precocious Cervical Ripening” has been replaced by the more inclusive “Premature Cervical Remodelling”. Despite this terminology shift, the core idea remains that a shorter CL is an early indicator of 'cervical insufficiency,' with cervical ripening often representing the initial change in gestational tissues. (241, 255, 256) This concept is crucial because the latency between cervical changes and myometrial activation provides a key opportunity for screening and identifying women at increased risk of premature labour. However, to develop effective interventions for women identified through screening, it is crucial to further explore the pathophysiology of premature CR and a shortened cervix. (201, 253)

Although several factors are commonly linked to a short cervix—such as the suspension of progesterone action, congenital cervical hypoplasia, previous cervical surgery and infection—the exact mechanisms underlying cervical "failure" and the development of a sonographically short cervix remain elusive. (86, 168, 257-260)

Research into the biochemical and molecular causes of weak cervical tissue during pregnancy has yielded inconclusive results due to several key challenges: (158, 261)

- **Inconsistent Definitions:** The concept of "cervical insufficiency" lacks a standardized definition, leading to variability in study outcomes.
- **Limited Human Data:** There is a notable scarcity of data on cervical tissue evaluation specifically in women with cervical insufficiency
- **Methodological Variations:** Existing studies often have methodological limitations that hinder definitive conclusions:
 - **Biopsy Location Variability:** The cervix is not a homogeneous structure and variations in the location of cervical biopsies can complicate result interpretation.
 - **Timing of Biopsy:** Many studies collect cervical tissue samples either in the non-pregnant state or postpartum, focusing on the repair stage rather than the critical softening or remodelling stages during pregnancy.

- **Inadequate Control Groups:** Control groups in these studies often consist of non-pregnant women undergoing benign hysterectomy or other non-comparable cohorts, rather than gestational age-matched pregnant individuals.

This review aims to address these issues by evaluating the existing evidence from both animal models and human studies.

A) INSIGHTS FROM ANIMAL STUDIES IN CERVICAL REMODELLING

The investigation of labour mechanisms in various animal models has provided invaluable insights, serving as a crucial bridge between theoretical understanding and practical applications in human obstetrics. However, these studies underscore the need for cautious interpretation due to anatomical differences, gravitational forces and hormonal variations between rodents and humans. Before correlating animal models of preterm birth to human preterm birth, it is crucial to understand species similarities and differences in parturition. (Table 1) (11, 37, 136, 139, 140, 150, 262) Rodents, despite some biological differences, share significant reproductive similarities with humans. Their practicality, due to the ability to use larger numbers at a lower cost and gestation-timed pregnant tissue samples can be obtained in a relatively short gestation period. (263) These models have been extensively used to compare term cervical ripening, infection-induced premature ripening and RU486-induced premature ripening (mifepristone).

Table 1: Advantages and limitations of animal models in cervical remodelling and preterm birth research

Animal model	Advantages	Limitations
Rodents	Cost-effective, can study large sample sizes, short gestation period, with rapid data collection and genetic manipulation tools available.	Significant anatomical and hormonal differences from humans
Sheep	Similar cervical structure and reproductive physiology to human, larger size facilitates surgical interventions and tissue collection.	Higher cost, complex management, less tools for genetic modifications.
Non-human primates	Closest to human reproductive biology	High cost of maintenance and ethical considerations; Fewer animals available for studies.
Guinea pigs	Similar cervical changes to humans	Longer gestation periods than rodents; Different reproductive physiology
Rabbits	Accessible and cost-effective	Differences in cervical structure and remodelling

These studies have provided crucial insights into the complex mechanisms involved:

I. MATRIX OF THE CERVIX:

- **RU486-Induced Ripening:** Premature cervical ripening induced by the progesterone receptor antagonist, mifepristone, accelerates processes typically associated with term ripening. (163) However, it operates through distinct mechanisms, including differences in collagen type I degradation and structural transformation. (213) RU486-treated cervixes result in more pronounced matrix disorganization in compared to those undergoing term cervical ripening. (163, 213, 264) It appears that mifepristone-induced ripening partially activates pro-inflammatory and tissue repair processes similar to those observed during postpartum repair. (163)
- **Infection-Induced Ripening:** Administration of LPS, a component of Gram-negative bacterial outer membranes, induces preterm cervical changes and labour within hours of treatment, suggesting a role of infection in premature cervical ripening. In LPS-treated mice, the collagen matrix exhibits similarities to term ripening. (264) Interestingly, despite

the upregulation of neutrophil collagenase MMP8 in the infection model, the overall collagen content remains unaffected. (163)

- **GAGs:** During normal gestation, expression of Has 2 genes, responsible for synthesizing HA, significantly increases near term. However, this increase in Has2 expression does not occur during premature induced ripening. (207, 264) Instead, an increase in Has1 expression is observed in preterm conditions. (141)
- **Collagen:** The changes in physical properties of the rat cervix during physiologic ripening cannot be attributed to increased collagenase activity. (223)

II. PROSTAGLANDIN (PG) EXPRESSION

PGs reduce cervical resistance by reorganizing collagen fibrils. (227) The expression of prostaglandin-cyclooxygenase-endoperoxide synthase (Ptgs) is upregulated in both premature parturition models, with Ptgs1 expression is heightened in RU486-induced premature cervical ripening, while Ptgs2 expression is notably induced in the infection model. (264) Nonsteroidal anti-inflammatory drugs, which block PG production, have proven effective in attenuating the progression of term and preterm labour in animal models. Research demonstrates that COX 1 activity is required for the appropriate timing of TL in mice, while COX 2 is induced during inflammation-mediated PTB. (265, 266)

III. CELLULAR RESPONSES

Cellular responses differ between term birth and various etiologic preterm births. In term cervical ripening, neutrophil depletion in term has no effect on the timing or success of parturition and general inflammatory response are not initiated in the cervix until shortly after parturition, suggesting that term cervical ripening does not require neutrophil activation. (161, 164) Moreover, in a transgenic mouse model, cervical ripening failed to occur despite the infiltration of neutrophils and macrophages into the tissue, suggesting that the presence of inflammatory cells alone may not be sufficient to initiate ripening. (225)

In RU486-induced premature cervical ripening is associated with a significant increase in tissue monocytes and eosinophils compared to TL. Conversely, LPS-induced ripening is characterized by an increase in neutrophils, with no notable change in monocytes or eosinophils. (264)

In rodents, there is an observed increase in transcripts encoding the chemotactic factor eotaxin1, followed by a significant rise in eosinophil presence on the day of birth. While the function of eosinophils in the cervix is not fully understood, recent studies suggest they may promote the differentiation of monocytes into alternatively activated macrophages during postpartum repair. (162, 163)

The role of lymphoid-derived cells in cervix function remains incompletely understood. (161, 267)

IV. PRO-INFLAMMATORY MARKERS

Inflammatory proteins also display different expression in term and preterm birth. In term birth, cytokine expression was not increased in cervixes during TL, but was increased in the postpartum period. (232) On the other hand, heightened gene expression of neutrophil chemoattractant Cxcl2 and pro-inflammatory cytokines show significant upregulation in both preterm models compared to untreated gestation, with LPS-induced inflammation demonstrating a manifold increase over RU486-induced. (264, 268)

V. COMPLEMENT ACTIVATION

Two mouse models of PTB have underscored the significance of complement activation in CR and PTB. Findings from both models revealed increase in cervical complement component C3 and C5a in PTB compared to gestational age-matched controls. Moreover, C5aR-deficient mice failed to undergo CR or PTB in response to LPS or mifepristone treatment, strongly suggest that C5aR is essential for the CR process preceding PTB. (215, 269)

VI. HORMONAL EFFECT

Progesterone and oestrogen play pivotal roles during parturition. In rodents, the onset of labour in this model is marked by a significant reduction in maternal progesterone levels (270-272). conversely, several animal and in vitro studies have emphasized the potential role of oestrogen in facilitating cervical ripening, labour and delivery. (142, 143) The increased expression level of ER α in the cervix being associated with PTL and delivery. (142-145, 273)

VII. EPITHELIAL CELLS

In a mouse model, vaginal bacteria inoculation combined with mild cervical injury induced by a common spermicide has been associated with higher rates of preterm birth compared to vaginal inoculation alone. (204, 274) Conversely, the use of a vehicle for vaginal progesterone alters cervical epithelial junctional proteins and reduces the rate of LPS-induced preterm birth. (275) This highlights the critical role of cervical epithelium in cervix barrier function against ascending infection and PTB.

VIII. NEUROMODULATION

When stimulated by microorganisms, toxins, or irritating stimuli, rat sensory nerves release neurotransmitters that initiate local inflammatory responses—a phenomenon known as neurogenic inflammation. This concept extends beyond immediate immune responses, impacting tissue remodelling processes such as ovulation, formation of the corpus luteum and cervical ripening. (276, 277) Additionally, neuropeptide production from cervix-related sensory neurons can be stimulated by oestrogen, highlighting the interaction of the endocrine and nervous systems (211) Factors influencing sensory nerve activity have the potential to significantly impact reproductive tissue dynamics and CR processes.

IX. MECHANICAL STIMULATION

Recent studies have highlighted an underappreciated role of mechanical forces in CR. Using proteome-wide technology, researchers explored mechano-related signalling molecules in the mouse cervix. They identified an increase in several cytoskeletal factors and signalling molecules, mirroring the escalating gravitational force exerted by the growing foetus on the cervix. This correlation suggests that fetal size and weight likely play a pivotal role in CR through mechano-transduction processes. (203)

X. LABOUR INITIATION

Labour initiation is a complex process governed by multiple factors and redundant pathways. Mice deficient in anthrax toxin receptor 2 (Antxr2) fail to give birth despite declining serum progesterone levels at term, highlighting the critical role of this receptor in birth initiation. (278, 279) Similarly, cervical ripening fails in mice lacking SRD5A1, underscoring the intricate

nature of parturition. (229, 230) Conversely, rodent models deficient in specific genes implicated in remodelling or parturition can still deliver at term, suggesting the existence of alternative mechanisms for initiating labour. (131, 132)

B) UNRAVELLING THE MOLECULAR MECHANISM LINKED TO PREMATURE CERVICAL REMODELLING

The interplay between endogenous and exogenous factors—such as infections, chemical exposures, mechanical damage, oxidative stress and endocrine disruptions— intersects with genetic predispositions to influence premature CR. This multifactorial nature of PTB emphasizes that CR involves various pathways influenced by different triggers. Notably, the molecular mechanisms governing CR vary significantly between preterm and term births, as well as among different causes of PTB (Table 2). Understanding both normal and abnormal CR processes is crucial for identifying individuals at risk and developing targeted interventions to mitigate preterm birth linked to cervical insufficiency.

Table 2: Molecular mechanisms linked to premature cervical remodelling

Molecular mechanism	Impact on Premature Cervical Remodelling	References
Cervical Epithelial Dysfunction	Compromises the cervix's barrier function, increasing susceptibility to infections and inflammation.	(197, 280, 281)
Inflammation	Involves immune response activation and cytokine release. Chronic inflammation accelerates CR, causing cervical shortening and insufficient cervix.	(204, 282) (281, 283)
Oxidative Stress	Imbalance between reactive oxygen species and antioxidants contributes to premature CR by inducing cellular senescence and sterile inflammation.	(283, 284)
Hormonal Disruption	Hormonal imbalances can precipitate the disruption of normal softening and ripening processes. Oestrogen enhances EMT, cellular migration and cervical inflammation.	(233, 235-237)
Genetic Predisposition	Genetic variations in genes related to connective tissue metabolism (e.g., Collagen Type I Alpha 1 Chain, TGF- β and IL-10) increase sensibility to environmental factors.	(73, 163, 255, 285)
Matrix Metalloproteinases	Altered MMP activity can lead to abnormal matrix remodelling and premature CR.	(141, 223)
Glycosaminoglycan	Changes in GAG composition and metabolism affect matrix hydration and collagen organization and CR.	(141, 162, 163, 207, 280)
Epithelial-Mesenchymal Transition	Persistent EMT can disrupt cervical barrier function and stimulate inflammations, promoting early remodelling	(161, 203, 218, 233, 280)
Complement Activation	Increased complement activation enhances inflammatory responses and matrix remodelling and can accelerate CR.	(215, 269)
Prostaglandins	Upregulation of PGs can enhance cervical matrix remodelling and inflammation, leading to premature CR.	(225, 227, 266)
Mechanical Forces	Mechanical stress can induce CR by activating mechano-sensing pathways in cervical tissues.	(203)

Legend: CR – cervical remodelling; EMT - epithelial-mesenchymal transition; MMP - matrix metalloproteinases; GAG – glycosaminoglycan

I. CERVICAL EPITHELIAL CELLS DYSFUNCTION

Throughout pregnancy, the cervix plays a pivotal role as a protective barrier, preventing microorganisms from ascending from the vaginal canal to the uterine cavity. Maintaining the barrier function of the cervical epithelium is crucial during the dynamic process of CR in pregnancy. Any compromise to this barrier heightens the risk of PTB. (280)

Various factors can compromise cervical health, including cervical surgeries, infections, inflammation, genetic predispositions and chemical exposures, all of which can breach this protective barrier and increase the risk of PTB. [25, 49, 115]. Drawing a parallel with gastrointestinal conditions, where compromised epithelial barriers contribute to disease states, sheds light on the vulnerability of the cervix. Damage to the cervical epithelium not only enables the ascent of infections and triggers intrauterine inflammation but also acts as a precursor to preterm birth. (139, 197, 201, 204, 274)

Recent research indicates that a non-reversible state of EMT may compromise cervical integrity, posing a potential risk for PTB. (161, 203, 218, 233, 234, 280) Persistent EMT, triggered by infection or other risk factors, can lead to ongoing inflammation and degradation of the cervical matrix, accelerating premature remodelling. (197, 218, 234) Furthermore, EMT induces the production of pro-inflammatory factors in various cell types and inflammation can, in turn, promote EMT processes. This complex interaction between inflammation and EMT is mutually reinforcing, with each process exacerbating the other. (161)

Additionally, EMT is influenced by the endocrine system. (233, 235-237) Evidence suggests that oestrogen enhances EMT and cellular migratory potential, as well as cervical inflammation, while progesterone during gestation is crucial for promoting tissue repair and maintaining balance to counteract the adverse effects of infection or inflammation in the cervix. (238, 239)

These insights underscore the significance of EMT and the critical role of epithelial functions in upholding barrier properties and immune surveillance, emphasizing the need for further investigation. (162, 201, 234)

These insights emphasize the importance of EMT and the critical role of epithelial functions in maintaining barrier properties and immune surveillance, underscoring the need for further investigation.

II. INFLAMMATION

A common denominator in PTB is inflammation at the maternal-fetal interface. Prolonged cervical inflammation can cause tissue damage, potentially contributing to CI and shortening, often culminating in PTB. (204, 281-283) Recent investigations have shown a potential connection between CI and heightened inflammation, with women experiencing CI presenting increased levels of IL-6 in the amniotic fluid. (286)

Research indicates that up to 40% of preterm births can be attributed to intrauterine infections, Research indicates that up to 40% of preterm births are attributed to intrauterine infections, typically caused by bacteria ascending from the vaginal and cervical regions. While infections may serve as the primary trigger for inflammation and preterm birth, exploring the role of infection is beyond the scope of this paper. (11, 176, 204, 274)

Various non-infectious stimuli can also induce sterile inflammation, heightening the risk of premature CR. (204) or example, sterile inflammation induced by reactive oxygen species (ROS) can amplify the inflammatory response, ultimately leading to the remodelling of the cervical ECM. (283, 284) Additionally, a non-reversible state of EMT can be induced by various factors, leading to persistent inflammation and degradation of the cervical matrix. (197, 218, 234)

○ Infection

Cervical infections play a critical role in determining pregnancy outcomes, capable of inflicting damage on the cervix and accelerate CR, ultimately leading to PTB. (204) Research indicates that up to 40% of preterm births can be attributed to intrauterine infections, commonly resulting from bacteria ascending from the vaginal and cervical regions. (11, 176) Previous studies using traditional 2D in vitro cultures and animal models, such as mice, have highlighted how infections and inflammation can damage the cervical epithelial layer, accelerate cervical ripening and lead to preterm birth. (201) It is hypothesized that the interaction between pathogens and cervical epithelial cells may trigger an inflammatory response, leading to premature CR. (201)

Infections induce cell-specific changes that contribute to cervical damage and remodelling during pregnancy. These changes include inflammation, EMT, cell death (apoptosis, necrosis and autophagy) and senescence. (197) Additionally, there may be a decrease in

the expression or degradation of tight junctions within this layer, which could contribute to the pathogenesis of infection-mediated PTB. (162, 264)

Experiments with LPS and TNF- α have shown increased levels of inflammatory cytokines, such as IL 6 and IL-8 and MMP-9 in both ectocervical and endocervical epithelial cells, disrupting the cervical epithelial barrier. This disruption of the cervical epithelial barrier leads to increased permeability and dysfunction, offering insight into how inflammation might contribute to CR. (169, 201) Additionally, infections have been shown to elevate apoptosis and senescence in cervical epithelial cells, thereby contributing to tissue remodelling during pregnancy. This is evidenced by the presence of higher apoptotic nuclei in human cervical stromal biopsies obtained from women in active labour compared to those not in labour. (287)

LPS mouse models have demonstrated a promotion of EMT-induced breakdown of the cervical epithelial barrier and loss of E-cadherin. (275) A non-reversible state of EMT induced in response to infection or other risk exposures may lead to persistent inflammation and degradation of the cervical matrix, ultimately resulting in preterm birth. (197, 218, 234)

Among pathogens linked to preterm birth, *Ureaplasma Urealyticum* is notably prevalent in cervical infections, with *Chlamydia Trachomatis*, *Enterococcus* spp. and *Mycoplasma Hominis* also frequently involved. (288-290). The efficacy of antimicrobial treatment in significantly reducing preterm delivery rates among patients with these infections underscores their infectious aetiology. (291) Furthermore, polymicrobial genital infections and specific alterations in vaginal flora and microbiota composition heighten preterm birth risks, whereas a cervical microbiome enriched with *Lactobacillus Crispatus* correlates with diminished risks of intra-amniotic complications and PPRM. (204)

Viral pathogens are also linked to PTB. For example, herpes simplex virus type 2 infection in mice models of pregnancy induced collagen remodelling, increased hyaluronic acid synthesis and altered hormonal responsiveness, thereby promoting premature cervical ripening and PTB risk. (292) Human papillomavirus (HPV) infections also have been linked to various adverse pregnancy outcomes, including PTB. (180) Furthermore viral infection and may render the cervix more susceptible to pathogenic bacterial infections, further escalating the risk of PTB. (204, 293)

- **Sterile inflammation**

A common denominator in PTB is inflammation at the maternal-fetal interface. Cervical inflammation, whether linked to infections or not, poses a significant risk of damaging the cervix and accelerating CR, often culminating in PTB. (204, 282) Consequently, some researchers characterize the normal maturation of the uterine cervix as a sterile inflammatory process that drives its remodelling, thereby facilitating dilatation. (281)

Cervical ripening shares striking similarities with the inflammatory process. Emerging evidence indicates that prolonged inflammation may inflict tissue damage, potentially contributing to the onset of CI and shortening of the cervix. (283) Infections serve as the primary trigger for inflammation and preterm birth. However, various non-infectious stimuli can also induce sterile inflammation, heightening the risk premature CR. (204)

Recent investigations supporting this evidence, shown a potential connection between CI and heightened inflammation. Studies have observed elevated levels of IL-6 in the amniotic fluid of women with CI, particularly in cases with shorter latency periods following rescue cerclage procedures. (286)

The induction of sterile inflammation in studies on oxidative stress further supports these findings. ROS have been shown to significantly upregulate inflammatory reactions in the cervix, ultimately leading to remodelling of the cervical ECM. (283, 284)

Additionally, a non-reversible state of EMT can be induced by various factors, leading to persistent inflammation and degradation of the cervical matrix. (197, 218, 234)

III. ENVIRONMENTAL FACTORS

Numerous endogenous and exogenous factors play pivotal roles in precipitating premature CR.

- **Oxidative stress**, induced by external factors such as cigarette smoke, has also emerged as a significant factor linked to premature CR. (233) A delicate balance between ROS and antioxidants is crucial for tissue remodelling, growth and overall maternal-fetal health. Imbalances in this redox equilibrium have been associated with premature senescence and inflammation, thereby increasing the risk of PTB. (284) The role of Oxidative Stress in women with cervical incompetence and short cervix remains inconsistent in existing literature. (283, 284)

- **Cervical trauma** and anatomical malformations have long been associated with preterm birth. (21, 86, 93, 294, 295)
- **Endocrine factors** such as the balance between progesterone and oestrogen, appears to extend to the cervix. (163, 203, 233) However, their specific effects on the uterine cervix during PTL and delivery are not fully understood.
- **Mechanical factors** also contribute to premature CR, with an increase in mechano-sensitive molecules suggesting a response to forces induced by the foetus. While we acknowledge the influence of fetal weight on CR, our understanding of mechanical stimulation remains limited. (203)

IV. GENETIC FACTORS

Recent research underscores the significant role of genetic factors in predisposing individuals premature remodelling. While cervical trauma and inflammation contribute to CI, genetic predispositions explain why only a subset of women with such exposures experience CI and subsequent PTB. (73, 294)

Studies indicate a genetic basis for CI, highlighting polymorphisms in genes involved in connective tissue metabolism and inflammation as key contributors to the condition. (163, 255) For example, Women with defects in genes responsible for collagen assembly or metabolism, including collagen 1A1 and TGF- β , are at heightened risk of PTB due to disruptions in connective tissue and ECM regulation. (285) Genetic anomalies affecting collagen and elastin synthesis, as observed in Ehlers–Danlos and Marfan syndromes, also increase susceptibility to cervical insufficiency. (163, 296)

Furthermore, variations in genes governing pro and anti-inflammatory responses play a role in CI risk. For example, polymorphisms in the promoter region of the IL-10 gene are more prevalent among women with CI compared to controls, indicating a genetic predisposition to altered inflammatory processes. (255, 294)

2.8 SCREENING FOR PRETERM BIRTH

Recognized as a leading cause of neonatal morbidity and mortality worldwide, PTB screening is a cornerstone of prenatal healthcare. Historically, preventive measures primarily addressed symptoms as they appeared (threatened PTB or PPROM), but recent advancements in PTB screening have shifted the focus toward identifying potential risk factors during the asymptomatic phase of pregnancy. This proactive approach employs various innovative methods to detect early signs of activation in the three critical components of the uterine environment:

- **Uterine Activity:** Using external tocodynamometers or electromyography to assess uterine contractions.
- **Cervical Changes:** Identifying changes in the cervix through digital examination or ultrasound.
- **Membrane or decidual activation:** Identifying markers such as fetal fibronectin, insulin-like growth factor binding protein-1 (IGFBP-1) and placental alpha microglobulin-1 (PAMG-1).

The strategic use of these screening methods aims not only to more effectively identify women at risk of preterm delivery but also to enable timely interventions that can reduce the risks associated with preterm birth. This approach is key to improving outcomes for both mothers and their babies. Over time, various screening strategies have been proposed and refined to enhance early detection and management of PTB.

2.8.1 CLINICAL HISTORY

The traditional approach to antenatal screening for spontaneous PTB has leaned on maternal characteristics, encompassing factors like age, race, smoking habits and obstetric history. Recognized risk factors include a prior history of sPTB, cervical dysplasia treatment and the presence of uterine anomalies. (246, 297-300) Notably, the most potent predictor of preterm birth is a previous preterm birth or late miscarriage, carrying a significant 32% likelihood of recurrence. (301, 302) Women with such histories, as well as those who have undergone

cervical excisional surgery, are typically classified as high-risk for preterm birth. (See chapter 2.4 Risk factors of spontaneous preterm birth).

However, it is crucial to recognize that only about 10% of sPTB occur in women with a history of preterm delivery and approximately 85% of PTB happen without any notable obstetric history. Relying solely on these identified risk factors has not significantly reduced the rates of spontaneous preterm delivery. (256, 303-305)

Attempts to develop risk scoring systems based on maternal factors to categorize women into high- or low-risk groups have yielded limited success, with low detection rates and high false-positive rates and prediction models based solely on maternal characteristics have demonstrated limited clinical utility. (306-309)

2.8.2 CERVIX EVALUATION TO PREDICT PRETERM BIRTH

As previously emphasized, the uterine cervix plays a crucial role in sustaining pregnancy and orchestrating the complex process of childbirth. Extensive research has highlighted the importance of cervical structural changes in increasing the risk of preterm birth. These alterations in cervical structure often precede spontaneous preterm delivery by several weeks, providing a valuable window of opportunity to identify women at high risk and implement timely measures to prevent or delay PTL. (10, 253).

Traditional methods, such as histopathological analysis through cervical biopsies, have provided important insights into cervical characteristics but are impractical during pregnancy due to inherent risks. (310) In contrast, non-invasive approaches like cervical length measurement through imaging or biomarker analysis have emerged as promising tools for assessing cervical integrity and identifying women at risk of preterm birth. (163, 311-320)

By incorporating these non-invasive screening methods into prenatal care, healthcare providers can more effectively identify and manage women at risk, enabling targeted interventions.

A) DIGITAL

Preterm screening through digital examination has long been a fundamental practice in obstetrics, aiming to identify cervical changes that may signify an impending preterm birth. (321)

Early cervical changes detected through palpation have been linked to an increased likelihood of preterm delivery. (322, 323) In 1964, the Bishop scoring system was introduced, correlating cervical dilation, effacement, consistency and position as assessed by digital examination with the timing of labour onset. (324) the identification of a shortened cervix and the patency of the internal os during vaginal examination has shown potential in predicting PTB. (241, 325, 326)

However, while digital examination has been a traditional method for evaluating cervical status, research has highlighted its limitations, particularly its subjective nature and the variability among examiners, which can lead to inconsistent interpretations and unreliable predictions of preterm birth risk. Despite these challenges, digital examination remains an important aspect of obstetric practice. (247, 321)

Nonetheless, ultrasonographic cervical assessment has emerged as a more precise and reliable alternative. Studies consistently demonstrate that ultrasound is superior to digital examination in evaluating cervical integrity and identifying preterm birth risk. (327-329)

B) ULTRASOUND

Ultrasound technology has revolutionized prenatal care, offering a window into the womb that enables healthcare providers to monitor the health and development of the foetus throughout pregnancy. Its role in PTB screening has become increasingly significant, providing crucial insights to clinical decisions. Research dating back to Anderson in 1990 has consistently demonstrated an inverse relationship between cervical length and the risk of PTB, with shorter cervical lengths indicating a higher risk of preterm delivery. (330)

This correlation has been reinforced by subsequent studies across both low- and high-risk patient groups, (246, 248, 331, 332) underscoring the value of cervical length measurement as a part of preterm birth screening protocols.

Beyond cervical length, ultrasound has been instrumental in identifying other potential indicators of PTL risk, such as cervical funnelling, the presence of intra-amniotic "sludge" and cervical angle. These parameters offer additional layers of insight, further enhancing the ability to predict and manage the risk of PTB. (139, 246, 247, 249-252, 256, 257, 310, 333-338) Recent advancements in ultrasound technology, including three-dimensional (3D) imaging and Quantitative Ultrasound (QUS) techniques, have expanded the capabilities of prenatal

care, providing even more detailed and accurate assessments of cervical health. These technological advancements continue to enhance our understanding of preterm birth risk, allowing for earlier and more precise interventions aimed at improving maternal and neonatal outcomes. (310)

I. CERVICAL LENGTH:

Transvaginal ultrasonography (TVUS) has emerged as the "gold standard" for cervical assessment, recognized for its unparalleled accuracy and efficacy. (339, 340) A multitude of investigations have validated a robust and consistent association between cervical length and the risk of preterm delivery. (244-246, 307, 330-332, 341-353) In light of these findings, TVUS measurement of cervical length emerges as the most specific and effective clinical tool for assessing the risk of PTB, encompassing both low-risk and high-risk asymptomatic women, however, critical questions remain regarding whom to screen. (246, 248, 310, 331, 332)

○ How to measure cervical length

In the realm of uterine cervix imaging, various approaches such as transabdominal, transvaginal and transperineal methods offer distinct vantage points for visualizing the uterine cervix. (168)

The pivotal advantage of TVUS lies in its ability to position the transducer close to the cervix, allowing for superior precision in orientation, anatomical delineation and image quality compared to other imaging modalities. Unlike other methods, TVUS's accuracy remains unaffected by external factors such as maternal obesity, cervical positioning, or interference from fetal anatomy and it features a low interobserver variation rate of 5-10%, which highlights the consistency and reliability of the results. (340) Additionally, TVUS is both safe and well-accepted by patients, with studies showing that over 90% of women experience minimal discomfort or embarrassment during the procedure. This high level of acceptability is significant given the intimate nature of the examination and reflects its design for patient comfort. Moreover, many patients prefer TVUS over digital examinations, further testament to its favourable reception. (332)

For these reasons, TVUS is considered the "gold standard" for cervical assessment in PTB prevention, offering the most reliable and effective method for screening and ensuring improved quality of care and patient outcomes. (339, 340)

Transabdominal ultrasound (TAUS) provides an alternative method for evaluating the uterine cervix during pregnancy, but it has several notable limitations:

- **Image Quality:** A US is often hindered by lower image quality, which complicates the visualization of critical landmarks such as the internal and external os. Studies indicate that the cervix is only visible in about half of the women scanned with an empty bladder. (331, 354) Although a distended bladder can improve visualization, it can also compress and artificially lengthen the cervix, leading to the underdiagnosis of a short cervix. (310, 331, 354).
- **Measurement Accuracy:** TAUS tends to overestimate CL by approximately 8 mm in women with a short cervix, resulting in a significant underdiagnosis rate of 57% and lower test specificity. (310, 355-357) It also has a poor detection rate for other ultrasound parameters like cervical funnelling and sludge. (242, 331)
- **External Factors:** the accuracy of TAUS is affected by factors such as segmental contractions, bladder compression, maternal obesity, cervical position and shadowing from fetal parts. (256, 340)
- Despite its limitations, TAUS is generally more acceptable to women due to its greater patient comfort, lower cost and reduced time requirements compared to TVUS. (331) Although TAUS may not be feasible for approximately 20% of women, a two-stage screening approach could mitigate the burden and costs associated with universal transvaginal cervical length screening. (358-360) evidence suggests that an initial TAUS with a CL measurement of <2.9 cm can effectively identify a shortened cervix, making TAUS a viable initial screening tool. If the cervix measures ≤ 2.9 cm or is poorly visualized on TAUS, a follow-up transvaginal CL assessment is recommended. (361) Additionally, a TA cervical length measurement of ≥ 35 mm can effectively rule out the possibility of a transvaginal cervical length <30 mm, potentially avoiding unnecessary transvaginal ultrasounds.
- Transperineal ultrasonography, also known as translabial ultrasound, is a non-invasive technique used for evaluating the uterine cervix. Developed before the widespread use

of transvaginal probe, it offers an alternative route for cervical assessment. . A significant limitation of this technique is its susceptibility to interference from bowel gas, which can obscure important cervical landmarks and hinder accurate visualization. Consequently, transperineal ultrasonography is typically employed as a secondary option, particularly in cases where transvaginal ultrasound is not feasible, such as in instances of severe vaginal bleeding or vaginismus. (256, 357)

- **TV CL measurement technique**

The Fetal Medicine Foundation (FMF) has developed comprehensive guidelines and certification processes for measuring cervical length using TVUS to ensure accuracy, consistency and effective clinical application. Before the examination, patients are adequately informed about the procedure to ensure comfort and cooperation. The examination itself is recommended to be carried out with the patient in a lithotomy position and with an empty bladder to facilitate the best possible view of the cervix. Utilizing a high-resolution transvaginal probe, the clinician gently inserts it into the vagina, aiming for the anterior fornix to obtain a clear mid-sagittal view of the cervix. It's crucial that both the internal and external os are visible for accurate measurement. The FMF suggests taking three straight measurements from the internal os to the external os and recording the shortest. Dynamic assessment, such as through the Valsalva manoeuvre, is optional, it can provide additional insights into cervical functionality. (362) Indeed, organizations such as the International Society of Ultrasound in Obstetrics and Gynaecology (ISUOG) endorse similar measurement techniques. (363)

- **When to screen**

Transvaginal CL screening is recommended between 16 and 24 weeks gestation. (252, 340, 345, 364-366). Studies consistently demonstrate that between 18 and 24 weeks' gestation, transvaginal measured CL serves as a robust predictor of PTB in asymptomatic women, regardless of prior risk. (252, 367)

Some single-centre studies suggest first-trimester CL screening might predict early spontaneous PTB. (368-370) However, the overall evidence for first and early second-trimester CL measurements has not consistently demonstrated sufficient predictive value

for preterm birth, particularly in asymptomatic women without a prior history of PTB. (340, 370-374). Most experts recommend against routine CL measurement before 16 weeks due to the underdevelopment of the lower uterine segment, which complicates differentiation from the endocervical canal. (329, 374)

After 24 weeks of gestation, the risk of sPTB in asymptomatic women continues to rise as cervical length decreases. (375) Gulersen et al. found that among asymptomatic women with singleton pregnancies screened between 23 and 28 weeks, those with a CL ≤ 10 mm had a significantly higher risk of preterm birth. (376) Despite this, routine cervical length screening beyond 24 weeks is not recommended for asymptomatic women, as no interventions have demonstrated effectiveness in improving outcomes at this stage. (340, 377-379) In contrast, cervical length evaluation after 24 weeks can be advantageous for women with threatened preterm labour. (380)

Serial CL measurements have been explored as an alternative to a single measurement, with some studies suggesting that CL shortening over time correlates with increased PTB risk. (381-389) This approach has been proposed as a potentially better predictor of sPTB than a single measurement. (385) Nonetheless, several studies have found no significant difference in predictive accuracy between serial measurements and a single measurement. (353, 367, 390-392) Due to increased costs associated with serial screenings and the lack of confirmed benefits, the more widely accepted recommendation is a single transvaginal CL measurement between 18 and 24 weeks for assessing PTB risk in asymptomatic pregnancies without other risk factors. (353, 367, 374) or high-risk women, serial measurements from 16 to 23 weeks and 6 days may significantly enhance predictive accuracy. (353, 367, 374)

In summary, a single CL measurement between 18 and 24 weeks is generally more effective for predicting PTB in low-risk asymptomatic pregnancies, while high-risk women may benefit from serial measurements between 16 and 23 weeks and 6 days.

○ **Cervical length distribution and cut-off**

Cervical function was once simply categorized as either competent or incompetent, but research has shown that the risk of PTB increases as CL decreases. (242-247)

A landmark study by Iams et al. revealed a bell curve distribution of cervical lengths, with the 50th percentile corresponding to 35 mm. They identified a heightened risk of sPTB with diminishing cervical length, establishing a clinically relevant threshold at the 10th percentile (< 26 mm), offering sensitivity of 37.3%, specificity of 92.2% and a negative predictive value (NPV) of 97.4%. (246) Subsequent studies have echoed these findings and a mid-trimester CL of ≤ 25 mm has been considered short, aligning with the 10th percentile for this gestational age group. (245, 385)

Recent research, however, suggests that the prevalence of a cervix < 26 mm is lower than previously estimated, ranging from 3.2% to 10.7%, with some studies reporting even lower prevalence ($< 1\%$). (245, 246, 339, 377, 378, 393-399) Based on this data, a CL of < 26 mm before 24 weeks of pregnancy corresponds to the 2nd-3rd percentile, while a CL of 30 mm aligns with the 10th percentile. (250, 400-404)

This evolving evidence highlights the importance of using population-specific percentiles CL assessment. Factors influencing the optimal cut-off include maternal characteristics, ethnic background, gestational age and the prevalence of PTB:

- **Population variability:** The mean CL at 24 weeks gestation is 34 mm in the USA, 38.3 mm in Iran, 39 mm in Switzerland and 37.9 mm in Turkey. (402, 405, 406)
- **Ethnicity:** The 5th percentile for CL between 16-24 weeks of gestation ranges from 27 mm in Chicago to 30 mm in an American Hispanic population. (402)
- **Previous PTB:** The incidence of a short cervix (defined as ≤ 20 mm) varies, with 2.2% in a low-risk group and 6.0% among women with a previous preterm delivery. (397).
- **Maternal characteristics:** Shorter mid-trimester CL is associated with higher maternal weight, younger maternal age, nulliparity and non-Caucasian ethnicity. (407)
- **Gestational age:** The distribution of CL varies with gestational age, meaning that a CL of < 26 mm correspond to different percentiles depending on the gestational age. (386)
- **Prevalence of PTB:** the effectiveness of CL cut-offs depends on the prevalence of PTB in a given population. In areas with high PTB rates, shorter CLs are better predictors of PTB, while in low-prevalence regions, the same cut-offs may lead to less accurate predictions and higher false-positive rates. (408)

For example, using CL normograms based on North American data may result in higher false-negative rates when predicting PTD in patients from other regions, such as

Switzerland or Iran. This highlights the need for normograms tailored to specific populations, taking into account both gestational age and population-specific percentiles. The predictive accuracy of CL for sPTB largely depends on the chosen cut-off value. (374) Thresholds have been proposed across a range from <15 mm to ≤ 30 mm. (339, 377, 378, 393, 395-397, 404, 409-413) Lower cut-offs typically improve specificity but may decrease sensitivity, while higher cut-offs increase sensitivity but may reduce specificity. (403) Despite differing recommendations, a CL of ≤ 25 mm remains the most commonly used and best-supported cut-off for predicting PTB across many studies and guidelines. (329, 339, 340, 345, 366, 377, 400, 401, 411, 414-416)

- **Resume:** TUCL measurement in the second trimester is the most reliable single predictor of sPTB. (168, 301, 310, 385, 417-419) A cervical length cut-off of 25 mm is widely accepted to define a short cervix. (329, 339, 340, 345, 362, 366, 377, 400, 401, 411, 414-416, 420) Studies indicate that TVUS CL has high specificity and positive likelihood ratios, although sensitivity and negative likelihood ratios are variable and generally low. This variability is attributed to the heterogeneous nature of spontaneous PTB, which involves multiple etiological factors. (247, 258)

II. FUNNELLING

In addition to CL, other ultrasonic markers have been proposed to evaluate the risk of preterm birth. (242) Funnelling, characterized by the opening of the internal cervical os, is linked to an increased risk of preterm birth in several studies. (246, 249-252) Some researchers suggest that funnelling might indicate early signs of isthmocervical insufficiency. (297, 421, 422)

However, funnelling alone is not considered a sufficient independent risk factor when cervical length is normal. In women with a long cervix, funnelling does not increase the risk of preterm delivery. Its clinical impact is limited and it provides little additional value in predicting preterm birth when used alone. (139, 333, 423-425) While funnelling combined with a short cervical length may improve screening sensitivity for preterm birth from 61% to 74%, logistic regression analyses show that funnelling, when used in conjunction with cervical length, does not significantly enhance the prediction of spontaneous delivery before 33 weeks. (251, 403, 424)

III. SLUDGE

Amniotic sludge, characterized by a collection of echogenic particles adjacent to the internal cervical os within the amniotic fluid, was first described in 2005. (338) Initially mistaken for blood clot, this debris is now recognized as an inflammatory exudate composed of fibrous material, white blood cells and bacteria. (426) It appears to serve as a marker of microbial invasion, representing an independent risk factor for histologic chorioamnionitis and microbial colonization of the amniotic cavity. (250, 256, 257, 337, 338)

The presence of 'sludge', emerges as one of the most potent and specific indicators of early sPTB, even proving to be an independent risk factor in the context of a short cervix. (139, 247, 250, 256, 257, 336-338)

The combination of sludge and a short cervix significantly amplifies the risk of preterm delivery before the 28th and 32nd weeks. (256, 337, 338) Studies have shown that amniotic fluid sludge independently predicts preterm birth in asymptomatic women and when combined with a short cervix, it enhances the prediction for delivery before 28 weeks. (139, 337, 427, 428) However, there is insufficient evidence to suggest that intra-amniotic sludge alone improves prediction accuracy beyond that provided by cervical length. (250, 257)

IV. CERVICO-UTERINE ANGLE

Another innovative method involves using the uterocervical angle (UCA) to assess the risk of PTD. Utilizing images obtained during the second trimester via TVUS, clinicians evaluate the angle formed by drawing lines along the longitudinal axes of the uterus and cervix.

Several studies have demonstrated a correlation between the uterocervical angle and the risk of preterm birth. (310, 333-336, 429) Sochacki-Wojcicka et al. proposed that angles between the uterus and cervix could be associated with gestational age at delivery. (335) A wide UCA (greater than 105°) detected during second-trimester ultrasound is believed to be linked with a higher risk of preterm birth. (334, 429, 430) It is theorized that an obtuse UCA facilitates the transmission of forces from the expanding uterus and adjacent organs to the cervix, potentially triggering its dilation. Conversely, an acute UCA may help maintain cervical integrity. (430, 431)

While recent research highlights the potential of the UCA as a screening tool for predicting preterm birth, it has not yet been widely adopted as a standard clinical indicator. Further validation is needed to establish its clinical utility. (430, 432)

V. OTHERS

Other ultrasonic markers have been investigated, but none have consistently demonstrated clinical utility. One such marker is the Cervical Gland Area, which is the sonographically hypoechoic or hyperechoic zone surrounding the endocervical canal. (256, 433-435)

Another notable marker is the "moon sign," which describes the detachment of fetal membranes from the decidua at the level of the internal cervical os. This phenomenon has been investigated for its potential association with PRRM and PTB. (436, 437)

Despite its potential significance, more studies are required to establish its clinical relevance. (438)

VI. NEW ULTRASOUND TECHNOLOGY IN DEVELOPMENT

QUS microstructural imaging provides a detailed assessment of the cervix by analysing its structural parameters, including length, echogenicity, texture and vascularity. This method evaluates key cervical tissue properties such as collagen content and vascularization, which are crucial for cervical function during pregnancy. By detecting abnormalities early, QUS imaging helps identify risks of preterm birth or other complications. (310)

Ultrasound imaging of the cervix using 3D technology offers a promising approach to assessing the risk of preterm birth. It enhances the evaluation of cervical characteristics, such as length, shape, volume and consistency. This method provides superior visualization and spatial orientation compared to traditional techniques, improving both measurement accuracy and reproducibility. Additionally, 3D imaging captures dynamic changes in the cervix over time, facilitating the longitudinal monitoring and management of pregnancies at risk for preterm birth. (310, 439-441)

C) OTHER IMAGING METHODS

I. MAGNETIC RESONANCE IMAGING

Magnetic Resonance Imaging (MRI) provides valuable insights into cervical health during pregnancy, offering high-resolution images that complement traditional ultrasound methods. MRI is particularly effective in accurately measuring cervical length and visualizing features such as sludge, which may indicate an increased risk of. (310, 442)

MRI also assesses cervical tissue properties through T2-weighted images, evaluating hydration and differentiation and uses Magnetic Resonance Elastography to measure tissue stiffness, potentially identifying PTB risk. (310) Additionally, combining Diffusion-weighted MRI with T2-weighted MRI creates apparent diffusion coefficient (ADC) maps. Elevated ADC values are associated with a higher risk of PTB, especially in patients with a short cervical length. (310, 443)

Furthermore, MRI can assess cervical tissue properties such as hydration through T2-weighted sagittal images. Signal intensity in these images correlates with gestational age and cervical stromal differentiation, providing valuable insights into CR and PTB risk. (444)

Despite these advantages, challenges such as limited MRI availability, higher costs and longer imaging times constrain its widespread clinical use. (310)

II. OPTICAL IMAGING

Optical imaging is a non-invasive technique that uses light to visualize biological tissues. It includes modalities like OCT, fluorescence imaging and diffuse reflectance spectroscopy.

In assessing PTB, optical imaging offers high-resolution visualization of cervical tissue and physiological changes linked to PTB risk. For example, OCT provides detailed cross-sectional images of the cervix, revealing microstructural features such as cervical length, collagen organization and vascularity, which can indicate PTB risk. (310, 317)

Fluorescence imaging techniques can visualize specific molecular targets or cellular processes in cervical tissues, offering insights into inflammation, infection, or structural changes associated with PTB. (310, 318)

2.8.3 BIOMARKERS TO PREDICT PRETERM BIRTH

Biomarkers, which reflect physiological or pathophysiological changes across different levels of biological organization, can provide valuable insights into the complex pathways that lead to preterm birth. They serve as critical indicators of disease onset and underlying biological processes, offering potential for early detection and understanding of preterm birth mechanisms. (139)

Despite extensive research across various biological fluids (saliva, amniotic fluid, urine, cervical mucus, serum/plasma), a singular, reliable biomarker for predicting sPTB remains elusive. IGFBP-1 and fetal fibronectin (fFN) in cervicovaginal secretions have emerged as prevalent tools in PTL prediction clinical practice. (445)

A) EXTRACELLULAR MATRIX DEGRADATION-RELATED BIOMARKERS

Fetal Fibronectin is an ECM protein found in cervicovaginal fluid until around the 24th week of pregnancy. After this point, its presence indicates choriodecidual disruption and is associated with an increased risk of sPTB. (139, 446-451) Additionally, it has been linked to clinical and histologic chorioamnionitis, suggesting a role in infection-related pathways that may lead to PTL. (160)

In clinical practice, fFN is a powerful biochemical marker for predicting sPTB due to its high NPV; only about 1% of women with a negative fFN test deliver within the following week. (11, 251, 450, 452) However, the test's sensitivity and positive predictive value (PPV) are lower, with false positives potentially caused by factors like sexual intercourse, cervical examinations, vaginal bleeding, or douching. (11, 451) Studies have demonstrated that combining fFN testing with cervical length measurements significantly enhances predictive accuracy and improves risk stratification for PTB. (451, 453, 454)

IGFBP-1 detected in cervicovaginal secretions and serves as a promising biomarker for predicting sPTB. As delivery approaches, fetal membranes detach from the decidua, leading to the leakage of IGFBP-1 into cervical secretions. This leakage serves as a current method for predicting sPTB. (139, 251, 445, 455) Combining IGFBP-1 with cervical length measurements yields greater specificity and PPV PTB. (456-458)

B) HORMONAL

Estrone-3-glucuronide (E3): Maternal salivary E3 levels correlate with serum levels and are associated with increased PTB risk. Despite its predictive potential, salivary estriol's routine use as a screening test for predicting sPTB in asymptomatic women remains limited (11, 139, 251, 459-461) Estradiol/estriol ratio have also been under investigations. (139)

CRH: Elevated maternal CRH levels during the mid-trimester are linked to higher risks of preterm birth. However, its predictive efficacy in low-risk populations is limited. (462, 463)

C) INFLAMMATORY MARKERS

Pro-inflammatory cytokines respond to infections at the maternal-fetal interface, prompting uterine contractility and cervical changes, making them potential screening tools for PTB. (139, 451, 464) Although amniotic markers are considered most effective, their diagnostic utility is limited by the scarcity of clinical settings performing amniocentesis. Consequently, cervical vaginal fluid (CVF) has become the preferred fluid for assessing risk biomarkers. (465, 466)

Among these markers, IL-6 exhibits the greatest predictive value. Elevated IL-6 concentrations in cervical fluid during PTL are associated with both intra-amniotic infection and sterile inflammation, highlighting its potential as a predictive marker for adverse outcomes. (139, 149, 204, 464, 465, 467-469).

Similarly, levels of IL-8 and monocyte chemoattractant protein-1 in CVF significantly increase in patients with intra-amniotic infections and/or inflammation, underscoring its potential as a predictive marker. (204, 221, 445, 465)

High levels of C-reactive protein, AMPs and metabolites in CVF have also shown promise as biomarkers for predicting PTB. (204, 465, 466, 470)

D) VAGINAL MICROBIOME

The correlation between the vaginal microbiome and preterm delivery offers insights into predicting delivery in high-risk asymptomatic women, though practical predictive tests are still in development. (261, 471, 472)

E) GENETIC MARKERS

Proteomic, Transcriptomic, Genomic and Metabolomic studies: Advanced "omics" approaches, including those from the Proteomic Assessment of Preterm Risk study, are being utilised to identify novel biomarkers for sPTB. (139, 451) Studies on genes involved in inflammatory mechanisms, such as IL6, NOS1 and TNF α , have been under study. (139) However, despite the potential of genetic screening tests, their clinical application is still limited by the complexity of interpreting results and the absence of targeted interventions for specific genetic variants. (451)

Epigenetics: Epigenetic modifications, such as nucleotide methylation, are also under study, as they can influence gene activity and provide a dynamic perspective on gene expression, particularly as it is affected by environmental factors. (473)

Fetal DNA: cfDNA has emerged as a promising predictive marker for PTB. (130, 148) (167) Studies have shown that elevated levels of cfDNA in the midtrimester are associated with an increased risk of sPTB later in gestation. (135, 146, 474)

F) OTHER BIOMARKERS

Numerous other markers have been investigated in the quest to predict PTB.

These include MMP-3, MMP-9, tissue inhibitor of metalloproteinases (TIMP) 1, TIMP-2, TIMP-3, TIMP-4 (475), alfa-fetoprotein (251) (457), Intercellular Adhesion Molecule 1 (251), serum ferritin (457, 476), serum granulocyte colony-stimulating factor (457), serum alkaline phosphatase (457, 477), cervicovaginal beta human chorionic gonadotropin measurement (251, 478), vitamin D-binding protein, triggering receptor expressed on myeloid cells-1 (475), Complement factor 3a and 5a in CVF (204), PAMG-1 (37), among others.

2.8.4 MODELS OF SCREENING FOR PRETERM BIRTH

While various markers have been explored for their potential in predicting PTB, none have emerged as the singular, flawless predictor. Recognizing the multifactorial nature of PTB risk, researchers have increasingly turned to the development of models that integrate multiple markers. By incorporating diverse markers—such as biochemical, ultrasonic and clinical indicators—into comprehensive predictive models, clinicians can enhancing both sensitivity and specificity in PTB screening. (251, 307)

One commonly evaluated risk-scoring system, developed by Creasy, encompasses factors such as maternal demographics, obstetric history, clinical parameters and lifestyle factors. Unfortunately, risk scores relying solely on maternal characteristics have shown limitations, with low detection rates and high false-positive rates. (306, 411)

An alternative method gaining traction is TUCL measurement in the second trimester. This screening test alone is associated with high specificity to predict PTB, but low sensibility and a poor area under the curve (AUC) (See chapter 2.8.2 Cervix evaluation to predict PTB – b) ultrasound).

Combining both clinical factors with CL improve the sensibility of the screening. (479)

Moreover, associations between short cervical length and other biomarkers like IGFBP-1 and fFN have been explored to enhance predictive value. (139, 399, 453, 454, 458, 480, 481)

A) ARTIFICIAL INTELLIGENCE

Artificial intelligence (AI) refers to the ability of a machine or software programme to perform tasks typically associated with human intelligence, including reasoning, learning, adaptation, understanding and interaction. There is significant potential for the use of AI in obstetric and gynaecological imaging, facilitating the training of ultrasound sonographers and providing instant image quality assurance. (482) It is also hoped that AI technologies will emerge as transformative tools in PTB screening, revolutionizing the prediction of PTB and promoting individualized care for high-risk women by enabling more precise, data-driven decision-making. (483, 484)

Woolery developed a prototype expert system for preterm birth risk assessment of pregnant women and concluded that the prototype was more accurate than traditional manual techniques in predicting PTB. AI offers a viable alternative for analysing large amounts of data and producing predictive algorithms. (485)

Lacerda de Andrade Júnior et al. conducted a retrospective observational study involving 524 unselected singleton pregnancies, each of which underwent a second CL assessment, with the objective of creating an AI screening algorithm for PTB. An AI predictive model was developed using a stacking-based ensemble learning method that incorporated a Neural Network approach, multivariate logistic regression and the best-performing AI algorithm. The study identified eight significant variables: previous spontaneous PTB before 37 weeks, smoking

history, maternal weight, cervical funnelling on TVUS, previous curettage, CL <30.9 mm, use of antibiotics during pregnancy and the straight CL/internal angle index inside the cervix. Comparing the results of the algorithm for predicting spontaneous PTB before 35 weeks' gestation with a CL of less than 25 mm, the sensitivity was 33.3% vs. 47.3%, specificity 91.8% vs. 92.8%, PPV 23.1% vs. 32.7%, NPV 94.9% vs. 96.0% and the AUC 0.32 vs. 0.81. (486)

Larger, multicentre, prospective studies utilizing AI technology and ultrasound findings are recommended to confirm these results, especially in high-risk cohorts and to provide statistical power to the findings.

2.9 Prevention of spontaneous preterm birth

PTB requires a comprehensive approach, incorporating both primary and secondary prevention strategies.

- **Primary prevention** focuses on addressing risk factors before and during pregnancy, which includes optimizing maternal health, promoting healthy nutrition, managing mental health disorders and controlling chronic conditions.
- **Secondary prevention** aims at individuals identified as at risk for sPTB, with increasing advocacy for universal screening due to PTB occurring even in the absence of obvious risk factors. (487)

Effective prevention strategies are vital for reducing preterm-related neonatal mortality and achieving global health goals. (163) While distinguishing between spontaneous and provider-initiated PTB is essential for tailoring interventions and addressing underlying causes or obstetric practices, this discussion will focus solely on strategies for preventing sPTB.

2.9.1 NON-PHARMACOLOGICAL APPROACH

Non-pharmacologic methods encompass a broad spectrum of interventions aimed at reducing the risk of preterm birth, including controlling risk factors, obtaining appropriate medical care, managing health conditions and adopting healthy lifestyle practices. Self-care practices, such as maintaining a healthy diet, exercise, minimizing exposure to environmental toxins and addressing issues such as domestic abuse, are essential in mitigating the risk of premature delivery. Optimizing medical conditions before conception, particularly for individuals with diabetes, hypertension, or asthma, can significantly improve pregnancy outcomes.

- **Bed rest** is a common intervention for the prevention and/or treatment of threatened PTB. Despite its prevalence, there is a lack of evidence supporting its effectiveness. Prospective randomized studies have not demonstrated a reduction in sPTB due to bed rest and potential harms include venous thromboembolism, bone demineralization and loss of employment. (139, 488)

- **Nutritional supplementation** has emerged as a promising avenue in the prevention of PTB. Studies highlight the importance of nutritional interventions, such as omega-3 long-chain polyunsaturated fatty acid supplementation, which has shown promise in reducing early birth, particularly among women with low omega-3 fatty acid levels. Zinc and folic acid supplementation also show potential benefits in reducing PTB rates and decreasing the risk of birth defects associated with prematurity. (488-490)
- **Smoking cessation** strategies have proven effective in reducing the incidence of preterm delivery, offering a pivotal opportunity in preventing PTB. (491-493) Various cessation strategies have proven effective in helping pregnant individuals quit smoking and subsequently reducing the incidence of preterm delivery. (20, 489, 493)
- **Assisted reproduction:** multiple gestations, often resulting from assisted reproduction, significantly increase the risk of PTB. Strategies to reduce this risk include emphasizing the reduction of the number of embryo transfers, with single embryo transfers emerging as an effective measure. (37, 112, 113)
- **Cervical cancer prevention:** Implementing cervical cancer prevention strategies, such as screening programmes and HPV vaccination, can reduce the incidence of cervical lesions and subsequent need for surgical interventions, serving as a preventive measure against PTB. (491)
- **Arabin pessary:** The cervical pessary, a longstanding silicone device, offers a minimally invasive alternative to traditional cerclage procedures for preventing sPTB. However, its efficacy in women with a short cervix during the mid-trimester remains debated. (139, 258, 378, 494-499) Initial studies showed promise, but subsequent trials reported mixed results, indicating a need for further investigation to determine the true effectiveness of the cervical pessary. (378, 495, 496, 500, 501) The Prospective Meta-analysis of Pessary Trials is currently being developed and aims to provide a clearer understanding of the role of pessary in the prevention of PTB. (502)

2.9.2 PHARMACOLOGICAL APPROACH

A) PROGESTOGEN

Progesterone plays a crucial role in maintaining pregnancy and has become a key strategy for reducing PTB. Its effectiveness is due to its ability to maintain uterine calm by suppressing

myometrial contractions, inhibiting oxytocin and preventing gap junction formation in myometrial cells. Additionally, progesterone affects the chorioamniotic membranes and the cervix, with its decreased action (functional progesterone withdrawal) being linked to cervical ripening and the onset of labour. (234, 258, 307)

The terms "progestogen" and "progestin" include both natural progesterone and synthetic compounds with similar effects. Over the past 20 years, numerous randomized controlled trials (RCTs) have investigated the use of progestogens, such as vaginal progesterone and 17-alpha-hydroxy-progesterone caproate, for preventing preterm birth. These studies have explored various administration routes, including oral, vaginal and intramuscular. (307)

- **Vaginal progesterone**

The landmark study by da Fonseca et al., representing the Fetal Medicine Foundation Second Trimester Screening Group in the United Kingdom, illuminated the protective role of vaginal progesterone in preventing preterm birth among women identified with a short cervix. In this pivotal trial, women with a cervical length of $\leq 15\text{mm}$, as determined by transvaginal ultrasound between 20 and 25 weeks of gestation, were randomized to receive either 200 mg of micronized progesterone or a placebo composed of safflower oil, from 24 to 34 weeks of gestation. The primary endpoint was the incidence of spontaneous preterm delivery before 34 weeks of gestation. Remarkably, the group treated with vaginal progesterone experienced significantly fewer preterm deliveries compared to the placebo group (19.2% vs 34.4%), with a similar rate of adverse events reported between the groups. (377)

A subsequent RCT, focused on a subgroup of 46 women with a CL < 28 mm from a larger study involving 611 women with a history of prior sPTB. These women received either vaginal progesterone or a placebo and the subgroup with a short CL receiving vaginal progesterone had a significantly lower occurrence of early PTB, reduced NICU admissions and shorter NICU stays, highlighting the targeted benefit of progesterone in women with a notably short cervix. (503)

The PREGNANT trial further corroborated the efficacy of vaginal progesterone in reducing preterm birth rates. This multicentre, randomized, double-blind, placebo-controlled study enrolled asymptomatic women with a singleton pregnancy and an ultrasound short cervix

(10-20 mm) at 19-23 6/7 weeks of gestation. Participants received either vaginal progesterone gel (90 mg) or a placebo daily until 36 6/7 weeks of gestation, delivery, or membrane rupture. The results demonstrated a significant reduction in preterm births before 33 weeks among those treated with progesterone (8.9% vs 16.1%; relative risk (RR): 0.55), indicating that treating 14 women with a cervical length of 10-20 mm could prevent one case of preterm birth before 33 weeks. Additionally, neonates born to mothers in the progesterone group showed a significantly lower incidence of respiratory distress syndrome, underscoring the dual benefit of vaginal progesterone in not only reducing the rate of preterm birth but also in enhancing neonatal outcomes. (395)

Subsequent research has provided substantial evidence supporting the effectiveness of progesterone in preventing preterm birth. (377, 395, 503-507) A meta-analysis by Romero in 2012 highlighted the benefits of treatment with vaginal progesterone. This comprehensive review demonstrated a significant decrease in the incidence of preterm birth at less than 33 weeks, less than 35 weeks and less than 28 weeks. Additionally, significant reductions were observed in respiratory distress syndrome, composite neonatal morbidity and mortality, admissions to the neonatal intensive care unit and the need for mechanical ventilation. Importantly, there were no significant differences in the rates of adverse maternal events or congenital anomalies between the groups treated with vaginal progesterone and those receiving a placebo, affirming the safety and efficacy of this intervention. (505)

The collective body of literature strongly supports the administration of vaginal progesterone to women diagnosed with an ultrasound short cervix as an effective and safe measure for preventing preterm birth and reducing neonatal morbidity and mortality in singleton pregnancies, irrespective of whether there is a history of sPTB and regardless of the specific cut-off used to define a short cervical length (<10 mm, 10-20 mm, or 21-25 mm). (508) However, this consensus was disrupted by the release of a significant multicentre trial, funded by the Medical Research Council in the United Kingdom, known as OPPTIMUM. (258) This large multicentre, randomised, double-blind trial concluded Vaginal progesterone was not associated with reduced risk of preterm birth or composite neonatal adverse outcomes and had no long-term benefit or harm on outcomes in children at 2 years of age. (509)

Romero et al. have introduced a revised iteration of their individual patient data meta-analysis (510), incorporating data from the OPPTIMUM trial. (511) This updated analysis now integrates findings from five trials involving a total of 974 women (498 receiving vaginal progesterone, 476 receiving a placebo). Notably, the inclusion of these additional data has yielded compelling evidence reinforcing the beneficial effect of vaginal progesterone among women with a short cervix of ≤ 25 mm in the mid-trimester. Specifically, the updated meta-analysis reveals a significant reduction in the risk of preterm birth associated with vaginal progesterone administration. Furthermore, this comprehensive review reaffirms the safety and efficacy of vaginal progesterone in reducing the risk of preterm birth, as well as neonatal morbidity and mortality, among women with a singleton gestation and a mid-trimester CL of ≤ 25 mm. Importantly, the analysis indicates that vaginal progesterone does not exert any deleterious effects on neurodevelopmental outcomes. (400, 511)

Compelling evidence underscores the efficacy of vaginal progesterone (VP) in reducing the risk of PTB and enhancing perinatal outcomes in singleton gestations characterized by a short cervix (≤ 25 mm), irrespective of whether there is a history of spontaneous PTB. (510) However, there lacks convincing evidence to advocate for prescribing VP solely based on a history of sPTB. A systematic review and meta-analysis incorporating data from ten RCTs revealed insufficient support for the use of VP in preventing recurrent preterm birth or improving perinatal outcomes among singleton gestations with such a history. (512) Furthermore, VP does not demonstrate efficacy in preventing preterm birth or enhancing perinatal outcomes in singleton gestations with a history of sPTB and a mid-trimester transvaginal sonographic cervical length exceeding 25 mm. (513) Hence, current evidence suggests that VP should be recommended exclusively for singleton gestations with a short cervix, regardless of any history of spontaneous PTB.

A novel formulation of micronized progesterone delivered via a Cyclogest pessary has demonstrated effectiveness in reducing the incidence of preterm birth, admissions to the NICU, as well as maternal systemic complications and associated side effects. (514) This promising development underscores the potential for exploring new therapeutic approaches in addressing the complex challenge of preterm birth prevention.

- **17-alpha hydroxyprogesterone caproate**

The differential effects of vaginal progesterone and 17-alpha hydroxyprogesterone caproate (17-OHP-C) in preventing preterm birth are evident. While vaginal progesterone has shown efficacy in reducing the rate of sPTB among women with a short cervix, 17-OHP-C has not demonstrated similar effectiveness in this population, as supported by multiple studies. (258, 404, 494, 504, 515, 516)

Meis et al. (517) reported a significant reduction in recurrent preterm delivery with weekly injections of 17-OHP-C among women at high risk, but subsequent research, including a recent RCT from 2015, found no prolongation of pregnancy in women with singleton gestations, a sonographic short cervix and other risk factors for preterm delivery. (515) This finding was further confirmed by a large-scale RCT in 2020 involving nearly 1700 pregnant women with a prior history of preterm birth, where no significant differences were observed in preterm birth rates or neonatal morbidity between 17-OHP-C and placebo groups. (518) Consequently, the US Food and Drug Administration advisory committee recommended the withdrawal of 17-OHP-C from the market, reflecting the accumulating evidence of its limited efficacy in preventing preterm birth. (307)

B) TOCOLYTICS

In the 1980s, various tocolytic agents were extensively investigated to combat premature labour. Despite these efforts, evidence that these agents effectively reduce preterm delivery rates or improve neonatal outcomes remains limited. While tocolytics can temporarily prolong pregnancy, allowing for steroid administration and maternal transfer to specialized care, they mainly address symptoms rather than underlying causes. (135, 487, 519) Common tocolytic agents include calcium channel blockers, beta-mimetics, magnesium sulphate, COX inhibitors and oxytocin-receptor antagonists, each working through different mechanisms. [69]

C) ANTIBIOTICS

Although research links infections to sPTB, the effectiveness of antibiotics in reducing this risk remains uncertain. Numerous randomized clinical trials have examined antibiotic therapy's impact on prolonging pregnancy and reducing neonatal complications, but results have been

inconsistent. Studies often show no significant benefit from antibiotics compared to placebo in terms of extending pregnancy or preventing sPTB-related issues such as respiratory distress syndrome or neonatal sepsis. (139, 520-523)

Antenatal periodontal treatment has emerged as a potential strategy to lower preterm birth rates. (524)

Additionally, while traditional treatments for BV have generally been ineffective in preventing preterm birth, early intervention with clindamycin appears promising. A meta-analysis of five randomized trials suggests that administering clindamycin before the 22nd week of gestation may reduce preterm birth rates. (525-527)

For cases of cervical insufficiency with intra-amniotic infection or inflammation, a combination of ceftriaxone, clarithromycin and metronidazole has resolved infections in 75% of cases. (528) While amniocentesis is the gold standard for detecting intra-amniotic infection, ultrasound has been used to identify potential infections based on the presence of "sludge." (529) However, a study by Ryan et al. in 2020, involving a large series of cases, failed to demonstrate the benefits of antibiotics in the presence of sludge. (487, 530)

Overall, the effectiveness of antibiotics in preventing preterm birth remains controversial, indicating a need for further research to clarify their role and efficacy. (489)

D) OTHER PHARMACOLOGICAL TREATMENTS

Antenatal CT administration do not directly impact the reduction of PTB, but it remains one of the most beneficial interventions for improving neonatal outcomes among patients who give birth prematurely. (139, 487, 531)

New therapeutic targets considering the inflammatory cycle and subsequent exacerbations have emerge. Heparin, known for its anti-inflammatory properties alongside its anticoagulant effects, stands out as a potential treatment option. (275)

2.9.3 SURGICAL APPROACH

A) CERCLAGE

Cervical cerclage, a surgical procedure involving the placement of a suture or tape around the cervix, aims to prevent cervical dilation and subsequent preterm birth. Initially described by Shirodkar in 1955, the technique involved dissecting the bladder to allow for the placement

of the suture close to the internal os. McDonald later simplified the procedure by eliminating bladder dissection, although this might result in the suture being positioned lower in the cervix. But other techniques and approaches has been described. (139, 532, 533)

The decision to perform cervical cerclage during mid-pregnancy typically hinges on one of three scenarios: history-indicated cerclage, ultrasound-indicated cerclage and physical exam-indicated cerclage. History-indicated cerclage is recommended for women with a poor prior obstetrical history, such as three or more prior PTB or second-trimester losses. Ultrasound-indicated cerclage is considered for patients with a short CL, while physical exam-indicated cerclage is based on cervical dilatation observed during manual examination. (139)

RCTs assessing the effectiveness of ultrasound-indicated cerclage have yielded contradictory results. (534-536) Althusius et al. reported that therapeutic cerclage with bed rest reduced preterm delivery before 34 weeks of gestation and compound neonatal morbidity in women with risk factors and/or symptoms of cervical incompetence and a cervical length of <25 mm before 27 weeks of gestation. (536) In contrast, Rust found that cerclage did not alter any perinatal outcome variables. Increased neonatal morbidity in these patients was believed to be associated with subclinical infection, PTB and abruption. (535) To et al. conducted a study involving 253 women with a CL $\leq$ 15 mm at mid-trimester, randomized to cerclage or expectant management, with similar rates of birth before 33 weeks in both groups. However, in women with a prior PTB and a short TVUS CL < 25 mm, cerclage reduced the incidence of preterm birth before 35 weeks compared to no cerclage. Therefore, cerclage indication in women with a mid-trimester short cervix should be reserved for those with a history of early PTB. (534)

A 2012 meta-analysis of RCTs comparing cervical cerclage to no treatment revealed a notable reduction in preterm births by approximately 20%. (537) However, another study indicated that the reduction in preterm birth was only observed when the cervical length was <15 mm (considered a secondary outcome). Additionally, this study suggested that cerclage might not be effective for women with prior sPTB whose cervical lengths fall between 15 and 24 mm. (494) Other study concluded that if cerclage is indeed effective, its benefits seem to be limited to a very specific group of women: those with a history of sPTB or second-trimester loss, whose mid-trimester cervical length ranges between 5 and 14 mm. (538)

A Cochrane review in 2017 concluded that cervical cerclage reduces the risk of preterm birth in women at high risk of preterm birth and probably reduces the risk of perinatal deaths. There was no evidence of any differential effect of cerclage based on previous obstetric history or short cervix indications. (539)

B) HYSTEROSCOPY

Surgery may be necessary to address certain uterine anomalies in patients, such as the excision of a uterine septum. (37) Studies have reported impressive outcomes following hysteroscopic septum resection, with rates of term deliveries reaching 75% and rates of live births as high as 85% post-resection. (540)

2.9.4 COMPARATIVE ANALYSIS OF INTERVENTIONS AND COMBINED TREATMENT STRATEGIES

I. PROGESTERONE VS CERCLAGE VS PESSARY

Both vaginal progesterone and cerclage were equally effective in reducing PTB and improving perinatal outcomes in women with a singleton gestation, prior spontaneous early PTB and a mid-trimester sonographic short cervix (<25 mm). (307, 541)

The SuPPoRT ("Stitch, Pessary, or Progesterone Randomised Trial") study, published in 2024, involved 386 pregnant women between 14+0 and 23+6 weeks' gestation, all of whom had a cervical length under 25 mm. Participants were randomized to receive one of the three interventions—progesterone, cerclage, or pessary. The study found that all three treatments had similar effects on the primary outcome of PTB before 37 weeks. Additionally, there were no significant differences observed for PTB before 34 and 30 weeks or in adverse perinatal outcomes. (542)

The optimal choice of treatment may therefore hinge on factors such as potential adverse events, cost and the preferences of both the patient and the clinician.

II. PROGESTERONE PLUS CERCLAGE

The combined treatment of cervical cerclage and vaginal progesterone may offer a greater reduction in preterm birth rates compared to using either therapy alone. (543-546)

III. PROGESTERONE PLUS PESSARY

A randomized clinical trial conducted on pregnancies with a short cervix at mid-pregnancy found that combining cervical pessary with daily vaginal progesterone offered no additional benefit in reducing preterm birth rates or adverse pregnancy outcomes compared to using daily vaginal progesterone alone. (547) However, a 2023 systematic review suggested that cervical pessary combined with vaginal progesterone may be both safe and effective in preventing preterm birth in singleton pregnancies and may also increase neonatal birth weight in multiple pregnancies when compared to vaginal progesterone alone. (548)

Moreover, an Arabin pessary coated with progesterone nanofibers is currently under development, offering a potential new approach to preterm birth prevention. Additionally, research indicates that micronized progesterone pessary is more effective than oral progesterone in reducing premature birth rates among women at high risk of early delivery. (514, 549)

3. PROJECT

To effectively reduce the incidence of PTB, it is imperative to deepen our understanding of its aetiology, refine screening methods and develop targeted interventions. Addressing PTB is challenging because it does not represent a single entity but a complex syndrome with multifaceted causes. Comprehensive and multidisciplinary approaches are essential to tackle this global health challenge. (135)

Historically, research on PTB has predominantly concentrated on the myometrium and fetal membranes. Despite the cervix's critical role in labour, its contribution to PTB pathogenesis has been relatively underexplored.

The concept of premature CR gained prominence in the 1990s when studies first identified a correlation between CL and PTB risk. (330) Subsequent research has reinforced this association, underscoring premature cervical shortening as a key predictor of PTB. (241, 246, 248, 256, 331, 332) Moreover, seminal work by Romero et al. characterized cervical ripening as a defining feature of the "premature parturition syndrome," underscoring the cervix's pivotal role in PTB pathogenesis. (253)

Early indicators of CR—such as shortened cervixes and patency of the internal os observed during routine vaginal examinations or via ultrasound - often precede spontaneous preterm delivery by several weeks. This suggests that CR is a primary molecular event in PTB rather than merely a consequence of myometrial activation. (10, 253).

Given this evidence, screening for early signs of CR presents a promising avenue for identifying high-risk women. Furthermore, a deeper understanding of the molecular and cellular mechanisms behind premature CR could lead to the development of novel prevention strategies.

In this context, our project aims to address a significant research gap by focusing on the cervix's role in PTB. This PhD thesis will investigate the cellular mechanisms underlying premature CR and evaluate its potential as a screening tool for identifying women at high risk of PTB. The research is organized into two interconnected studies:

- **Study I – cellular dynamics of cervical remodelling in preterm and term labour:** This study investigates the cellular mechanisms underlying CR during preterm and TL. It focuses

on comparing the density and distribution of key cellular components, including inflammatory cells (neutrophils and mononuclear cells) and fibroblasts, in cervix tissues samples collected from women experiencing PTL and TL. The findings aim to provide insights into the inflammatory and structural processes driving cervical changes in labour.

- **Study II - Second-Trimester Cervical Length as a Predictor of Preterm Birth:** This study evaluates the utility of TUCL measurements as a predictive tool for PTB, considering it a potential marker of early CR. By analysing TUCL distribution and establishing optimal cut-off values, the study assesses its diagnostic performance in identifying women at risk for PTB. Additionally, it explores the integration of TUCL with maternal risk factors to improve predictive accuracy, aiming to refine strategies for early PTB risk detection and prevention. In conclusion, the objective of this research is to investigate two complementary aspects of CR in predicting PTB. Study I examines the histological processes and cellular dynamics of CR to gain a deeper understanding of its mechanisms, while Study II evaluates the utility of second-trimester TUCL measurements as a predictive tool for PTB, considering TUCL as an indicator of early CR. Together, these studies aim to enhance our understanding of CR and potentially contribute to the improvement of PTB prediction and prevention strategies.

3.1 STUDY I: CELLULAR DYNAMICS OF CERVICAL REMODELLING IN PRETERM AND TERM LABOUR

3.1.1 OBJECTIVE

This study aims to investigate the cellular processes involved in the early stages of CR during spontaneous PTL and TL through detailed histopathological analysis and quantitative assessments. The focus is to understand the role of inflammatory cells (neutrophils and mononuclear cells) and fibroblasts in the cervix during the initiation of labour.

- **Quantify inflammatory cell densities:** The study seeks to quantify the density of inflammatory cells —neutrophils and mononuclear cells— during the initial stage of labour.
- **Compare inflammatory profiles between preterm and term labour:** Compare inflammatory profiles, specifically the distribution of neutrophils and mononuclear cells, between PTL and TL groups.
- **Explore the role of fibroblasts in labour:** Investigate the presence and distribution of fibroblasts within cervical tissue samples during the initial stage of labour.
- **Compare fibroblasts profiles between preterm and term labour:** Determine whether fibroblast densities vary significantly between preterm and term labour.
- **To provide insights into the pathophysiology of preterm birth:** the ultimate goal is to provide deeper insights into the cellular mechanisms driving CR in PTB, facilitating the development of targeted interventions to improve maternal and neonatal outcomes.

3.1.2 METHODS

Study design: We conducted a prospective observational study spanning from October 2018 to March 2021, with a 12-month hiatus due to the COVID-19 pandemic, at two Obstetrics and Gynaecology wards in Porto: Pedro Hispano Hospital and São João University Hospital.

Participants: Pregnant women were recruited and categorized into two groups: PTL, TL. A small set of preterm not in labour (PTnotL) was also included. Gestational age was determined by crown-rump length measurement in the first trimester. PTB was defined as delivery occurring between ≥ 24 weeks and < 37 weeks of gestation. The term group comprised pregnant women with a gestational age of > 39 weeks to exclude early term deliveries that

might share similar triggers with late PTL. For the labour groups, we included pregnant women in the early stage of labour, defined as cervical ripening <4 cm, as evidence suggests that the labour process is comparable in term and preterm deliveries, though the initial trigger may differ. The PTnotL group encompassed women with medically indicated prematurity and unripe cervices (defined as Bishop Score <5), with cervical biopsy performed prior to caesarean section or labour induction. Exclusion criteria included multiple pregnancies, signs of infection, cervical insufficiency and presence of cervical dysplasia in the histologic examination.

Procedures: Eligible participants underwent a random transvaginal cervical biopsy using appropriate forceps (approximately 5x5x5mm). No complications arose from the procedures. Biopsy specimens were fixed in 4% formaldehyde for up to 24 hours before embedding in paraffin, sectioned and stained with haematoxylin and eosin for histopathological analysis. The histopathological examination focused on evaluating the density of inflammatory and fibroblast lineage cells.

Cellular analysis was performed using QuPath®, a digital image analysis software. For each sample, two regions of interest (ROIs) measuring 750 × 1500 µm (1.125 mm²) were selected to encompass representative areas of the endocervix and/or exocervix, including both epithelial and connective tissue components. Within these areas, the cell detection function was applied to identify all cells. The 'Train Object Classifier' feature was then used to classify cells as neutrophils, lymphocytes, plasma cells and fibroblasts. Other cells, including vascular cells and those that cannot be classified based on morphology, were grouped under the category 'other'. For the purposes of analysis lymphocytes and plasma cells were considered together as mononuclear cells. Representative cells were manually identified by the authors to set the classifier that enabled the programme to automatically recognize each type of cell in every ROI.

To ensure objectivity, the authors were blinded to sample identities during the review and refinement of the software's output.

Subsequently, the classifications made by the software were reviewed by the authors, who identified and corrected any misclassifications through additional cell identification, thus improving the classifier. This iterative process was repeated for ROIs in all samples to ensure accurate and consistent cellular identification across the dataset (Figure 1).

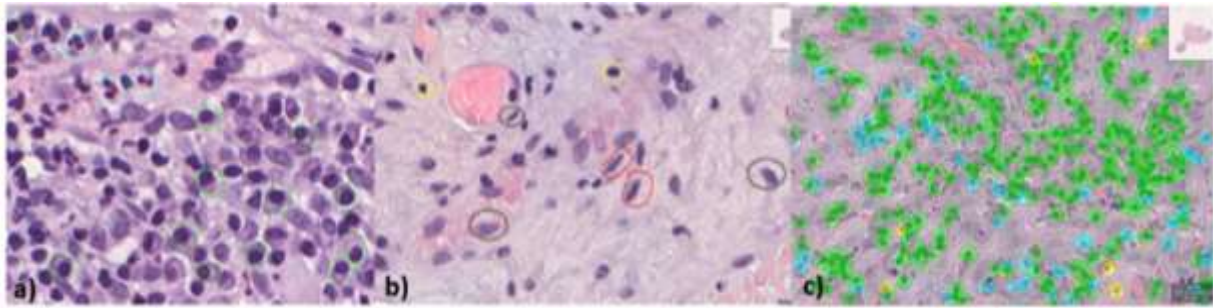


Figure 1: QuPath® cell classification.

Legend: a) and b) - manual identification of the cells; c) – automatic identification of the cells; Blue for neutrophils, Green for plasma cells, Yellow for lymphocytes, Red for fibroblast lineage and Grey for other cells.

Statistical Analysis: Continuous variables were described as median and range (minimum, maximum) given the small number of samples and the asymmetric distribution of the inflammatory infiltrate.

Statistical analysis was performed using SPSS® software. Kruskal-Wallis test was employed to determine statistical significance between women groups for different cell distribution patterns. It was also employed to evaluate differences in demographic and clinical variables between the groups. Mann-Whitney test and Wilcoxon signed-rank test were used to evaluate differences in median inflammatory cell counts between endocervix and exocervix, using independent and paired analysis, respectively. A p-value of <0.05 was considered statistically significant.

Ethical Considerations:

This study was conducted after obtaining approval from the local Ethics Committee (Ref. No. 81-17) and each participant provided informed consent. All procedures were conducted in accordance with ethical standards and guidelines.

3.1.3 RESULTS

A total of 34 singleton pregnant women participated in the study, distributed into three groups: 14 in PTL, 14 in TL and 6 in PTnotL. Reasons for early termination in the PTnotL group included severe cardiac disease, uterine dehiscence and severe growth restriction.

Demographic and clinical characteristics of the study groups are summarized in Table 3.

Table 3: Maternal and clinical characteristics of the study I population

Parameters	PTL (n=14)	TL (n=14)	PTnotL (n=6)	p value
Age (years) median (range)	31 (19, 42)	33 (24, 44)	32 (21, 39)	p=0.767
BMI (kg/m²) median (range)	21 (17, 38)	23 (17, 26)	26 (21, 35)	p=0.146
Education (n)				p=0.167
< 9 th grade	1	4	2	
10-12 th grade	3	3	1	
≥ graduation	4	6	2	
Parity median (range)	0 (0, 1)	1 (0, 2)	1 (0, 3)	p=0.271
Nulliparous (n)	8	6	3	
1 (n)	5	5	1	
2 (n)	0	3	1	
3 (n)	0	0	1	
Prior preterm birth (n)	0	0	0	
Weeks of gestation median (range)	33 (26, 36)	39 (39, 40)	35 (34, 36)	p<0.001
New born weight (g) median (range)	2540 (980-3340)	3420 (2990-3645)	2400 (1600-2650)	p<0.001
Sampled mucosa (n)				p=0.934
Endocervix	2	2	0	
Exocervix	3	4	1	
Both	9	8	5	

Legend: BMI – body mass index; PTL - preterm spontaneous labour; TL - term spontaneous labour; PTnotL - preterm not in labour

No differences were noted between groups regarding mother's age, parity and body mass index. None had a prior history of preterm birth. Throughout both the parturition and postpartum periods, no signs of infection were observed in any of the participants.

The cervical samples were collected during a phase of cervical effacement and dilation, which made it difficult to visually identify the transformation zone. As a result, there was variability in whether the biopsy included endocervical or exocervical tissue, depending on the sample. Out of the 34 samples analysed, 26 contained endocervix, 30 had exocervix and 23 included both endocervix and exocervix. In general, all samples had some degree of inflammatory infiltrate, with intensity ranging from mild to moderate. Quantitatively, there was a median of 380 inflammatory cells/mm² per sample, ranging from a minimum count of 33 inflammatory cells/mm² to a maximum of 1226 inflammatory cells/mm². It predominantly

consisted in chronic inflammation (median mononuclear cells/ mm^2 = 295 (33, 793) vs. median neutrophils/ mm^2 = 80 (0, 997).

Inflammation was more pronounced in the endocervix, with a median of 813 (159, 1898) inflammatory cells/ mm^2 per sample, compared to the exocervix, which had a median of 175 (32, 1115) cells/ mm^2 ($p < 0.001$). This observation persists when considering only the 12 samples with both endocervix and exocervix using a paired analysis ($p < 0.001$). These differences are also significant when analysing neutrophils and mononuclear inflammation separately.

Next, we compared cells' density between PTL and TL. When considering inflammation in general including both mononuclear cells and neutrophils, irrespective of endocervix and exocervix, there was a tendency for a higher number of inflammatory cells/ mm^2 in PTL (median=454 cells/ mm^2 , range:104, 1226) compared to TL (median=326 cells/ mm^2 , range:33, 879), but this was not significant ($p=0.154$)(Figure 2).

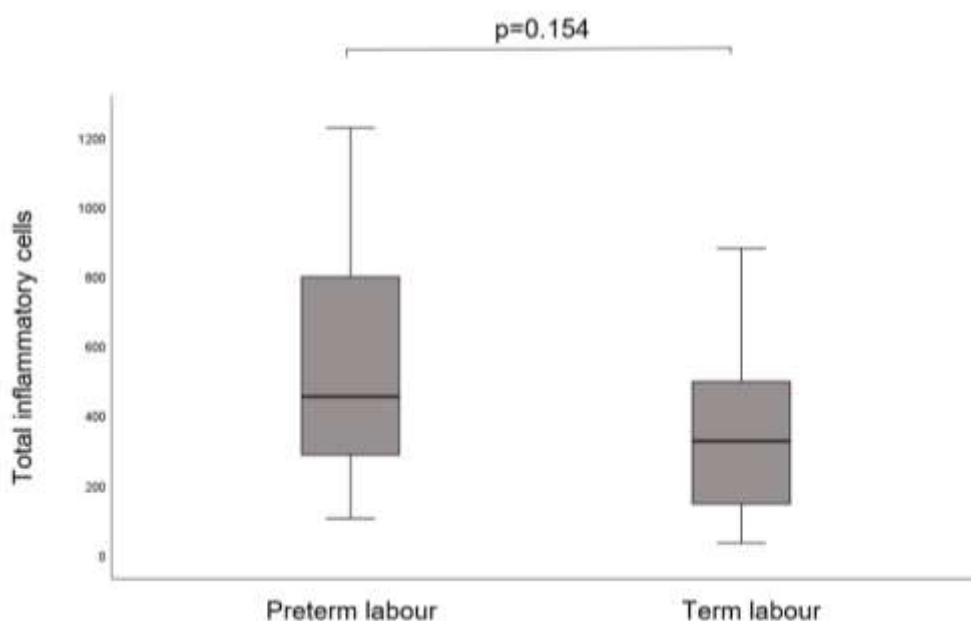


Figure 2: Box plot depicting inflammatory cell counts in cervical mucosa from preterm labour and term labour groups.

Legend: PTL – preterm labour; TL – term labour. The graph shows a slightly higher, but statistically insignificant, inflammatory cell density in the PTL group compared to the TL group, as assessed by the Mann-Whitney test. The analysis included a total of 28 biopsy samples, consisting of 14 samples from the PTL group and 14 samples from the TL group. Of these, 17 samples contained both endocervical and exocervical tissue.

A more detailed analysis focusing on the exocervix, highlighted that inflammation in this location was significantly higher in PTL compared to TL (PTL: median 283 cells/mm² vs. TL: 113 cells/mm², $p=0.027$). This was true both for neutrophil ($p=0.021$) and mononuclear cells ($p=0.028$) (Figure 3 and 4). In contrast, in the endocervix there was no significant differences in any kind of inflammation between PTL and TL.

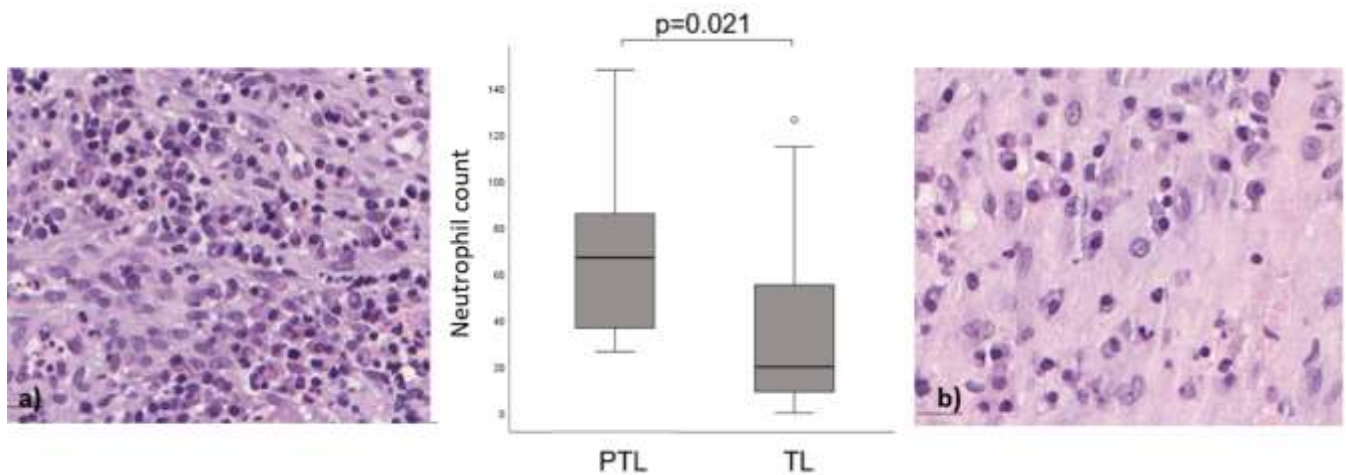


Figure 3: Neutrophil count quantification in the exocervix.

Legend: a) Histological representative image of preterm sample with abundant inflammatory cells including frequent neutrophils; b) Histological representative image of term sample with lower numbers of inflammatory cells, including neutrophils. Box-plot graph comparing the quantitative levels of neutrophil counts between preterm spontaneous labour (PTL) and term spontaneous labour (TL) (Mann-Whitney test). The analysis included a total of 24 biopsy samples, with 12 samples from the PTL group and 12 samples from the TL group.

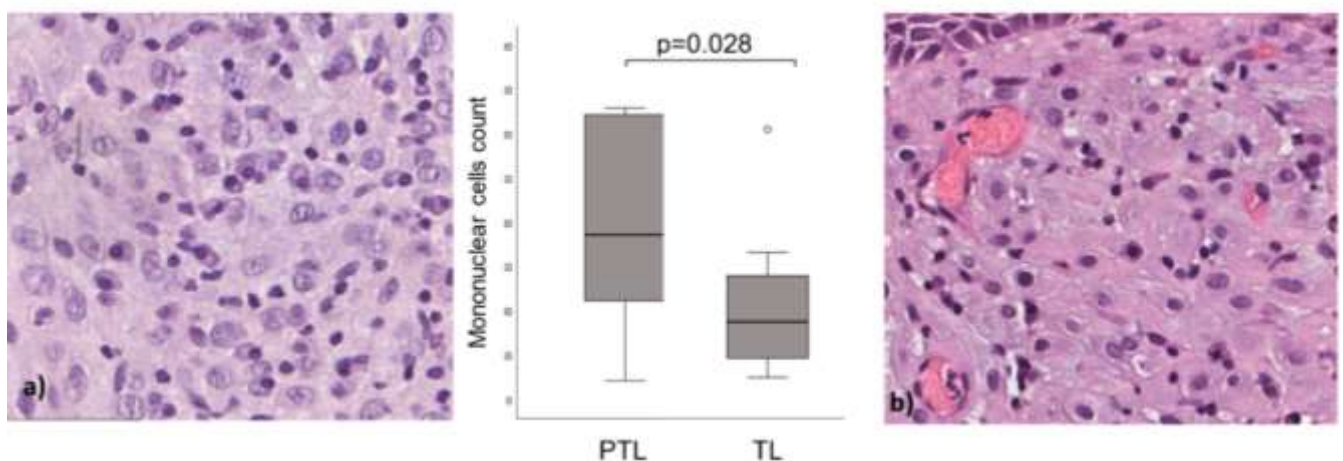


Figure 4: Mononuclear cell count quantification in the exocervix.

Legend: a) Histological representative image of preterm sample with abundant mononuclear cells (lymphocytes and plasmocytes); b) Histological representative image of term sample showing lower numbers of mononuclear cells. Box-plot graph comparing the quantitative levels of neutrophil counts between preterm spontaneous labour (PTL) and term labour (TL) (Mann-Whitney test). The analysis included a total of 24 biopsy samples, with 12 samples from the PTL group and 12 samples from the TL group.

To further support that PTL associated with inflammation in the exocervix, we analysed a group of preterm pregnancies not in labour. When comparing the preterm groups, neutrophil inflammation was significantly decreased in the PTnotL exocervix (median 10 neutrophils/mm², range: 3, 95) compared to PTL (median 66 neutrophils/mm², range: 26, 147) ($p=0.035$). In contrast, there was no significant difference in mononuclear cell density (PTnotL: median 99 cells/mm², range: 56,160 vs. PTL: 185 cells/mm², range: 20, 1041, $p=0.111$) (Figure 5).

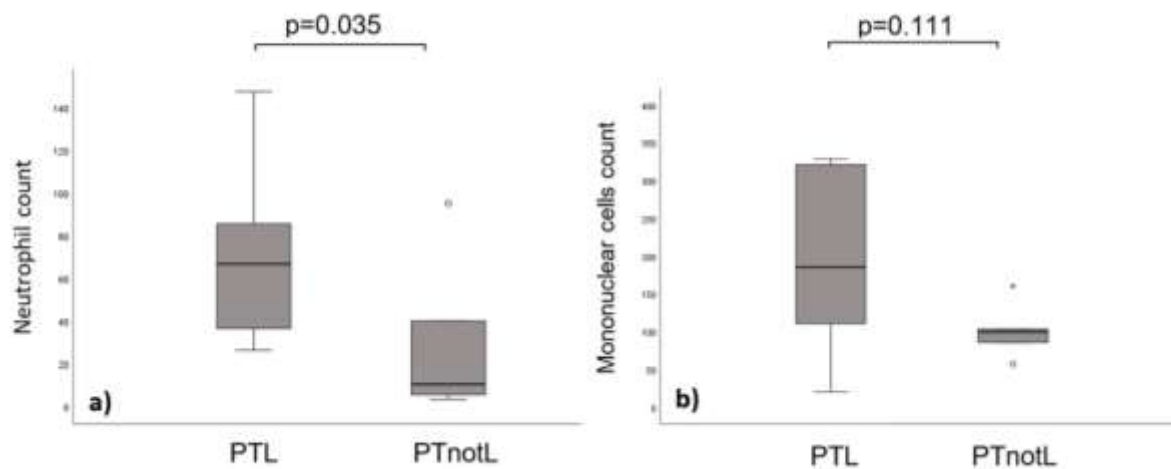


Figure 5: Inflammatory cell quantification in the exocervix, comparing preterm spontaneous labour and preterm not in labour.

Legend: a) neutrophil count b) mononuclear cell count (Mann-Whitney test). PTL - preterm spontaneous labour; PTnotL - preterm not in labour. The analysis included a total of 18 biopsy samples, with 12 samples from the PTL group and 6 samples from the PTnotL group.

Regarding fibroblasts lineage the ROI had a median of 105 cells/mm² per sample and were significant more frequent in the exocervix ($p<0.001$). No significant difference was observed between PTnotL and PTL groups. Globally, PTnotL inflammatory cells density was similar to PT samples.

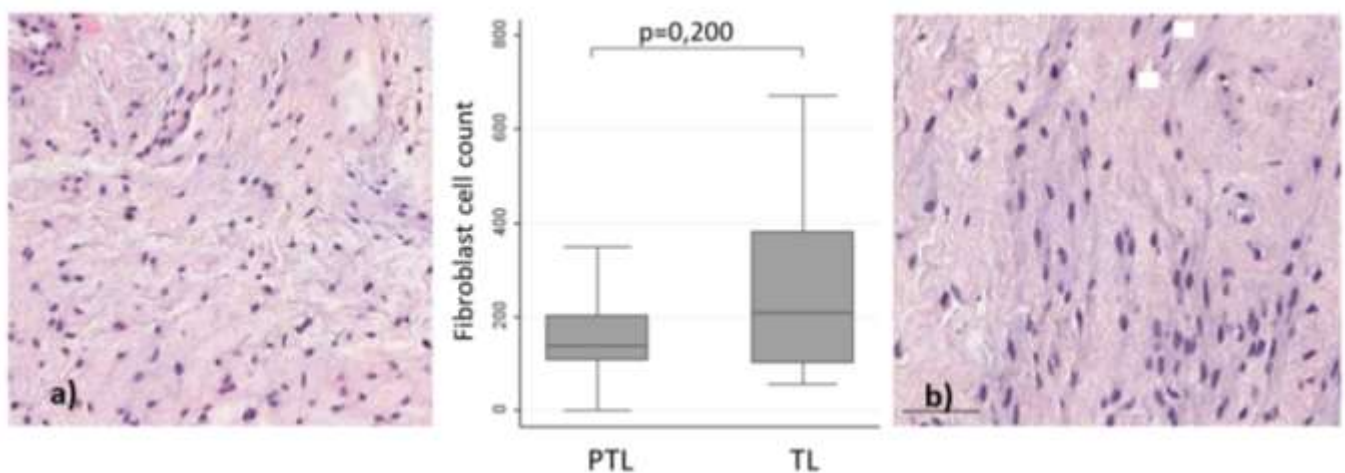


Figure 6: Fibroblast count quantification in the exocervix.

Legend: a) Histological representative image of fibroblasts in a preterm sample; b) Histological representative image of fibroblasts in a term sample. Box-plot graph comparing the quantitative levels of fibroblast counts between preterm spontaneous labour (PTL) and term spontaneous labour (TL) (Mann-Whitney test). The analysis included a total of 24 biopsy samples, with 12 samples from the PTL group and 12 samples from the TL group.

3.2 STUDY II – PRETERM BIRTH SCREENING USING CERVICAL LENGTH

3.2.1 OBJECTIVE

This study aims to evaluate the utility and effectiveness of second-trimester TUCL measurement as a predictive tool for PTB in a cohort of Portuguese pregnant women. By evaluating the distribution of TUCL measurements, determining optimal cut-off values and exploring associations with maternal risk factors, the research seeks to enhance the prediction of PTB risk. Additionally, the study compares the diagnostic performance of TUCL with established clinical guidelines, aiming to improve strategies for early PTB detection and prevention.

- **Evaluate the distribution of cervical length and prevalence of short cervix in the study population:** Determine the distribution of CL measurements and the prevalence of a short cervix (defined as $CL \leq 25$ mm—the most commonly applied cut-off value in clinical practice) in this population.
- **Assess the utility of TUCL measurement in predicting PTB:** Assess the effectiveness of TUCL as a tool for identifying women at higher risk of PTB, including an analysis of the relationship between CL and PTB in the cohort.
- **Determine optimal TUCL cut-off values for PTB prediction in the study population:** Identify the most accurate TUCL cut-off point for predicting PTB in this population.
- **Compare the diagnostic performance of different TUCL cut-offs:** Compare the diagnostic accuracy of TUCL cut-offs commonly used in clinical practice (e.g., ≤ 25 mm) with newly proposed cut-offs derived from the study population.
- **Identify and Validate a Multivariable Model for PTB Prediction:** Develop a comprehensive logistic regression model incorporating TUCL along with key maternal risk factors such as age, history of previous PTB and cervical surgery. The goal is to determine how the inclusion of these factors improves the prediction of PTB compared to TUCL alone.

3.2.2 METHODS

Study design: This was an observational, retrospective cross-sectional cohort study conducted at the Obstetrics and Gynaecology Department of São João University Hospital in Portugal. The study period included pregnant women who underwent second-trimester ultrasound examinations between January 2013 and October 2017.

Participants: All singleton pregnant women who underwent a routine second-trimester ultrasound between the 18th and 22nd weeks of gestation, as determined by crown-rump length before the 14th week, at São João University Hospital from January 2013 to October 2017 were included in the study. Exclusion criteria were applied to:

- Medical induce preterm birth (including premature rupture of membranes).
- Women who had a cervical cerclage performed prior to the second-trimester ultrasound.
- Diagnosed with chorioamnionitis
- Deliveries occurring before the 24th week of gestation.

Data Collection: Data were extracted from the Obstetrics and Gynaecology department database (Obscare® software). Information collected included maternal demographics (age, parity), obstetric history (previous preterm birth, cervical surgery) and outcomes of interest (gestational age at delivery, mode of delivery and PTB status).

- Transvaginal Ultrasound Cervical Length Measurement: All ultrasound examinations at CHSJ were performed by obstetricians accredited by the FMF for cervical assessment. Transvaginal ultrasound images were reviewed to identify patients with images complying with FMF recommendations, which include using a transvaginal probe to visualize the sagittal view of the cervix occupying 75% of the image and identifying the internal os, external os and cervical canal. Cervical length measurement was performed in a straight line between the external and internal os. (Figure 7)



Figure 7: Transvaginal ultrasound measurement of the cervical length

Legend: a) normal cervix; b) “short cervix”

Outcome Measures of the Study:

- **Primary Outcome - Prevalence of Short Cervix:** The primary outcome of this study was to determine the prevalence of short cervix ($\leq 25\text{mm}$) in Portuguese pregnant women during the second trimester.
- **Secondary Outcomes**
 - **Diagnostic Performance of TUCL Cut-offs:** The study evaluated the diagnostic ability of TUCL in predicting PTB, measured in terms of sensitivity, specificity, PPV, NPV and the AUC from receiver operating characteristic (ROC) analysis.
 - **Comparison of Optimal TUCL Cut-off:** Comparison of the optimal TUCL cut-off with the widely used 25mm cut-off in terms of diagnostic performance metrics and reclassification of risk groups.
 - **Multivariable Model Validation:** Development and validation of a multivariable logistic regression model incorporating TUCL as a predictor of PTB, adjusted for maternal age, previous history of PTB and cervical surgery.

Statistical Analysis: Descriptive statistics were used to summarize maternal and clinical characteristics. Frequencies of preterm birth were calculated across different groups of cervical length measurements. Diagnostic accuracy parameters (sensitivity, specificity, PPV, NPV and AUC) were evaluated for different transvaginal ultrasound cervical length cut-offs. Univariate and multivariate logistic regression analyses were conducted to identify predictors of preterm birth and associations with cervical length. Likelihood ratio tests were used to select the optimal transvaginal ultrasound cervical length cut-off.

Stratified analysis was performed to evaluate the association between short cervix and preterm birth in subgroups based on maternal age, history of preterm birth and previous cervical surgery. Effect modification among strata was assessed using homogeneity tests.

Comparison of Cervical Length Cut-offs: The diagnostic performance of the optimal transvaginal ultrasound cervical length cut-off was compared with the commonly used 25 mm cut-off using multivariate models.

All statistical tests were two-tailed and p-values < 0.05 were considered statistically significant. Adjusted estimates were calculated using the Cochran-Mantel-Haenszel method. SPSS® software was used to perform the statistical analysis.

Ethical Considerations: This study was conducted after obtaining approval from the local Ethics Committee (Ref. No. 81-17) and each participant provided informed consent. All procedures were conducted in accordance with ethical standards and guidelines.

3.2.3 RESULTS

A) PARTICIPANT CHARACTERISTICS

During the study period from January 2013 to October 2017, 8,016 women underwent routine second-trimester ultrasound and delivery at São João University Hospital. Of these, 3,476 were excluded for various reasons: delivery before 24 weeks (n=5), medical induction of PTB (n=241), cervical cerclage prior to ultrasound (n=19), chorioamnionitis diagnosis (n=3), absence of cervical length measurement (n=958) and non-compliance with FMF measurement standards (n=2,426). The latter primarily involved transabdominal measurements (n=1,275), which did not meet FMF guidelines. Some women had multiple exclusion criteria.

The final cohort consisted of 4,481 women, with a mean age of 30.7 ± 5.5 years. Of these, 45.4% were primigravidae and 56.2% had no previous deliveries. The majority had no significant medical (86.5%) or obstetric (96.7%) history. Only 1.6% had a previous spontaneous PTB and 0.9% had a history of cervical surgery, the two major known risk factors for PTB.

Among the cohort, spontaneous delivery occurred in 64.3% of cases and 76.4% of women had vaginal deliveries. The prevalence of spontaneous PTB in the original cohort was 6.9%. After applying the exclusion criteria, this prevalence decreased to 4.0% (n=179), mainly due to the

exclusion of medical inductions. Of these PTB, 96.0% (n=149) occurred between 34 and 37 weeks and 0.7% (n=30) occurred before 34 weeks. Maternal and clinical characteristics are summarized in Table 4.

Table 4: Maternal and clinical characteristics of the study II population

Maternal features	Age (years) Mean: 30.7 ± 5.5 Min: 14 Max: 50 BMI (kg/m ²) Mean: 24.6 ±4.9 Obesity (>30): 8.0% Years of study < 4 th year: 0.7% 4 th to 12 th : 64.6% >12 th : 34,7% Smoker: 12.9% Alcohol/drugs: 0.2%	Obstetric background	None: 96.7% Pre-eclampsia: 0.9% Fetal death: 0.6% Fetal malformation: 0.4% 2 ^o trimester abortion: 0.1% Fetal growth restriction: 3.9% Fetal malformation: 1.6% Urinary infection: 3.2% Other infections: 4.4% Hypertensive syndrome: 3.2% Gestational diabetes: 9.6% Surgery on 1 ^o /2 ^o trimester: 0.2%
Medical background	None: 86.5% Uterine malformations: 0.3% Psychiatric Disorders: 1.4% Sexual transmitted disease: 1.0% Cardiac or renal disorder: 1.0% Diabetes: 0.7% Hypertension: 2.6% Hypothyroidism: 5.2% Neoplasia: 0.8%	Obstetric data	Primigravidae: 45.4% Nulliparous: 56.2% Assist reproduction: 2.7% Labour induction: 35.7% Vaginal delivery: 76.4% Male newborn: 50,6%
Risk factors of PTB	Spontaneous PTB: 1.6% Cervical surgery: 0,9% Short cervical length: 3.0%	Time of delivery	Mean: 39.2 weeks Min: 24.2 weeks Max: 42 weeks Term delivery: 96.0% Preterm delivery: 4.0% Early PTB (< 34 th w): 0.7% Late PTB (≥ 34 th w): 3.3%

Legend: Min – minimum; Max – maximum; BMI – body mass index; PTB – Preterm birth; W – weeks;

B) DISTRIBUTION OF CERVICAL LENGTH:

The mean gestational age at the time of ultrasound was 21 weeks + 3 days, with 0.2% at 18 weeks, 0.5% at 19 weeks, 12.4% at 20 weeks, 66.5% at 21 weeks and 20.5% at 22 weeks.

The mean TUCL measurement was 33.8 ± 4.8 mm, ranging from 3.0 mm to 53.0 mm. The 3rd, 5th and 10th percentiles of TUCL were 25.0 mm, 27.0 mm and 29.0 mm, respectively. The frequency of term and preterm births across different TUCL intervals is shown in Table 5.

Table 5: Distribution of preterm and term births across different cervical length intervals

Cervical Length	Early PTB (< 34weeks)	PTB (<37weeks)	Term birth (≥ 37 weeks)	Total
< 15mm	4 (13.3%)	5 (2.8%)	11 (0.3%)	16 (0.4%)
15.1 to 20mm	3 (10.0%)	4 (2.2%)	11 (0.3%)	15 (0.3%)
20.1 to 25mm	0 (0%)	6 (3.4%)	74 (1.7%)	80 (1.8%)
25.1 to 30mm	3 (10.0%)	30 (16.8%)	506 (11.8%)	536 (12.0%)
≥ 30 mm	20 (66.7%)	134 (74.9%)	3700 (96.5%)	3834 (85.6%)

Legend: PTB – Preterm birth

C) ASSOCIATION BETWEEN CERVICAL LENGTH AND PRETERM BIRTH

TUCL was significantly lower in women with PTB (mean 31.6 mm, 95% confidence interval 30.7–32.5 mm) compared to those with term births (mean 33.9 mm, 95% confidence interval 33.8–34.0 mm, $p < 0.001$). Despite high specificity for predicting PT, TUCL diagnostic ability was limited by a very low sensitivity, with an AUC close to 0.5, for all different cut-offs studied as depicted in Table 6.

Table 6: Cervical length measurement sensitivity and specificity to predict preterm birth and cumulative incidence of the different cut-offs

Cut-off	Sensitivity	Specificity	AUC	PPV	NPV
15mm	2.8	99.7	0.51	10.9	0.97
20mm	6.2	99.5	0.53	11.5	0.94
25mm	10.6	97.3	0.54	3.9	0.91
30mm	30.7	80.1	0.55	1.5	0.86

Legend: AUC – area under the curve; PPV – positive predictive value; NPV – negative predictive value

D) SUBGROUP ANALYSIS

Univariable analysis (Tables 7 and 8) identified maternal age ≥ 40 years, previous history of PTB and cervical surgery as significant predictors of PTB. These factors were also associated with shorter TUCL, acting as confounders.

As expected, in univariable analysis treatment with progesterone/pessary associated with shorter TUCL and thus, also with PTB, when considering the total study cohort. Within the group of women with a diagnosis of short cervix, 68% (n=93) underwent treatment and 13% (n=18) decline it. There was, however, no significant difference between the frequency of PTB among women who did or declined progesterone or pessary (14% vs. 22.2% of PTB, respectively, $p=0.472$) and so treatment was not included in our multivariable model.

Table 7: Demographics characteristics comparing preterm and term birth

		PTB (n): 179 (4.0%)	Term birth (n): 4302 (96.0%)	p Value
Maternal age	Mean (years)	31.4 ± 6.0	30.7 ± 5.5	0.098 ¹
	< 40 years	161 (89.9%)	4122 (95.8%)	<0.001 ²
	≥40 years	18 (10.1%)	180 (4.2%)	
Body mass index	Mean (kg/m ²)	2397 ± 5.3	24.6 ± 4.9	0.055 ¹
Education	≤ twelfth grade	111 (62.4%)	2807 (65.4%)	0.404 ²
	> twelfth grade	67 (37.6%)	1485 (34.6%)	
Addictions	Smoking	21 (11.7%)	556 (12.9%)	0.640 ³
	Drugs	0 (0%)	4 (0.1%)	1.000 ³
	Alcohol	1 (0.6%)	4 (0.1%)	0.184 ³
GA at TUCL	Mean (weeks)	21.5 ± 0.5	21.5 ± 0.6	0.592 ¹
Type of pregnancy	Spontaneous	165 (95.4%)	4041 (97.2%)	0.150 ²
	Medical assisted	8 (4.6%)	115 (2.8%)	
Gravid	Primigravidae	83 (46.5%)	1953 (45.4%)	0.789 ²
Parity	Nulliparous	101 (56.4%)	2417 (56.2%)	0.949 ²
Obstetric history	Preterm birth	17 (9.5%)	55 (1.3%)	<0.001 ³
	Second trimester miscarriage	1 (0.6%)	4 (0.1%)	0.184 ³
	Recurrent pregnancy loss	2 (1.1%)	16 (0.4%)	0.160 ³
Maternal background	Conization	10 (5.6%)	31 (0.7%)	<0.001 ²
	Mullerian anomalies	3 (1.7%)	11 (0.3%)	0.016 ³
	Chronic hypertension	9 (5.0%)	100 (2.3%)	0.021 ²
	Diabetes	3 (1.7%)	28 (0.7%)	0.125 ³
	Hypothyroidism	7 (3.9%)	227 (5.2%)	0.429 ²

Table 7: Demographics characteristics comparing preterm and term birth (continue)

		PTB (n): 179 (4.0%)	Term birth (n): 4302 (96.0%)	p Value
Obstetrical complications	Malformations and cromossomopathies	6 (3.4%)	64 (1.5%)	0.059 ³
	Fetal growth restriction	9 (5.0%)	167 (3.9%)	0.439 ²
	Hypertensive syndromes	0	143 (3.3%)	0.040 ³
	Gestational diabetes	24 (1.4%)	405 (9.4%)	0.751 ²
	Short interpregnancy intervals	1 (0.6%)	4 (0.1%)	0.184 ³
	Surgical procedure during pregnancy	0 (0%)	7 (0.2%)	1.000 ³
	Urinary infections or asymptomatic bacteriuria	8 (4.5%)	135 (3.1%)	0.321 ²
	Others infections during pregnancy	10 (5.9%)	188 (4.4%)	0.438 ²
Treatment with progesterone or Arabin pessary		25 (13.9%)	116 (2.7%)	0.000 ²

Legend: GA – gestational age; PTB – preterm birth; TUCL – transvaginal ultrasound cervical length;

¹ T test; ² Chi square test; ³ Fisher test

Table 8: Cervical length description according to demographics characteristics

			Cervical length (M±SD)	p Value ¹
Maternal age	< 40 years old		33.8 ± 4.8	0.546
	≥ 40 years old		34.0 ± 5.0	
Education	≤ twelfth grade		33.8 ± 4.8	0.568
	> twelfth grade		33.9 ± 4.9	
Addictions	Smoking	No	33.9 ± 4.8	0.089
		Yes	33.5 ± 5.3	
	Drugs	No	33.8 ± 4.8	0.443
		Yes	35.5 ± 2.8	
	Alcohol	No	33.8 b± 4.8	0.170
		Yes	30.5 ± 3.1	
Type of pregnancy	Spontaneous		33.8 ± 4.8	0.664
	Medical assisted		33.6 ± 6.4	
Gravid	Primigravidae		33.5 ± 4.8	0.000
	Multigravidae		34.1 ± 4.9	
Parity	Nulliparous		33.4 ±4.8	0.000
	Multiparous		34.3 ± 4.8	
Obstetric history	Preterm birth	No	33.9 ± 4.8	0.000
		Yes	31.8 ± 5.0	
	Second trimester miscarriage	No	33.8 ± 4.8	0.001
		Yes	26.8 ± 8.4	
	Recurrent pregnancy loss	No	33.8 ± 4.8	0.412
		Yes	32.3 ± 7.9	

Table 8: Cervical length description according to demographics characteristics (continue)			Cervical length (M±SD)	p Value ¹
Maternal background	Conization	No	33.9 ± 4.8	0.000
		Yes	30.8 ± 5.6	
	Mullerian anomalies	No	33.8 ± 4.8	0.993
		Yes	33.9 ± 3.2	
	Chronic hypertension	No	33.8 ± 4.8	0.540
		Yes	34.1 ± 6.2	
	Diabetes	No	33.8 ± 4.8	0.120
		Yes	35.9 ± 4.6	
	Hypothyroidism	No	33.8 ± 4.9	0.659
Yes		34.0 ± 4.5		
Obstetrical complications	Malformations and cromossomopathies	No	33.8 ± 4.8	0.821
		Yes	34.0 ± 4.9	
	Fetal growth restriction	No	33.9 ± 4.8	0.000
		Yes	32.4 ±4.9	
	Hypertensive syndromes	No	33.8 ± 4.8	0.448
		Yes	33.5 ± 4.8	
	Gestational diabetes	No	33.8 ± 4.8	0.967
		Yes	33.8 ± 45.0	
	Short interpregnancy intervals	No	33.8 ± 4.8	0.871
		Yes	34.3 ± 2.4	
	Surgical procedure during pregnancy	No	33.8 ± 4.8	0.934
		Yes	34.0 ± 3.3	
	Urinary infections or bacteriuria	No	33.8 ± 4.8	0,112
		Yes	33.12 ± 4.8	
Other infections during pregnancy	No	33.9 ± 4.9	P= 0.880	
	Yes	33.2 ± 4.6		
Treatment with progesterone or Arabin pessary		No	34.2 ± 4.3	P= 0.000
		Yes	24.1 ±7.2	

Legend: M – mean; SD – standard deviation; ¹T test

Multivariable logistic regression analysis, incorporating maternal age ≥ 40 year's old, previous history of PTB and cervical surgery, was done to evaluate the impact of TUCL measurement as a predictor of preterm birth (Table 9). The estimated OR for the effect of TUCL on PTB, controlling for covariates, was 0.92 (95%CI 0.90, 0.95; $p < 0.001$), highlighting the significant negative association between TUCL and PTB. The diagnostic ability of the multivariable model improved, showing an AUC of 0.65 (Figure 8).

Table 9: Multivariable logistic regression analysis to evaluate the impact of cervical length transvaginal ultrasound measurement as a predictor of preterm birth

Outcome	Odds Ratio	95% confidence Interval		P Value
Cervical length	0.925	0.90	0.95	0.000
Maternal age	2.265	1.32	3.90	0.003
Previous preterm birth	6.754	3.72	12.16	0.000
Cervical surgery	0.178	0.81	0.40	0.000
Constant	14.557	2.61	81.31	0.002

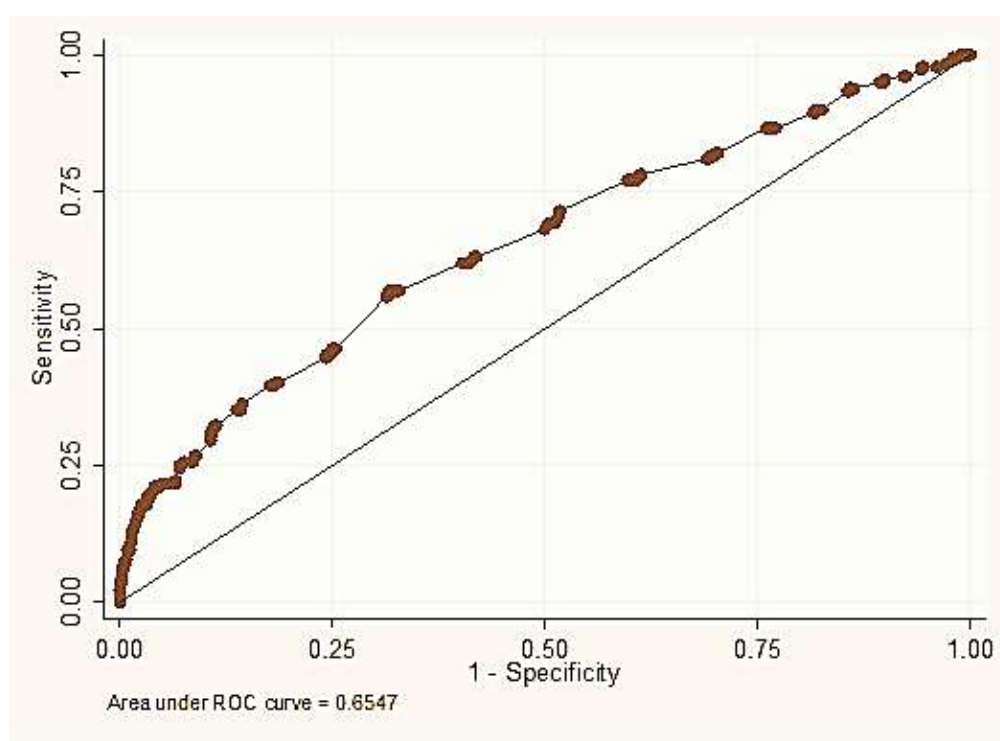


Figure 8: Graphic representations of sensitivity, specificity and area under curve of the multivariable model

E) OPTIMAL CERVICAL LENGTH CUT-OFF

To select the optimal TUCL cut-off, we ran several multivariable logistic regression models, that included the main effects of maternal age ≥ 40 years old, previous history of PTB and cervical surgery as covariates and TUCL subgroup defined using different cut-offs from 8 and 50mm. Plotting the log likelihood from these models against TUCL showed that a TUCL cut-off of 20mm (95%CI 19.5, 22mm) best discriminated two TUCL subgroups with differential odds of PTB (Figure 9). Women with a short cervix, defined by $TUCL \leq 20$ mm, had an OR of 12.2 (95%CI 5.8, 25.4, $p < 0.001$), compared to those with $TUCL > 20$ mm.

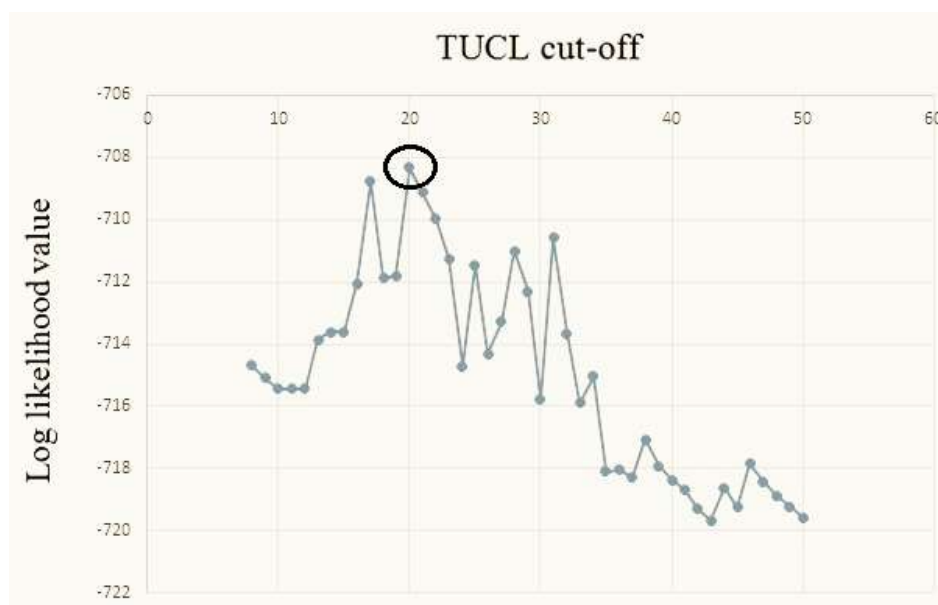


Figure 9: Plotting to determine the best transvaginal ultrasound cervical length cut-off

Legend: transvaginal ultrasound cervical length (TUCL) cut-off based on multiple log likelihood of the logistic regression model (including maternal age ≥ 40 years old, previous history of PTB and cervical surgery), using TUCL cut-offs between 8 and 50mm. The circle represents TUCL cut-off that best discriminated the risk of PTB.

Considering maternal age ≥ 40 years old, previous history of PTB and cervical surgery, as the main risk factor for PTB, a stratified analysis was done by separately evaluating women with at least one of these factors ($n=297$) versus women that did not present any of these ($n=4184$) (Table 10). The significant association of a short cervix ($TUCL \leq 20$ mm) with PTB was maintained in both groups, with an OR 16.2 (95%CI 2.7, 97.1, $p < 0.001$) for women with risk factors and an OR 9.8 (95%CI 4.1, 23.7, $p < 0.001$) for women without them. There was no

effect modification between groups ($p = 0.614$, homogeneity test). Adjusted OR for women with short cervix ($TUCL \leq 20\text{mm}$), considering this stratification, was 11.4 (95%CI 5.1, 25.4, $p < 0.001$).

I. COMPARISON OF CERVICAL LENGTH CUT-OFFS:

Comparison of the 20 mm and 25 mm TUCL cut-offs showed no significant difference in AUCs (20 mm TUCL: AUC 0.59, 95% confidence interval 0.56–0.62; 25 mm TUCL: AUC 0.60, 95% confidence interval 0.57–0.64; $p = 0.157$). However, the 20 mm cut-off performed better in predicting PTB. When using the 20 mm cut-off, 15 women (0.33%) were reclassified into different predicted probability groups compared to the 25 mm cut-off. Of the 4 women upgraded to a higher probability group (from 0.50–0.75 to >0.75), 75% ($n = 3$) had PTB. Conversely, among the 8 women reclassified to a lower probability group (from 0.25–0.50 to <0.25), only 1 had PTB. (Table 10)

As described in table 10, from the 4 women upgraded to a higher probability (from 0.50–0.75 in the 25mm model to >0.75 in the 20mm model), 75% ($n = 3$) had a PTB. On the other hand, from the 8 women reclassify to a lower predicted probability (from 0.25–0.50 in the 25mm model to <0.25 in the 20mm model), only 1 had PTB.

Table 10: Distribution of the pregnant women according to group probability of preterm birth

		Group probability - TUCL20				Total reclassified
		<0.25	0.25-0.50	0.50-0.75	≥0.75	
Group probability - TUCL25	<0.25	4456	1	0	0	0.02%
	PTB% (n)	3.8% (168)	0%	0%	0%	
	0.25-0.50	8	8	2	0	55.6%
	PTB% (n)	12.5% (1)	50.0% (4)	50.0% (1)	0%	
	0.50-0.75	0	0	1	4	80.0%
	PTB% (n)	0%	0%	100% (1)	75.0% (3)	
	≥0.75	0	0	0	1	0%
	PTB% (n)	0%	0%	0%	0 %	

Legend: Distribution of the pregnant women according to group probability of preterm birth (PTB) using transvaginal ultrasound cervical length (TUCL) measurement and presence/absence of risk factors (maternal age ≥40 years old, previous PTB and cervical surgery). Cross-classification of the TUCL 25mm cut-off group (most used cut-off) versus 20mm cut-off group (our best cut-off) and frequency of PTB to each subgroup.

4. DISCUSSION

The association between cervical shortening and preterm birth has been extensively documented, underscoring the cervix's critical role in the pathophysiology of PTL. Early changes in cervical tissue are pivotal in initiating premature delivery. (10, 139, 141, 241-252, 255-257) However, the specific histological and molecular alterations underlying early CR remain poorly understood. Studies investigating the biochemical causes of weakened cervical tissue have yielded inconclusive results, likely due to the multifactorial and complex nature of CR. (158, 191)

A comprehensive understanding of the normal physiological process of CR during TL is essential to fully understand the pathophysiological processes involved in prematurity. In 1978, Liggins proposed that cervical ripening resembles an inflammatory reaction, with immune cell migration promoting collagenase and elastase activities, which are crucial for the ripening process. (550) However, this classic model has been challenged by animal studies and the fact that it was based on postpartum samples, which may reflect changes related to the common pathway and postpartum repair rather than the initial events preparing the cervix for labour. (161, 163, 263, 280)

The significance of cervical neutrophils in driving labour-related changes has been debated, as not all studies show an increase in neutrophil density during CR. (164, 551) Emerging research suggests that these cells primarily contribute to active dilation and postpartum tissue repair, rather than initiating cervical ripening itself in term CR. (161, 163, 164) Interestingly, depleting neutrophils doesn't affect the timing of parturition (163) and myeloperoxidase (MMP) activity, a marker of functional neutrophils, is not detectable until postpartum, challenging long-held assumptions about the role of MMPs in remodelling the cervix. (163) Furthermore, Sakamoto et al. found no correlation between clinical cervical ripening and IL-8 concentrations, a marker of inflammation, in cervical tissue. (221) In a transgenic mouse model, cervical ripening failed to occur despite the infiltration of neutrophils and macrophages into the tissue, suggesting that the presence of inflammatory cells alone may not be sufficient to initiate ripening. (225)

Recent evidence suggests that the process of CR in term births is primarily attributed to changes in collagen organization. (163, 213) The key events driving this transformation involve a reduction in cross-linked collagen and matricellular proteins, leading to increased tissue compliance without compromising its integrity, thus preparing the cervix for the subsequent phase. (163, 191) In contrast, while inflammatory cells and markers may not be crucial for term remodelling, they play a significant role in preterm remodelling. This information leads to the conclusion that the mechanisms initiating CR differ between term and preterm births. (264)

Neutrophils serve as frontline cells in the innate immune system and play a crucial role in preterm birth, a condition frequently linked to infection. (551, 552) Moreover, sterile inflammation, where inflammation occurs without infection, is also prevalent in preterm birth scenarios and alarmins contribute to preterm delivery by promoting leukocyte migration and inducing inflammation at the maternal-fetal interface, despite the presence of infection. (171, 551) On the other hand, mononuclear cells seem to shape the immune response in the cervix and contribute to postpartum repair but don't actively participate in the initial stages of CR. (136)

A key issue in studying CR is the scarcity of human cervical tissue data. Animal studies have been valuable, but differences between human and animal processes due to anatomical and hormonal factors must be acknowledged. Study I addresses this gap by utilizing cervical biopsies from pregnant women in the early stages of labour, providing insights into the cellular mechanisms driving CR.

In our study, we included two distinct groups of women and obtaining biopsies in the initial stage of labour: one group experiencing sPTB (less than 37 weeks of gestation) and the other experiencing term birth (more than 39 weeks of gestation). Women between 37 and 39 weeks were excluded to avoid overlapping mechanisms of delivery, as late preterm and early term can share similar labour processes.

Additionally, a third group consisting of women with preterm pregnancies not in labour was included. This allowed us to differentiate between cellular changes associated with premature labour and those typical of the stage of prematurity, regardless of labour status.

One of the objectives of this study was to assess the cellular density of inflammation in cervical tissue samples obtained from pregnant women in preterm and TL in order to understand their role in term and premature remodelling.

We found no significant difference in the global inflammatory infiltrate between PTB and TL, but a detailed analysis of different areas of the cervix revealed that while the endocervix did not show significant differences, the exocervix had more pronounced inflammation in PTB. This could be due to the generally higher inflammation in the endocervix that is characteristic of this location, coupled with a reduced sample size, leading to insufficient statistical power to detect differences.

Evaluating the exocervix, neutrophils were increased in spontaneous PTB compared to TL, supporting the evidence that inflammation may have a role in prematurity, but not in term delivery. When comparing spontaneous PTL with PTnotL, we observed that neutrophils were significantly higher in PTL compared to PTnotL. Even though we only had a small set of samples, this suggests that neutrophils are involved in the premature labour process itself and not only a consequence of prematurity. These findings reinforce the notion that neutrophils are essential to the CR process leading to PTL, supported by studies linking inflammation—including sterile inflammation—to preterm birth. (551, 552)

Mononuclear cell inflammation was more pronounced than neutrophils in all three groups. Their role as barrier and first line in defence may justify their abundance in the cervix. These cells were increased in PTL compared to TL. Conversely, there was no significant difference in mononuclear cells between the premature groups, suggesting that while these cells contribute to cervical inflammation, they may not be as critical in triggering labour as neutrophils. The role of mononuclear cells appears to be more pronounced in postpartum repair than in the remodelling process leading to labour initiation. This results are consistent with literature evidence that mononuclear cells play a significant role in the immune response but not in CR. (136)

Cervical remodelling is a complex process involving various components other than inflammatory cells. Fibroblasts, due to their pivotal role in matrix production, are also of interest in CR physiology. A study analysing postpartum samples concluded that fibroblasts are activated or recruited during the ripening process and are implicated in both tissue degradation/remodelling and the attraction of neutrophils. (553, 554) Despite their

significance, prior research has largely overlooked the changes fibroblasts undergo during labour.

Our analysis indicated a more pronounced presence of fibroblasts in the exocervix, consistent with the higher abundance of stroma in this region. However, there were no significant differences between TL and PTL, suggesting that there is no influx of these cells in triggering term or preterm CR. It is important to highlight that we are evaluating cell density and not function. Evidence suggests that remodelling at term is primarily due to changes in collagen crosslinking rather than alterations in matrix production. (163, 213) Therefore, further research is needed to evaluate the function of these cells rather than their presence (for example, how they contribute to crosslinking in term or neutrophil chemotaxis in PTL) to fully understand the specific mechanisms governing fibroblast dynamics during remodelling.

While our study benefitted from utilizing labour samples more representative of the pathways that lead trigger labour, there are inherent limitations:

- **Small Sample Size:** One of the primary limitations of this study is the relatively small sample size. A small sample size reduces statistical power and increases the risk of type II errors, limiting the generalizability of the findings. This constraint may have prevented the detection of subtle but potentially significant differences between groups.
- **Observational Study Design:** Being an observational study, it establishes associations rather than causality. This design does not allow for manipulation of variables or control over potential confounders that could influence the outcomes observed.
- **Generalizability:** Conducted at two hospitals in Porto, Portugal, findings may not be generalizable to other populations.
- **Biopsy Timing and Location:** Variability in the timing and location of cervical biopsies could introduce bias. Biopsies were randomly taken, resulting in potential sampling bias as not all samples may represent all areas of the cervix.
- **Methodological Constraints:** The study employed digital image analysis to quantify cell densities, which relies on morphological characteristics rather than specific markers or functional assays (e.g., immunohistochemistry). While efficient for large-scale quantification, this approach might overlook nuanced cellular behaviours or phenotypes that could influence remodelling dynamics.

- **Statistical Considerations:** While appropriate statistical tests were employed, the study's reliance on non-parametric tests (e.g., Mann-Whitney, Kruskal-Wallis) due to the data's asymmetric distribution and small sample sizes might limit the ability to detect smaller but clinically significant differences between groups.
- **COVID-19 Pandemic Interruption:** The study had a 12-month hiatus due to the COVID-19 pandemic, which could have disrupted participant recruitment, data collection continuity and potentially introduced biases related to changes in healthcare delivery or participant behaviours during the pandemic period.

While we aimed to understand the molecular basis of premature CR and contribute to the development of targeted therapies, it is also crucial to determine the optimal methods, timing and target populations for screening for premature remodelling. The exploration of ultrasonographic measurement of the cervix commenced in 1978, marking its inception in medical literature. This initial step was rapidly succeeded by significant advancements, including the visualization of cervical dilation in 1979 and the identification of an “incompetent” cervix in 1983. (555-557) These early breakthroughs set the stage for extensive research into the application of TVUS for assessing PTB risk. (352)

By 1990, the landmark study by Andersen et al. revealed that a sonographic CL below the 50th percentile at 30 weeks of gestation significantly increased the risk of PTB. (330) The momentum of research continued to build, with notable contributions such as the 1996 study by Iams et al., which detailed the relationship between cervical length and preterm delivery risk in a large group of asymptomatic, low-risk women. (246) Following closely, in 1997, Crane and colleagues presented findings that highlighted an increased preterm risk associated with a shortening of the upper cervical segment below 10 mm, enriching the growing body of evidence. (341)

The late 1980s onwards witnessed an influx of studies, particularly from the Fetal Medicine Foundation, confirming the predictive accuracy of cervical length measurement for early preterm delivery risk. (245, 332, 342) In a seminal 2000 study, Hassan et al. analysed a cohort of 6,877 women, affirming the significant predictive power of a short cervix identified via second-trimester sonography for early spontaneous preterm delivery. (331)

Subsequently, a multitude of investigations have validated and reinforced these initial findings, establishing a robust and consistent association between cervical length and the risk

of preterm delivery. (244, 307, 330, 343-353) Recognized for over three decades, the shortened cervix in the second trimester has been found to be the best single predictor SPTB. (168, 301, 310, 385, 417-419) Table 11 presents a summary of data from studies evaluating mid-trimester cervical length.

Globally, there is a consensus on the importance of cervical length screening among women at high risk for PTB. (297, 345, 479, 558). Extensive literature supports the notion that women with a history of prior spontaneous PTB and a short CL are at the highest risk of experiencing PTB again. (340, 385) However, the role of cervical length screening in women with no history of spontaneous preterm delivery remains less established. (389) Emerging evidence suggests that a short cervix in the second trimester is a robust predictor of spontaneous PTB regardless of obstetrical history. (394, 403) Several systematic reviews have underscored the effectiveness of TUCL in predicting spontaneous preterm delivery in asymptomatic patients with singleton gestations. (252, 353, 412, 559)

Table 11: Summary of data reported by studies evaluating mid-trimester cervical length

Study	N	GA (weeks) at screening	Mean TUCL (mm)	TUCL SD (mm or Range)	PTB rate	Prevalence TUCL ≤25mm
Iams 1996 (560)	2915	24	35.2	8.5	4.3 ¹	10
Hibbard 2000 (561)	841	18-22	38.5	8.0	11.2	
Leung 2005 (403)	2952	18-22	37.9	6.5	3.7	1.8
Fonseca 2007 (377)	24189	20-25	34.0	0-67		1.7
Silva 2010 (562)	26106	20	37.5	8.0	8.8	
Hassan 2011 (563)	32091	19-24				2.6
Goya 2012 (564)	11 875	18-22	34.0	3-68	6.0	6
Orzechowski 2014 (565)	1569	18-24			4.3	1.3 ⁴
Kuusela 2015 (393)	2122	16-24	38.3	6.4	4.2	0.5
van der Ven 2015 (566)	11943	16-22	44.1	7.8	3.9	
Son 2016 (567)	17590	18-24			4.8	0.89
Peixoto 2016 (568)	996	20 - 24+6	37.0	10.7		
Peixoto 2017 (569)	2018	20-24+6	37.6 ² 37.9 ³		13.6	2.7
Souka 2018 (570)	10506	20-24			3.90	5.2
Wang 2021 (571)	1222	22-24	37.9 ² 39.3 ³		7.3	0.33

Legend: GA – gestational age; TUCL - transvaginal ultrasound cervical length; SD - standard deviation; PTB – preterm birth; ¹ Delivery < 35weeks; ² PTB; ³ Term birth; ⁴ TUCL <25mm

In light of these findings, TUCL emerges as the most specific and effective clinical tool for assessing the risk of PTB, encompassing both low-risk and high-risk asymptomatic women. (246, 248, 310, 331, 332, 572) However, critical questions remain regarding how, when, which cut-offs and who to screen.

I. How?

TVUS has emerged as the "gold standard" for cervical assessment, recognized for its unparalleled accuracy and efficacy. (339, 340, 374)

II. When?

A single CL measurement between 18–24 weeks appears superior for predicting PTB in low-risk asymptomatic pregnancies, while high-risk women may benefit from serial measurements from 16 to 23 weeks 6 days. (353, 367, 374)

III. Cut-off?

Short cervix ≤ 25 mm has the best predictive accuracy of PTB and it is the most commonly used cut-off. (329, 339, 340, 345, 366, 377, 400, 401, 411, 414, 415) A pivotal study by Iams et al. heightened risk of sPTB with diminishing cervical length, establishing a clinically relevant threshold at the 10th percentile (< 26 mm). (246) Subsequent studies have echoed these findings and a mid-trimester cervical length of ≤ 25 mm has been considered short, aligning with the 10th percentile for this gestational age group. (245, 385) However, recent research suggests that the prevalence of women with a cervix < 26 mm is lower than previously thought (245, 246, 339, 377, 378, 393–399) Globally, there is ongoing debate regarding the optimal threshold for cervical length that should trigger therapeutic or prophylactic interventions. Various thresholds, ranging from < 15 to ≤ 30 mm, have been proposed.

IV. Who? Universal screening pros and cons

Universal screening refers to the systematic evaluation of an entire population or specific group for a particular condition, disease, or risk factor, regardless of individual risk status. The principles of universal screening are designed to ensure effective and equitable identification of health conditions early in their development. (392, 573) Here are the key principles:

- **Condition should be an important health problem** - preterm birth is a significant global health challenge, contributing to high neonatal and child mortality rates. To address this, the Sustainable Development Goal target 3.2 aims to reduce neonatal mortality to 12

or fewer deaths per 1,000 live births worldwide. Numerous organizations and initiatives, such as the International Preterm Birth Collaborative, the Global Alliance to Prevent Prematurity and Stillbirth, Born Too Soon and World Prematurity Day, emphasize the global commitment to preventing preterm birth and reducing its impact. (574)

- **A recognizable early stage should exist** - a short cervix is an early indicator of potential preterm delivery, especially in asymptomatic women. PCCR is a strong predictor of spontaneous preterm delivery and a potential target for screening. (325, 392)
- **Suitable screening test should be available** - TUCL measurement stands out as a reliable and validated test for PTB screening. (575)
- **Screening should be acceptable to the population** – TUCL screening is widely accepted among pregnant women. Research indicate that TVU is well-tolerated, with less than 2% of women experiencing severe discomfort during the procedure. In comparison to other medical exams like cervical smears, TVU is often considered less burdensome. (392, 576) Approximately 75% of women with singleton pregnancies, without a history of spontaneous PTB, consent to undergo TVU CL screening between 18-24 weeks of gestation. (577) These findings underscore the acceptability, of TVU CL screening, indicating its potential for successful implementation as a universal screening programme while being widely acceptable to the majority of women. (577, 578) Incorporating second trimester TUCL universal screening into routine clinical practice is readily accepted. (579)
- **Effective treatment should be available** - vaginal progesterone therapy has been associated with an impressive approximate 40% reduction in the risk of preterm birth in this population. (303, 307, 377, 395, 400) American College of Obstetricians and Gynaecologists (ACOG) and the Society for Maternal-Fetal Medicine (SMFM) recommend the administration of progesterone to women with short cervix. (366, 388) Alongside vaginal progesterone, alternative treatment options such as cerclage and vaginal pessary are also applicable (See 2.9. Prevention of spontaneous preterm birth).
- **Screening programme should be cost-effective** - several cost-effectiveness and decision analyses have consistently highlighted that TUCL screening coupled with vaginal progesterone administration is a cost-effective intervention for preventing preterm birth and its associated perinatal morbidity and mortality. (387, 580-584) Cahill et al. conducted a comprehensive decision analysis, comparing the cost-effectiveness of four distinct

strategies aimed at mitigating preterm birth risks. These strategies included: universal ultrasound screening via cervical length measurements coupled with vaginal progesterone treatment; ultrasound screening specifically targeted at women with heightened preterm delivery risk, followed by vaginal progesterone treatment; treatment with 17OHP-C among high-risk women without prior ultrasound screening and no screening or treatment. Their analysis revealed that universal ultrasound screening alongside vaginal progesterone treatment emerged as the dominant strategy. Remarkably, the study projected that implementing universal screening in the USA could potentially save nearly \$13 billion annually and prevent 95,920 preterm births. Even when various assumptions were altered, such as the cost of vaginal progesterone or ultrasound screening, universal screening remained the preferred strategy in 99% of scenarios. (387)

Similarly, in the study by Werner et al. (581) the cost-effectiveness of ultrasound measurement of cervical length between 18 and 24 weeks was compared against no screening. Within this model, women identified as high risk due to a cervical length <15 mm were administered vaginal progesterone, resulting in a remarkable 45% reduction in the risk of preterm delivery before 34 weeks, as supported by data from Fonseca's study. Once again, ultrasound screening emerged as the dominant strategy. For every 100,000 women screened, potential savings of over \$12 million could be achieved, with the ability to prevent 22 neonatal deaths or long-term neurological deficits. Consistent with the findings of the Cahill study, universal screening remained generally cost-effective even when various assumptions were altered, encompassing factors such as the cost of vaginal progesterone or ultrasound screening, predictive values related to a short cervix, or the efficacy of progesterone. (581)

Subsequent studies have shown that the incidence of a short cervix (≤ 25 mm) is lower than initially anticipated, which may affect the cost-effectiveness of universal screening. (377, 394-398) In light of these findings, Werner et al. undertook a re-evaluation of the cost-effectiveness of universal screening and even with short cervix incidences being as low as 0.8%, their model demonstrated that universal screening remained cost-effective compared to no screening. (580)

Parry et al. (389) calculated that approximately 400 ultrasound examinations would be needed to prevent 25 preterm births before 34 weeks. (508) Despite the increased costs

associated with exams and treatment, many studies have concluded that universal screening is a more cost-effective strategy. (387, 583-585)

Several factors can influence the cost-effectiveness of universal screening for PTB, including the prevalence of PTB, the incidence of a short cervix, the cut-off used to define a short cervix and the effectiveness of progesterone in reducing PTB risk. For example, a sensitivity analysis indicated that TUCL screening may no longer be considered cost-effective if the prevalence of TVU CL ≤ 20 mm falls below 0.31% or if progesterone demonstrates a lower efficacy. (586)[531] but many cost-analyses studies have concluded that TUCL screening is cost-effective, even if we assume a low incidence of short cervical length and a modest impact of treatment with progesterone. (339, 408, 567, 580, 583, 586-591)

An alternative approach to mitigate the cost of the examination is the two-step method. A review assessing the cost-effectiveness of a screening programme using transabdominal ultrasound as the initial screening in low-risk women and TUCL screening in higher-risk populations. (360, 592)

In summary, while universal TUCL screening involves initial costs, the evidence supports its cost-effectiveness and potential to reduce preterm birth rates.

- **Programme should have continuous quality assurance** - in response to this need, certification programmes have been established to train and certify healthcare professionals, as FMF certification programmes. (362, 593)
- **Benefits should outweigh harms** - it is crucial to address potential harms, including false-positive results, overdiagnosis, unnecessary interventions and psychological distress. Studies have demonstrated the safety of endovaginal ultrasound in pregnant women, even in cases of PPROM. (392, 594) These findings underscore the safety, acceptability, reproducibility and accuracy of transvaginal ultrasound cervical length screening. (578)
- **Equitable access to screening services** - accurate assessment of cervical length using ultrasound requires not only appropriate technology but also training and experience in obtaining and interpreting images. Currently, not all facilities offering obstetrical ultrasound have the expertise, capacity and equipment necessary to provide cervical length screening. (595)

- **Cases of real universal screening implementation** - several successful examples of universal transvaginal ultrasound cervical length screening programmes have been implemented, demonstrating the feasibility of such initiatives. (567, 577, 596, 597)

One of the most compelling cases of successful universal screening implementation comes from Israel, where national guidelines were introduced recommending second trimester routine TUCL measurement. The introduction of these guidelines led to a notable decrease in PTB prevalence, particularly among singleton pregnancies. (598)

The ECHOCOL Study: The study comprised two parts: a before-and-after multicentre study and an analysis of the ECHOCOL prospective cohort. **The Before-and-after** analysis revealed a significant decrease in PTB rates between the periods before and after the implementation of routine CL screening. This reduction persisted even after adjusting for various confounding factors through multivariate analysis, indicating that the observed decrease in PTB rates was likely attributable to the screening intervention. **The Prospective Cohort Study** provided real-world data that further confirmed the effectiveness of routine CL measurement in identifying pregnancies at risk of PTB. By closely monitoring cervical length, healthcare providers could intervene early, thereby reducing the likelihood of preterm delivery. (597)

In summary, universal TUCL screening aligns with the general principles outlined by the World Health Organization for an effective screening tool. The International Federation of Gynaecology and Obstetrics has updated its guidelines to recommend universal TUCL screening. (420) Additionally, the ACOG and the SMFM recognize the potential of such a screening strategy, indicating its consideration in clinical practice. (340, 366) However, most other societies have not updated their guidelines accordingly. (366, 388, 599)

In light of the contradictory information regarding CL distribution, prevalence of short cervix and the optimal cut-off values, we designed a retrospective study to assess these parameters among Portuguese pregnant women. Our study included 4,481 singleton pregnancies and we documented an inverse association between TUCL measurements and PTB, consistent with findings from many other studies. (246, 330, 339, 352, 393, 508, 559, 596)

As reported in recent studies, our prevalence of short CL was lower than in initial studies, with a CL ≤ 25 mm representing the 3rd percentile of our population. (246, 409, 600) Additionally, while TUCL testing demonstrated high specificity for predicting PTB, it exhibited low

sensitivity and a modest AUC, aligning with findings from other studies. (246, 409, 600) These results are expected, as spontaneous PTB is a heterogeneous condition with multiple etiological pathways. Only a subset of PTB cases is attributable to cervical factors. Other causes, such as infection, uterine overdistension and placental or hormonal factors, are not detected by this screening method, limiting its overall efficacy. (247, 258)

To address the multifactorial nature of PTB, we developed a multivariable model to enhance screening efficacy. Our model combined TUCL measurements with factors such as maternal age over 40 years, history of PTB and prior cervical surgery. This approach improved predictive accuracy, as evidenced by an increased AUC, indicating better discrimination between those at risk and not at risk for PTB. Similarly, a study by Celik et al. demonstrated that combining cervical length measurements with obstetric history enhances the prediction of spontaneous PTB, achieving higher sensitivity and specificity than either factor alone. (479)

Nowadays, there is no debate that second trimester TUCL is the most powerful screening instrument available, but the best cut-off to separate normal from short cervix is still controversial. (330, 343, 352, 371, 577, 601) As most parameters in medicine, there is no biologic TUCL cut-off and define “short” is not an easy task. Lower cut-offs present with a good specificity but low sensitivity, but higher value leads to an increase in the false positive rate. (246, 602) Most guidelines recommend a 25mm cut-off as it corresponds to the 10th percentile of the initial published trials. (246, 330, 331, 340, 366, 377, 400, 401, 602) However, studies that are more recent had showed a lower prevalence of short cervix defined as TUCL ≤ 25 mm (table 11). (409, 567, 577, 600, 603) Our results follow this new tendency, as the prevalence of TUCL ≤ 25 mm was only 3% and the 10th percentile of our population 28.9 mm and so we test what would be the best cut-off for our population and we concluded that it was 20mm (Figure 3). When comparing 25 to 20 cut-off, the vast majority of pregnant women are categorized into the low-risk group (<0.25 probability) for PTB, regardless of whether the 25 mm or 20 mm TUCL cut-off is used. A small number of women are reclassified into higher probability groups when the 20 mm cut-off is applied, indicating that a lower TUCL threshold may identify additional women at increased risk for PTB and PTB rates increase with higher probability groups, suggesting that the classification system aligns with actual PTB occurrences. The reclassification of women into higher-risk categories with the 20 mm cut-off, along with corresponding higher PTB rates in these groups, may support the consideration

of a lower TUCL threshold in clinical practice to better identify women at risk for PTB in order to reduce the false positive rate.

Our study on the applicability and usefulness of second trimester TUCL for predicting PTB in Portuguese pregnant women has several strengths and contributions to the field. However, there are some limitations to consider:

- **Study Design and Analysis:**

Retrospective Design: the study relies on existing data and may not capture all relevant variables or ensure the accuracy of recorded data, potentially introducing information bias.

Cross-sectional Analysis: While useful for assessing prevalence and associations, a cross-sectional design limits the ability to establish causality or temporal relationships between variables.

- **Selection Bias and Exclusion Criteria:**

Exclusion Criteria: The study excluded a significant number of participants (3476 out of 8016) due to various reasons such as incomplete data, transabdominal measurements instead of transvaginal and other medical reasons. This could introduce selection bias if the excluded cases differ systematically from those included.

Single-Centre Study: Conducted at a single hospital (São João University Hospital), which may limit the generalizability of the findings to other populations or settings.

- **Generalizability and External Validity:**

Population Characteristics: The cohort characteristics (e.g., predominantly low-risk pregnancies, relatively low prevalence of short cervix) may not be representative of other populations.

Geographic Limitation: Findings may not be applicable to regions with different healthcare systems, prenatal care practices or demographic profiles.

- **Measurement and Interpretation of TUCL:**

Variability in Measurement: Despite efforts to standardize TUCL measurements using FMF guidelines, there can still be variability in measurements based on operator skill and technique, potentially affecting the accuracy and reliability of the results.

Definition of Short Cervix: The study uses a 25mm TUCL cut-off to define a short cervix, which while justified by data may not be universally applicable and could lead to different clinical outcomes in other populations.

- **Clinical Implications:**

Treatment Impact: The study mentions the use of vaginal progesterone or Arabin Pessary for women with short cervixes, but it does not fully explore the impact of these treatments on PTB rates or outcomes, which is crucial for evaluating the clinical significance of TUCL screening.

This project offers valuable insights into the histological and cellular changes associated with PTL, as well as the impact of screening for early CR through TUCL measurement. Addressing the limitations of the studies could enhance the applicability of its findings to broader clinical practice.

5. RECOMMENDATION FOR FUTURE RESEARCH

PTB remains one of the most significant challenges in obstetrics and while considerable progress has been made in understanding its underlying mechanisms, much remains to be explored. This research highlights the crucial role of CR in the pathogenesis of PTB, with particular focus on the inflammatory processes that distinguish preterm from TL. As our understanding deepens, it is clear that a multifaceted approach to research will be necessary to address the complex biological processes involved in PTB and to develop more effective screening and therapeutic strategies. Below are several key recommendations for future research to further elucidate the role of cervical function and remodelling in preterm birth.

A) FOCUS ON WOMEN WITH SHORT CERVIX IN THE SECOND TRIMESTER

Investigating women with a short cervix during the second trimester could provide valuable insights into the cellular and molecular alterations that initiate premature CR. This early stage is crucial for understanding the mechanisms leading to preterm birth.

Recent studies have identified specific molecular profiles associated with a short cervix. For instance, a study found that a short cervix is linked to decreased cervicovaginal lipid metabolites, particularly sphingolipids, which are essential for stabilizing cell membranes and protecting against environmental exposures.(604)

By focusing on women with a short cervix, researchers can identify early biomarkers and therapeutic targets to address the underlying causes of premature CR.

B) ELUCIDATING MOLECULAR AND CELLULAR MECHANISMS OF CERVICAL REMODELLING

Recent animal studies have highlighted distinct molecular and cellular pathways may drive CR, depending on the timing and triggers of labour. Future research should prioritize the investigation of these pathways to identify reliable markers of premature CR. Focusing on processes such as EMT, inflammation triggers beyond infection, endocrine imbalances, environmental toxicants and genetic polymorphisms will be crucial.

C) STUDYING INFLAMMATORY CELLS AND THEIR ROLE IN LABOUR INITIATION

The inflammatory response is increasingly recognized as a key factor in PTB. Future studies should focus on the role of inflammatory cells, particularly neutrophils and mononuclear cells, in the initiation of labour. Investigating how these cells contribute to CR, labour onset and PTB will provide insights into the underlying pathophysiology. Immunohistochemically evaluations of tissue samples should be expanded to assess the functional activation of these inflammatory cells.

D) ADVANCEMENTS IN NON-INVASIVE SCREENING METHODS

While TUCL remains the gold standard for screening PTB risk, its predictive accuracy is limited by suboptimal AUC performance. To overcome these limitations, research should focus on developing and validating new, non-invasive screening markers. Preliminary studies about to be published suggest that evaluating the cervical angle (assessed by measuring the cervical length in two segments and calculating the angle between these segments) could improve diagnostic accuracy.

E) INTEGRATING MOLECULAR BIOLOGY, GENETICS AND BIOENGINEERING

A multidisciplinary approach that integrates expertise from obstetrics, molecular biology, genetics and bioengineering will be crucial to advancing our understanding of PTB. Collaboration across these fields will allow for innovative research that considers the genetic and molecular underpinnings of CR. Genetic polymorphisms may offer insights into why certain women are at greater risk of PTB and bioengineering approaches could help develop new diagnostic tools or therapeutic devices, such as cervical support systems or tissue-engineered therapies. This cross-disciplinary approach will be key to advancing both our scientific knowledge and clinical management of PTB.

6. CONCLUSIONS

In conclusion, this thesis has made significant strides in enhancing our understanding of the role of cervical function and remodelling in the aetiology of PTB, a critical obstetric complication with profound implications for both maternal and neonatal health. Through a comprehensive exploration of cervical length measurement, particularly TUCL, we have reinforced the importance of early detection and intervention in managing PTB risk. Our findings contribute to the growing body of evidence supporting the utility of cervical length screening as a valuable tool for identifying at-risk pregnancies and guiding clinical decision-making.

This research has also shed light on the complexities of CR and its underlying cellular mechanisms, offering new insights into the timing and nature of labour onset. By examining inflammatory profiles and fibroblast activity in cervical tissue, we have identified key biomarkers that may help predict and prevent preterm delivery. These insights not only advance the scientific understanding of PTB but also pave the way for more targeted and individualized approaches to maternal care.

While challenges remain in the implementation of universal cervical length screening, particularly regarding cost-effectiveness and feasibility in diverse healthcare settings, the findings of this thesis advocate for a more integrated approach that combines cervical screening with other maternal risk factors. By refining screening thresholds and improving our understanding of the cervix's role in PTL, we can enhance the predictive accuracy of these interventions.

Ultimately, the findings presented here may have significant clinical implications. By addressing some of the gaps in our knowledge about cervical function and remodelling, this thesis lays groundwork that may be useful for the development of more effective guidelines and strategies aimed at reducing the incidence of preterm birth. The broader impact of these efforts is the potential to improve neonatal outcomes, reduce healthcare costs and promote better maternal-fetal care on a global scale. Through the continued pursuit of research in this field, we can look forward to a future where preterm birth prevention is more successful, leading to healthier mothers and children worldwide.

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APPENDIX

I. ARTICLE

Félix J, Bartosch C, Matias A. The impact of routine transvaginal ultrasound measurement of the cervical length on the prediction of preterm birth: a retrospective study in a tertiary hospital. *Rev Bras Ginecol Obstet*. 2021 Apr;43(4):264-74. doi: 10.1055/s-0041-1726053.



The Impact of Routine Transvaginal Ultrasound Measurement of the Cervical Length on the Prediction of Preterm Birth: A Retrospective Study in a Tertiary Hospital

O impacto da medição por rotina do comprimento cervical por ecografia transvaginal na predição de parto pré-termo: Um estudo retrospectivo num hospital terciário

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Abstract

Preterm birth (PTB) is a major obstetric problem associated with high rates of neonatal morbidity and mortality. The prevalence of PTB has not changed in the last decade; thus, the establishment of a screening test and effective treatment are warranted. Transvaginal ultrasound measurement of the cervical length (TUCL) has been proposed as an effective method to screen pregnant women at a higher risk of experiencing PTB. **Objective** To evaluate the applicability and usefulness of second-trimester TUCL to predict PTB in a cohort of Portuguese pregnant women.

Methods Retrospective cross-sectional cohort study including all singleton pregnant women who performed their second-trimester ultrasound (between weeks 18 and 22 + 6 days) from January 2013 to October 2017 at Centro Hospitalar Universitário São João.

Results Our cohort included 4,481 women. The prevalence of spontaneous PTB was of 4.0%, with 0.7% occurring before the 34th week of gestation. The mean TUCL was of 33.8 mm, and percentiles 3, 5 and 10 corresponded to TUCLs of 25.0 mm, 27.0 mm and 29.0 mm respectively. The multiple logistic regression analysis, including maternal age, previous PTB and cervical surgery showed a significant negative association between TUCL and PTB, with an odds ratio (OR) of 0.92 (95% confidence interval [95%CI]: 0.90–0.95; $p < 0.001$). The use of a TUCL of 20 mm is the best cut-off, when compared with the 25-mm cut-off, improving the prediction of risk.

Keywords

- ▶ preterm birth
- ▶ preterm birth screening
- ▶ transvaginal ultrasound cervical length
- ▶ cervical length cut-off

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Conclusion The present study showed an inverse association between TUCL and PTB, and that the inclusion of other risk factors like maternal age, previous PTB and cervical surgery can improve the screening algorithm. Furthermore, it emphasizes that the TUCL cut-off that defines short cervix can differ according to the population.

Resumo

O parto pré-termo (PPT) é uma grande complicação obstétrica que se associa a elevadas taxas de morbimortalidade neonatal. A sua prevalência não tem alterado na última década, sendo essencial determinar uma forma de rastreio e tratamento eficaz. A medição ecográfica transvaginal do comprimento cervical tem sido proposta como um método eficaz de rastreio das grávidas com risco aumentado de PPT.

Objetivo Avaliar a aplicabilidade e utilidade da medição ecográfica transvaginal do comprimento cervical na previsão de PPT numa amostra de grávidas portuguesas.

Método Estudo de coorte retrospectivo incluindo todas as grávidas com gestação unifetal que realizaram ecografia do 2º trimestre (de 18 a 22 semanas + 6 dias) no Centro Hospitalar Universitário de São João entre janeiro de 2013 e outubro de 2017.

Resultados A nossa amostra incluiu 4.481 mulheres. A prevalência de PPT espontâneo foi de 4,0%, sendo que 0,7% ocorreu antes das 34 semanas de gestação. A média do comprimento cervical por ecografia transvaginal foi 33,8 mm, e os percentis 3, 5 e 10 da amostra corresponderam a comprimentos cervicais de 25,0 mm, 27,0 mm e 29,0 mm, respetivamente. A regressão logística múltipla, que incluiu a idade materna, PPT anterior e antecedentes de conização, demonstrou uma associação estatisticamente significativa entre o comprimento cervical e o risco de PPT, com um risco relativo de 0,92 (intervalo de confiança de 95% [IC95%]: 0.90–0.95; $p < 0.001$). A utilização de um valor de referência de comprimento cervical de 20 mm, quando comparado com o valor de referência de 25 mm, melhora a previsão do risco de PPT.

Conclusão Este estudo demonstra uma associação entre o comprimento cervical avaliado por ecografia transvaginal e o risco de PPT, e salienta que a inclusão de outros fatores de risco, como idade materna, PPT anterior e antecedentes de conização podem melhorar o algoritmo de rastreio. Realça ainda que o valor de comprimento cervical utilizado para definir “colo curto” varia de acordo com a população em estudo.

Palavras-chave

- ▶ parto pré-termo
- ▶ rastreio parto pré-termo
- ▶ comprimento cervical por ecografia transvaginal
- ▶ valor de referência do comprimento cervical

Introduction

The World Health Organization (WHO) defines preterm birth (PTB) as a delivery that occurs before the 37th week of gestation. It can occur spontaneously or due to medical induction (iatrogenic). Poorly understood to date, spontaneous PTB is a heterogeneous syndrome with multiple underlying pathophysiologic events and causes.¹

Approximately 11% of infants worldwide are born too soon, corresponding to 15 million premature newborns every year.^{1–3} The prevalence ranges from 5% in European developed countries to 18% in certain African countries, but these international differences may reflect variations in definitions rather than a true epidemiological difference. For example, the method to determine the gestational age and different viability limits can influence this rate. In Portugal, the prevalence of singleton live preterm newborns is of 7.4%.⁴

Despite all advances in medicine, PTB is still an important health problem, and the leading cause of neonatal mortality. Prematurity is associated with multiple neonatal complications and long-term morbidity.^{5,6}

Fetal development is a continuum, and the risk of perinatal complications is inversely related to the gestational age at delivery. For this reason, some experts recommend a sub-classification of PTB into early PTB (< 34 weeks) and late PTB (between 34 weeks and 36 weeks + 6 days), as the negative impact is different in the two groups.⁷ Infants born before the 32nd week of gestation represent less than 2% of all premature births, but they contribute to 50% of the overall perinatal mortality.⁸

Preterm birth is such a major economic and social burden that its reduction is one of the Millennium Development Goals established by the United Nations.⁹ Unfortunately, despite all efforts, the rate of prematurity has not changed in the past 30 years, and, in 2016, the WHO included PTB as one of the top-10 priority research areas.^{10,11}

During the last years, many risk scores have been proposed to predict PTB, but they all have a low sensitivity and poor positive predictive value (PPV).^{12,13} The history of previous spontaneous PTB, for example, is the most significant risk factor known, but only 10% to 15% of PTBs occur after a previous event.^{14,15}



Fig. 1 Transvaginal ultrasound measurement of the cervical length (TUCL). (A) Normal cervix; (B) short cervix.

As the majority of spontaneous PTBs occur in low-risk pregnancies, Andersen et al.¹⁶ (1990) proposed the use of transvaginal ultrasound measurement of the cervical length (TUCL) as a predictor of PTB. Since then, the technique has been well standardized, and its reproducibility, confirmed.^{17,18}

The risk of experiencing PTB is inversely correlated to the cervical length, but the ideal cut-off for clinical use is still controversial.^{16,19–23} By definition, a cervical length below the 10th centile for gestational age is considered “short.” This value varies according to the gestational age, the population distribution of TUCL, and the prevalence of PTB. In the initial trials, the 10th centile was of 25 mm; therefore, this cut-off has been widely used.^{15,24–27} Since then, many cut-offs (from 15 mm to 30 mm) have been proposed, but none is consensual.

The main objective of the present study was to evaluate the applicability and usefulness of second-trimester TUCL to predict PTB in Portuguese pregnant women. We analyzed the distribution of TUCL in our cohort and determined the prevalence of short cervix using different cut-offs. Furthermore, we developed models to estimate the best TUCL cut-off in our cohort and improve its usefulness.

Methods

The present was an observational, retrospective cross-sectional cohort study carried out at the Obstetrics and Gynecology Department of Centro Hospitalar Universitário São João (CHUSJ), Portugal, after approval by the hospital's ethics committee (CES 81-17).

We included all singleton pregnant women who underwent the second-trimester ultrasound (the 18th week to the 22nd week + 6 days, determined by the crown-rump length before the 14th week) from January 2013 to October 2017 in this hospital. Delivery in the same institution and the existence of delivery data were also inclusion criteria. We excluded all women that had induced PTBs for medical reasons (including premature rupture of membranes), cervical cerclage performed prior to screening, diagnosis of chorioamnionitis, and deliveries before the 24th week.

The ultrasound exams at CHSJ are performed by obstetricians with accreditation from the Fetal Medicine Foundation (FMF) for cervical assessment. However, because the universal screening of TUCL is not mandatory, all ultrasound images available through the Astraia software (Astraia Software, GmbH, Munich, Germany) were reviewed in order to identify patients with an ultrasound image that complied with standard the rules of the FMF, which recommends the use of a transvaginal probe with the identification of the sagittal view of the cervix, occupying 75% of the image. Identification of the internal os, external os and cervical canal is essential. The measurement is performed in a straight line between the external and internal os. Care should be taken to distinguish between the cervical canal and the lower uterine segment (→ Fig. 1).

At our hospital, all pregnant women with TUCLs ≤ 25 mm are considered to have a short cervix, and vaginal progesterone or the Arabin (Dr. Arabin GmbH & Co., Witten, Germany) pessary is suggested.

Maternal characteristics, medical history, obstetric history and delivery data were obtained from the database of the Obstetrics and Gynecology Department through the Obscare software. This data was compiled using the Statistical Package for the Social Sciences (SPSS, IBM Corp., Armonk, NY, US) software, version 24, for the statistical analyses. The continuous variables were expressed as means $\pm$ standard deviations (SDs), and frequencies and percentages were used to describe the categorical variables.

The frequencies of PTB were calculated according to different groups of cervical length measurements. The diagnostic ability of different TUCL cut-offs was evaluated in terms of sensitivity, specificity, PPV, negative predictive value (NPV), and the area under the curve (AUC).

An exploratory univariate analysis of clinical and demographic global data was first performed to determine the variables that predicted PTB and those associated with TUCL. All of the hypothesis tests conducted were two-tailed, and they included the Student *t*-test, the Chi-squared (χ^2) test, and the Fisher exact test, as appropriate. Then, we developed a multivariate logistic regression model aiming to predict

PTB as the outcome, using the TUCL as the explanatory variable, and including the main effects of maternal age, previous PTB and cervical surgery. For all of these analyses, values of $p < 0.05$ were considered statistically significant.

To select an optimal TUCL cut-off, we used the maximum likelihood and a confidence interval based on a likelihood ratio test. The likelihoods were calculated for a series of our multivariate logistic regression model using all TUCL cut-offs between 8 mm and 50 mm. The 95% lower and upper confidence bounds were determined as parameter values that reduce the maximum likelihood by $\chi^2(0.05,1)/2 = 1.92$. Using this optimal TUCL cut-off, we then assessed the potential differential effects across subgroups of risk factors using a stratified analysis. The effect modification among strata was checked using a test of homogeneity. Adjusted estimates were calculated using the Cochran-Mantel-Haenszel method.

In Portugal, the most used cut-off is $TUCL \leq 25$ mm. In order to compare our optimal cut-off with the 25-mm cut-off, we classified each woman into groups of predicted probabilities derived from corresponding multivariate models using each cut-off. We then cross-classified these groups and compared them to the observed proportions of events in each group.

Results

During the aforementioned period, 8,016 women underwent a routine second-trimester ultrasound and delivery at CHUSJ. In total, 3,476 women were excluded from this group for the following reasons: delivery before the 24th week ($n = 5$);

medically-induced PTB ($n = 241$); cervical cerclage prior to ultrasound ($n = 19$); diagnosis of chorioamnionitis ($n = 3$); absence of cervical length measurement ($n = 958$); and images of the cervical length measurement that did not comply with FMF recommendations ($n = 2,426$). Within the latter group, the major reason for exclusion was a transabdominal measurement ($n = 1,275$), instead of the transvaginal approach preconized by the FMF. Some women presented more than one exclusion criteria.

Our final cohort consisted of 4,481 women with a mean age of 30.7 ± 5.5 years. Primigravidae represented 45.4% of the sample, and 56.2% had no previous delivery. Most of them had no medical (86.5%) or obstetric (96.7%) relevant background. Only 1.6% of these women had a previous spontaneous PTB, and 0.9% had history of cervical surgery, the 2 major known risk factors for PTB.

Spontaneous delivery occurred in 64.3% of the cases, and 76.4% of the women underwent vaginal delivery. The prevalence of spontaneous PTB prevalence of the original cohort (8,016 women) was of 6.9% (553), and, after applying the exclusion criteria (with the sample reduced to 4,481 women) the prevalence dropped to 4.0% ($n = 179$), mainly due to the exclusion of medically-induced PTB. In total, 96.0% ($n = 149$) of the cases of PTB occurred between the 34th and 37th weeks, and 0.7% ($n = 30$) occurred before 34th week of gestation. The maternal and clinical characteristics of our cohort are described in **table 1**.

The mean gestational age at the time of the ultrasound was 21 weeks + 3 days, with a distribution of 0.2% (8) at 18 weeks, 0.5% (22) at 19 weeks, 12.4% (555) at 20 weeks,

Table 1 Maternal and clinical characteristics of the study population

Maternal features	Medical background	Risk factors for Preterm birth
Age (years) Mean $\pm$ standard deviation: 30.7 ± 5.5 Min: 14 Max: 50 Body mass index (kg/m^2) Mean $\pm$ standard deviation: 24.6 ± 4.9 Obesity (> 30): 8.0% Years of schooling < 4th grade: 0.7% 4th to 12 th grades: 64.6% > 12 th grade: 34.7% Smokers: 12.9% Alcohol/drug users: 0.2%	None: 86.5% Uterine malformations: 0.3% Psychiatric disorders: 1.4% Sexually-transmitted diseases: 1.0% Cardiac or renal disorders: 1.0% Diabetes: 0.7% Hypertension: 2.6% Hypothyroidism: 5.2% Neoplasia: 0.8%	Spontaneous preterm birth: 1.6% Cervical surgery: 0.9% Short cervical length (≤ 25 mm) → On 2 nd -trimester ultrasound: 3.0% Obstetric background None: 96.7% Preeclampsia: 0.9% Fetal death: 0.6% Fetal malformation: 0.4% 2 nd T abortion: 0.1%
Obstetric interurrences	Actual obstetric data	Time of delivery
Fetal growth restriction: 3.9% Fetal malformation: 1.6% Urinary infection: 3.2% Other infections: 4.4% Hypertensive syndrome: 3.2% Gestational diabetes: 9.6% Surgery on 1 st /2 nd trimesters: 0.2%	Primigravida: 45.4% Nullipara: 56.2% Assisted reproduction: 2.7% Labor induction: 35.7% Vaginal delivery: 76.4% Male newborn: 50.6%	Mean: 39.2 weeks Minimum: 24.2 weeks Maximum: 42 weeks Term delivery (≥ 37 week): 96.0% Preterm delivery (< 37 week): 4.0% Early preterm birth ($< 34^{\text{th}}$ week): 0.7% Late preterm birth ($\geq 34^{\text{th}}$ week): 3.3%

Table 2 Distribution of preterm and term births across different cervical length intervals

Cervical length	Early preterm birth (< 34 weeks): n (%)	Preterm birth (< 37 weeks): n (%)	Term birth (≥ 37 weeks): n (%)	Total: n (%)
< 15 mm	4 (13.3)	5 (2.8)	11 (0.3)	16 (0.4)
15.1 to 20 mm	3 (10.0)	4 (2.2)	11 (0.3)	15 (0.3)
20.1 to 25 mm	0 (0)	6 (3.4)	74 (1.7)	80 (1.8)
25.1 to 30 mm	3 (10.0)	30 (16.8)	506 (11.8)	536 (12.0)
≥ 30 mm	20 (66.7)	134 (74.9)	3,700 (96.5)	3,834 (85.6)

Table 3 Sensitivity and specificity of the cervical length measurement to predict preterm birth and cumulative incidence of the different cut-offs

Cut-off	Sensitivity	Specificity	Area under the curve	Positive predictive value	Negative predictive value
15 mm	2.8	99.7	0.51	10.9	0.97
20 mm	6.2	99.5	0.53	11.5	0.94
25 mm	10.6	97.3	0.54	3.9	0.91
30 mm	30.7	80.1	0.55	1.5	0.86

66.5% (2979) at 21 weeks, and 20.5% (917) at 22 weeks. The mean TUCL was of $33.8 \text{ mm} \pm 4.8 \text{ mm}$ (range: 3.0 mm to 53.0 mm). Percentiles 3, 5 and 10 corresponded to TUCls of 25.0 mm, 27.0 mm and 29.0 mm respectively. **Table 2** presents the frequency of term and preterm births across different TUCL intervals. Among pregnant women with PTB, the TUCL was significantly lower (mean: 31.6 mm; 95%CI: 30.7–32.5 mm) compared to the measurements of those with term birth (mean: 33.9 mm; 95%CI: 33.8–34.0 mm; $p < 0.001$).

Even though the TUCL alone showed a high specificity to predict PTB, its diagnostic ability was limited by a very low sensitivity, with an AUC close to 0.5 for all different cut-offs studied, as depicted in **table 3**.

The univariate analysis (**Tables 4** and **5**) showed that maternal age ≥ 40 years, history of PTB, and cervical surgery were the main significant predictors of PTB. Additionally, history of PTB and previous cervical surgery were also associated with shorter TUCL, thus acting as confounders.

The multivariate logistic regression analysis, incorporating maternal age ≥ 40 years old, history of PTB, and previous cervical surgery, evaluated the impact of the TUCL as a predictor of PTB (**Table 6**).

The estimated odds ratio (OR) for the effect of the TUCL on PTB, controlling for covariates, was of 0.92 (95%CI: 0.90–0.95; $p < 0.001$), which highlights the significant negative association between TUCL and PTB. The diagnostic ability of the multivariate model improved, showing an AUC of 0.65 (**Fig. 2**).

As expected, in the univariate analysis, the treatment with progesterone/pessary was associated with shorter TUCL, and thus, also with PTB, when considering the total study cohort.

Within the group of women with a diagnosis of short cervix, 68% ($n = 93$) underwent treatment, and 13% ($n = 18$) declined it. There was, however, no significant difference between the frequency of PTB among women who accepted or declined progesterone or the pessary (14% versus 22.2% of PTB respectively; $p = 0.472$); therefore, the treatment was not included in our multivariate model.

To select the optimal TUCL cut-off, we ran several multivariate logistic regression models using different cut-offs (from 8 mm and 50 mm) associated with other variables like maternal age ≥ 40 years, history of PTB, and previous cervical surgery. Plotting the log likelihood from these models against the TUCL showed that a cut-off of 20 mm (95%CI: 19.5–22 mm) best discriminated 2 TUCL subgroups with differential odds for PTB (**Fig. 3**). Women with a short cervix, defined by $\text{TUCL} \leq 20 \text{ mm}$, had an OR of 12.2 (95%CI: 5.8–25.4; $p < 0.001$) compared with those with $\text{TUCL} > 20 \text{ mm}$.

Considering maternal age ≥ 40 years, history of PTB and previous cervical surgery as the main risk factors for PTB, a stratified analysis was performed by separately evaluating women with at least 1 of these factors ($n = 297$) versus women who did not presented any of them ($n = 4,184$ [93.4%]) (**Table 7**). The significant association of a short cervix ($\text{TUCL} \leq 20 \text{ mm}$) with PTB was maintained in both groups, with an OR of 16.2 (95%CI: 2.7–97.1; $p < 0.001$) for women with risk factors, and an OR of 9.8 (95%CI: 4.1–23.7; $p < 0.001$) for women without them. There was no effect modification between the groups ($p = 0.614$, homogeneity test). Considering this stratification, the adjusted OR for women with short cervix ($\text{TUCL} \leq 20 \text{ mm}$) was of 11.4 (95%CI: 5.1–25.4; $p < 0.001$).

Table 4 Demographics comparing preterm and term births

		Preterm birth – n: 179 (4.0%)	Term birth – n: 4302 (96.0%)	p-value
Maternal age	Mean ± standard deviation	31.4 ± 6.0	30.7 ± 5.5	0.098 ^a
	< 40 years old	161 (89.9%)	4122 (95.8%)	< 0.001 ^b
	≥40 years old	18 (10.1%)	180 (4.2%)	
Body mass index	Mean (kg/m ²)	23.97 ± 5.3	24.6 ± 4.9	0.055 ^a
Schooling	≤ 12th grade	111 (62.4%)	2807 (65.4%)	0.404 ^b
	> 12th grade	67 (37.6%)	1485 (34.6%)	
Addictions	Smoking	21 (11.7%)	556 (12.9%)	0.640 ^c
	Drugs	0 (0%)	4 (0.1%)	1.000 ^c
	Alcohol	1 (0.6%)	4 (0.1%)	0.184 ^c
Gestational age at cervical length measurement	Mean ± standard deviation (weeks)	21.5 ± 0.5	21.5 ± 0.6	0.592 ^a
Type of pregnancy	Spontaneous	165 (95.4%)	4,041 (97.2%)	0.150 ^b
	Medical assisted	8 (4.6%)	115 (2.8%)	
Gravidity	Primigravida	83 (46.5%)	1,953 (45.4%)	0.789 ^b
Parity	Nulliparous	101 (56.4%)	2,417 (56.2%)	0.949 ^b
Obstetric history	Preterm birth	17 (9.5%)	55 (1.3%)	< 0.001 ^c
	Second trimester miscarriage	1 (0.6%)	4 (0.1%)	0.184 ^c
	Recurrent pregnancy loss	2 (1.1%)	16 (0.4%)	0.160 ^c
Maternal background	Conization	10 (5.6%)	31 (0.7%)	< 0.001 ^b
	Mullerian anomalies	3 (1.7%)	11 (0.3%)	0.016 ^c
	Chronic hypertension	9 (5.0%)	100 (2.3%)	0.021 ^b
	Diabetes	3 (1.7%)	28 (0.7%)	0.125 ^c
	Hypothyroidism	7 (3.9%)	227 (5.2%)	0.429 ^b
Obstetrical complications	Malformations and cromossomopathies	6 (3.4%)	64 (1.5%)	0.059 ^c
	Fetal growth restriction	9 (5.0%)	167 (3.9%)	0.439 ^b
	Hypertensive syndromes	0	143 (3.3%)	0.040 ^c
	Gestational diabetes	24 (1.4%)	405 (9.4%)	0.751 ^b
	Short interpregnancy intervals	1 (0.6%)	4 (0.1%)	0.184 ^c
	Surgical procedure during pregnancy	0 (0%)	7 (0.2%)	1.000 ^c
	Urinary infections or asymptomatic bacteriuria	8 (4.5%)	135 (3.1%)	0.321 ^b
	Others infections during pregnancy	10 (5.9%)	188 (4.4%)	0.438 ^b
Treatment with progesterone or Arabin pessary		25 (13.9%)	116 (2.7%)	0.000 ^b

Notes: ^at-test; ^bChi-squared test; ^cFisher test.

A simple comparison of the 20-mm and 25-mm cut-offs, based on AUCs of multivariate models, showed no statistical difference (20 mm: AUC = 0.59 [95%CI: 0.56–0.62] versus 25 mm: AUC = 0.60 [95%CI: 0.57–0.64]; $p = 0.157$). However, a better performance in the prediction of PTB of the 20-mm compared to the 25-mm cut-off was highlighted by comparing the distribution of women according to the prediction probabilities derived from the corresponding multivariate models. Globally, 15 (0.33%) women were

reclassified to a different predicted-probability group when the multivariate model included the 20-mm instead of the 25-mm cut-off. As described in **table 6**, out of the 4 women upgraded to a higher probability (from 0.50–0.75 in the 25-mm model to > 0.75 in the 20-mm model), 75% ($n = 3$) experienced a PTB. On the other hand, out of the 8 women reclassified to a lower predicted probability (from 0.25–0.50 in the 25-mm model to < 0.25 in the 20-mm model), only 1 (12.5%) had PTB.

Table 5 Cervical length description according to demographics

			Cervical length (mean $\pm$ standard deviation)	p-value ^a
Maternal age	< 40 years old		33.8 $\pm$ 4.8	0.546
	$\geq$ 40 years old		34.0 $\pm$ 5.0	
Schooling	$\leq$ 12th grade		33.8 $\pm$ 4.8	0.568
	> 12th grade		33.9 $\pm$ 4.9	
Addictions	Smoking	No	33.9 $\pm$ 4.8	0.089
		Yes	33.5 $\pm$ 5.3	
	Drugs	No	33.8 $\pm$ 4.8	0.443
		Yes	35.5 $\pm$ 2.8	
	Alcohol	No	33.8 $\pm$ 4.8	0.170
		Yes	30.5 $\pm$ 3.1	
Type of pregnancy	Spontaneous		33.8 $\pm$ 4.8	0.664
	Medically-assisted		33.6 $\pm$ 6.4	
Gravidity	Primigravida		33.5 $\pm$ 4.8	0.000
	Multigravida		34.1 $\pm$ 4.9	
Parity	Nulliparous		33.4 $\pm$ 4.8	0.000
	Multiparous		34.3 $\pm$ 4.8	
Obstetric history	Preterm birth	No	33.9 $\pm$ 4.8	0.000
		Yes	31.8 $\pm$ 5.0	
	Second-trimester miscarriage	No	33.8 $\pm$ 4.8	0.001
		Yes	26.8 $\pm$ 8.4	
	Recurrent pregnancy loss	No	33.8 $\pm$ 4.8	0.412
		Yes	32.3 $\pm$ 7.9	
Maternal background	Conization	No	33.9 $\pm$ 4.8	0.000
		Yes	30.8 $\pm$ 5.6	
	Mullerian anomalies	No	33.8 $\pm$ 4.8	0.993
		Yes	33.9 $\pm$ 3.2	
	Chronic hypertension	No	33.8 $\pm$ 4.8	0.540
		Yes	34.1 $\pm$ 6.2	
	Diabetes	No	33.8 $\pm$ 4.8	0.120
		Yes	35.9 $\pm$ 4.6	
	Hypothyroidism	No	33.8 $\pm$ 4.9	0.659
		Yes	34.0 $\pm$ 4.5	
Obstetrical complications	Malformations and cromossomopathies	No	33.8 $\pm$ 4.8	0.821
		Yes	34.0 $\pm$ 4.9	
	Fetal growth restriction	No	33.9 $\pm$ 4.8	0.000
		Yes	32.4 $\pm$ 4.9	
	Hypertensive syndromes	No	33.8 $\pm$ 4.8	0.448
		Yes	33.5 $\pm$ 4.8	
	Gestational diabetes	No	33.8 $\pm$ 4.8	0.967
		Yes	33.8 $\pm$ 45.0	
	Short interpregnancy intervals	No	33.8 $\pm$ 4.8	0.871
		Yes	34.3 $\pm$ 2.4	
	Surgical procedure during pregnancy	No	33.8 $\pm$ 4.8	0.934
		Yes	34.0 $\pm$ 3.3	
	Urinary infections or asymptomatic bacteriuria	No	33.8 $\pm$ 4.8	0.112
		Yes	33.12 $\pm$ 4.8	
	Other infections during pregnancy	No	33.9 $\pm$ 4.9	0.880
		Yes	33.2 $\pm$ 4.6	
Treatment with progesterone or Arabin pessary	No		34.2 $\pm$ 4.3	0.000
	Yes		24.1 $\pm$ 7.2	

Note: ^at-test.

Table 6 Multivariate logistic regression analysis to evaluate the impact of transvaginal ultrasound measurement of the cervical length as a predictor of preterm birth

Outcome	Odds ratio	95% confidence interval		p-value
Cervical length	0.925	0.90	0.95	0.000
Maternal age	2.265	1.32	3.90	0.003
Previous preterm birth	6.754	3.72	12.16	0.000
Cervical surgery	0.178	0.81	0.40	0.000
Constant	14.557	2.61	81.31	0.002

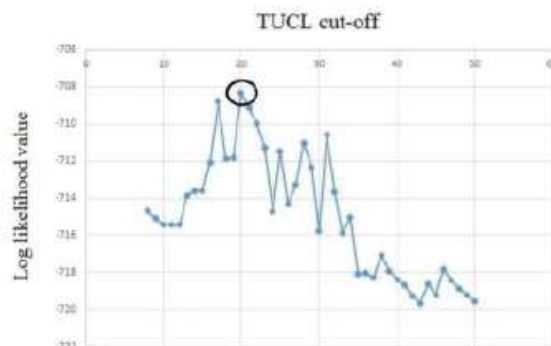


Fig. 3 Plotting to determine the best TUCL cut-off based on multiple log likelihood of the logistic regression model (including maternal age ≥ 40 years, history of PTB, and previous cervical surgery), using cut-offs between 8 mm and 50 mm. The circle represents the cut-off that best discriminated the risk of experiencing PTB.

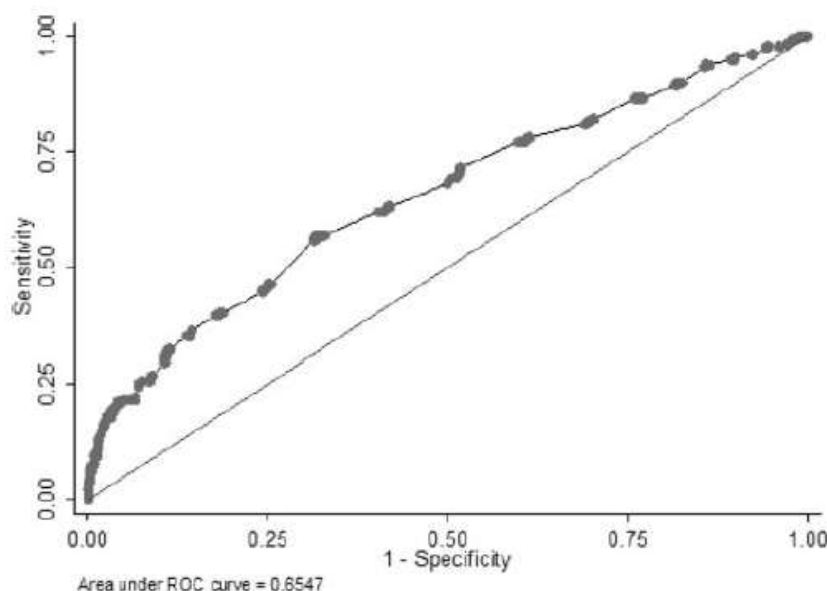


Fig. 2 Graphic representations of sensitivity, specificity and area under the curve (AUC) of the multivariate model.

Table 7 Distribution of the pregnant women according to group probability of preterm birth using transvaginal ultrasound measurement of the cervical length and presence/absence of risk factors (maternal age ≥ 40 years, previous PTB and cervical surgery). Cross-classification of the 25-mm cut-off group (most used cut-off) versus the 20-mm cut-off group (our best cut-off) and frequency of PTB in each subgroup

		Group probability – TUCL: 20 mm				Total reclassified
		< 0.25	0.25-0.50	0.50-0.75	≥ 0.75	
Group probability – TUCL: 25 mm	< 0.25	4,456	1	0	0	0.02%
	PTB% (n)	3.8% (168)	0%	0%	0%	
	0.25-0.50	8	8	2	0	55.6%
	PTB% (n)	12.5% (1)	50.0% (4)	50.0% (1)	0%	
	0.50-0.75	0	0	1	4	80.0%
	PTB% (n)	0%	0%	100% (1)	75.0% (n3)	
≥ 0.75	0	0	0	0	1	0%
	PTB% (n)	0%	0%	0%	0%	

Abbreviations: PTB, preterm birth; TUCL, transvaginal ultrasound measurement of the cervical length.

Discussion

Good practice of disease screening recommends that the condition be an important health problem and facilities for diagnosis and treatment be available, as already published in 1968 by the WHO.²⁸

Preterm birth fulfills the first prerequisite, as it represents a major obstetric complication. Our cohort presents 4% (179) of spontaneous PTB, and this prevalence is similar to that of other studies regarding screening and treatment.^{27,29,30}

A second assumption needed to implement a screening process is the existence of a test able to detect the high-risk population, and the TUCL satisfies this requirement. An inverse association between the TUCL and PTB, which was also observed in our study, has been widely documented.^{16,21,26,27,29–32} The TUCL in our cohort showed a high specificity to predict PTB, but low sensitivity and a poor AUC, results similar to those of other studies. Iams et al.²⁷ reported that $TUCL \leq 25$ mm had a sensitivity of 37% and a specificity of 92%, but more recent studies obtained even lower sensitivities, such as 2.4% in the study by van der Ven et al.,³³ and 8.0% in the one by Esplin et al.³⁴

Our multivariate model showed an improvement in the AUC value, highlighting that a combined screening including maternal age > 40 years, history of PTB and previous cervical surgery should be considered for screening, instead of the TUCL alone. The Society for Maternal-Fetal Medicine Publications Committee, in their 2012 guidelines, concluded that the most effective approach was to initiate treatment in low-risk women with a $TUCL \leq 20$ mm, or high-risk pregnant women with a $TUCL \leq 25$ mm, supporting that other risk factors should be included in the screening algorithm.³⁵

Nowadays, there is no debate that second-trimester TUCL is the most powerful screening instrument available, but the best cut-off to separate normal from short cervixes is still controversial.^{16,19–23} As most parameters in medicine, there is no biological TUCL cut-off, and defining “short” is not an easy task. Lower cut-offs present good specificity but low sensitivity, but higher values (like 29 mm) lead to an increase in the false-positive rate.^{27,36} Most guidelines recommend a 25-mm cut-off, as it corresponds to the 10th percentile in the initial published trials.^{7,15,16,27,36–40} However, more recent studies showed a lower prevalence of short cervixes defined as $TUCL \leq 25$ mm, averaging 2.5%,^{14,33,34,41,42} Our results follow this new tendency, as the prevalence of $TUCL \leq 25$ mm was of only 3% (134), and the 10th TUCL percentile in our cohort corresponded to 29.0 mm.

TUCL distribution can be influenced by many factors; therefore, the ideal cut-off can change in different populations. That said, we concluded that the best cut-off for our cohort was 20 mm (–Fig. 3). This value enabled us to improve the prediction of the risk of experiencing PTB mainly by reducing the false-positive rate (8 women were reclassified as low probability, and only 1 (15.5%) of them experienced a PTB).

In parallel to studies on the efficacy of TUCL screening, cost-analysis studies were also conducted, which concluded that TUCL screening is cost-effective even if we assume a low

incidence of short cervical length and a modest impact of the treatment with progesterone.^{25,31,43–45}

The Federation of Gynecology and Obstetrics Working Group on Best Practices in Maternal Fetal Medicine recommended universal transvaginal cervical length screening and vaginal progesterone when $TUCL < 25$ mm.⁴⁶ Subsequently, studies^{41,42,47} using this recommendation showed a reduction in the PTB rate when universal screening was applied. Son et al.,⁴² for example, obtained a 20% reduction in the rate of PTBs after implementing TUCL screening, even with a very low prevalence short cervixes ($TUCL \leq 25$ mm: 0.89%). The negative impact of PTB is so huge that every approach able to reduce it has a positive impact and should be considered.

Conclusion

Preterm birth represents a major health problem, and strategies to prevent are important. The present study showed an inverse association between TUCL and PTB, and emphasized that other factors like maternal age, history PTB and previous cervical surgery can improve the screening algorithm. The value that defines a short cervix can differ in each population, and, for our cohort, the best cut-off was 20 mm. Even though TUCL has a low diagnostic performance, it is the best screening method available to predict PTB, and TUCL screening has been shown to reduce the PTB rate.

Contributors

All authors were involved in the design and interpretation of the analyses, contributed to the writing of the manuscript, and read and approved the final manuscript.

Conflict of Interests

The authors have no conflict of interests to declare.

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II. ARTICLE

Félix J, Bartosch C, Matias A. Unlocking the cervix: biological mechanisms and research gaps in preterm birth. *Cureus*. 2024 Nov 1;16(11):e72835. doi: 10.7759/cureus.72835.

Unlocking the Cervix: Biological Mechanisms and Research Gaps in Preterm Birth

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Abstract

Preterm birth (PTB), defined by the WHO as delivery before 37 completed weeks of gestation, remains a significant global health challenge and the leading cause of neonatal mortality. Despite extensive efforts to prevent PTB, rates have remained stable, largely due to an incomplete understanding of its underlying pathophysiology. While research has traditionally focused on the myometrium and foetal membranes, the cervix's critical role in maintaining pregnancy and initiating labour is increasingly recognized but still often underexplored. This review aims to consolidate current knowledge on cervical function and remodelling during pregnancy and labour while identifying key research gaps and potential intervention strategies. A comprehensive literature review spanning 50 years was conducted using PubMed, identifying 114 studies that met the inclusion criteria. These studies examined cervical function during labour, spontaneous PTB, and the molecular mechanisms involved in cervical remodelling (CR). The review highlights significant molecular differences in cervical remodelling between term and preterm births. Critical processes such as extracellular matrix (ECM) remodelling, immune cell activity, and genetic predispositions vary depending on the specific triggers and gestational age at the onset of labour. Inflammatory responses are especially heightened in preterm births, particularly those driven by infections. However, much of the current data are derived from animal models, which may not accurately represent human physiology. In conclusion, a deeper understanding of the complex molecular mechanisms driving cervical remodelling is crucial to addressing PTB. Future research should prioritize human tissue studies to bridge the gap between animal models and human physiology. Developing targeted interventions that address cervical insufficiency and improve pregnancy outcomes is essential for reducing PTB rates.

Categories: Obstetrics/Gynecology, Epidemiology/Public Health

Keywords: cervical extracellular matrix, inflammatory response, labour genetic predisposition, molecular mechanism of cervical remodelling, preterm labour

Introduction And Background

Background

Preterm birth (PTB) is defined by the World Health Organization (WHO) as birth before 37 completed weeks of gestation or fewer than 259 days from the first day of a woman's last menstrual period [1]. The global prevalence of PTB is estimated at around 9.9%, with significant regional variations and a higher burden in low- and middle-income countries [2-4].

PTB is a major concern due to its severe impact on neonatal mortality and long-term health complications, resulting in approximately 3.1 million neonatal deaths annually. It is associated with acute conditions such as respiratory distress syndrome and necrotizing enterocolitis, as well as chronic disorders, including cardiovascular and neuropsychiatric issues [5-8]. Beyond immediate neonatal care, PTB imposes substantial economic and healthcare costs and perpetuates a generational cycle of preterm births [6,9,10].

While global health initiatives, including the Millennium Development Goals, have aimed to reduce preterm birth rates, progress has been limited [11,12]. PTB is a multifactorial syndrome with many underlying causes, such as infections, maternal health conditions, and genetic factors, which makes it difficult to address with a single approach. Additionally, the aetiology of PTB is still not fully understood, which hinders the development of effective screening methods and therapeutic interventions. Ongoing research is crucial to better understand the diverse mechanisms behind PTB and to develop targeted, successful strategies.

Addressing PTB is challenging due to its multifaceted causes [13]. Research from the 1990s identified premature cervical remodelling (CR) as a key factor in PTB risk [14]. Romero et al. highlighted cervical ripening as a hallmark of "premature parturition syndrome," emphasizing CR as a primary molecular event in PTB [15]. This underscores the cervix's crucial role in PTB development and suggests that targeting CR could be a promising approach for screening and intervention strategies. This review aims to summarize current knowledge of cervical function in both normal and premature labour and its role in triggering PTB, while identifying critical gaps for future research.

How to cite this article

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Review

Methods

Our literature review examined clinical studies, systematic reviews, and literature reviews on the role of the cervix in labour, particularly premature labour. We searched the PubMed database for full-text studies published in English or Portuguese over the past 50 years, using the search terms: "role of cervix in spontaneous preterm birth," "cervix in labour," "cervical biopsies in labour," and "cervical biopsies in preterm labour."

The inclusion criteria encompassed studies investigating the function of the cervix during labour, research addressing the role of the cervix in spontaneous preterm birth, animal studies exploring cervical roles in preterm birth, and studies utilizing cervical biopsies to examine molecular and cellular processes during cervical remodelling in labour. In contrast, we excluded articles focused on multiple pregnancies, research on induced preterm birth, studies investigating preterm birth with known causes (e.g., infection, hydramnios, foetal malformation), and biopsy studies primarily aimed at detecting cervical lesions.

Initially, 467 articles met the inclusion criteria and underwent abstract review. After applying the exclusion criteria, the final number of articles included in the review was 114 (Figure 1).

PRISMA

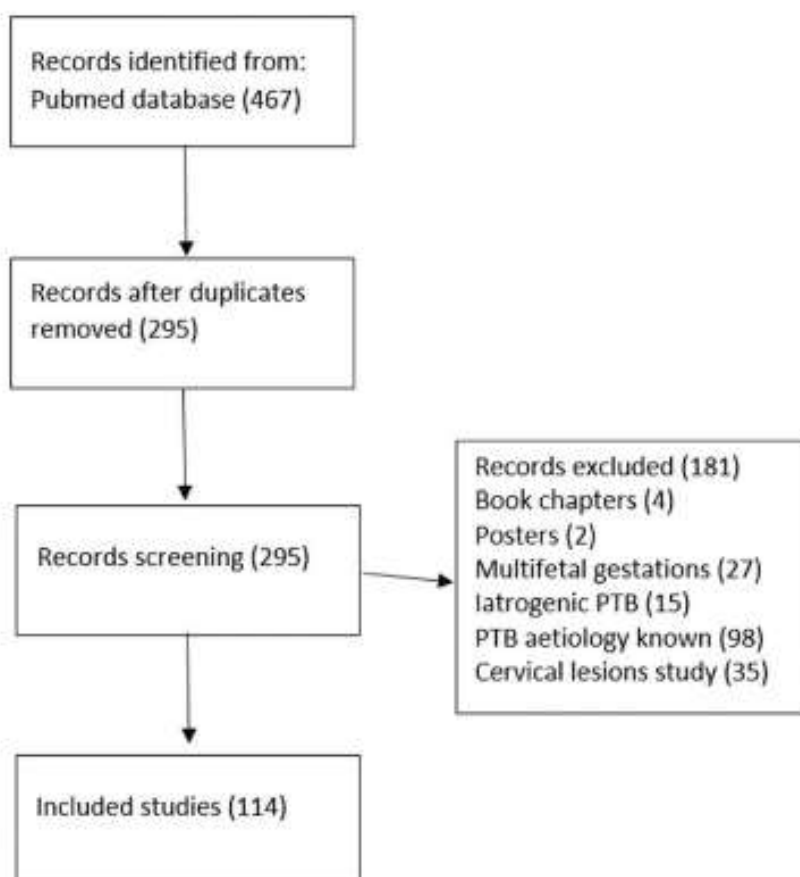


FIGURE 1: Study flowchart (PRISMA)

Cervical function, structure, and remodelling during pregnancy

The cervix acts as a vital mechanical barrier throughout pregnancy, remaining closed until term and undergoing substantial remodelling for successful delivery. Understanding CR is crucial, as deviations from normal patterns increase the risk of PTB. Despite extensive research, knowledge of cervical tissue mechanics

remains limited due to the challenges of studying cervix samples during pregnancy, a period often considered a "protected state." Advances in animal studies, imaging technologies, and molecular biology have offered valuable insights into these processes and their implications for pregnancy outcomes.

The cervix is composed of multiple tissue layers, each with a specific function. Its epithelial outer surface, facing the vagina, is lined with stratified squamous epithelium, which protects it from physical trauma and infections. The inner epithelial portion, the endocervix, is lined with columnar epithelial cells that secrete mucus, which plays a key role in fertility by controlling the passage of sperm [16,17]. Beneath these epithelial layers lies the stroma, a structure made up of dense extracellular matrix (ECM) and various cell types. The ECM, consisting of fibrillar collagens, elastin fibres, proteoglycans, glycosaminoglycans (GAGs), and matricellular proteins, is essential for the cervix's mechanical properties and structural integrity [18-20]. Cells such as fibroblasts, smooth muscle cells, immune cells, glandular cells, and vascular cells each play specialized roles in matrix production, tone regulation, and immune response [16,21]. Additionally, the cervix is highly vascularized and innervated, ensuring adequate blood flow and sensory function. Notably, unlike the rest of the uterus, the cervix exhibits increased innervation throughout pregnancy [22].

Traditionally, the cervix has been viewed as a homogeneous structure, with the ECM comprising 85-90% of its content and cells contributing only 10-15% [20,23,24]. However, recent research has revealed a more intricate and heterogeneous architecture, with regional variations in collagen dynamics and cellular composition [23-25]. Optical coherence tomography has highlighted the presence of circumferential collagen fibres in the middle stroma, which are crucial for maintaining cervical integrity and resisting dilation [22]. Additionally, differences in collagen cross-linking and smooth muscle distribution, especially at the internal os, suggest specialized functions and higher contractility in these regions [25-29]. This evidence underscores the need to recognize location-specific differences in cervical architecture and cell distribution, as these significantly influence the cervix's role during pregnancy and labour [20].

During pregnancy, the cervix serves a dual function: it acts as a formidable barrier against microbial invasion and mechanical stress from the expanding uterus while also undergoing a controlled softening and dilation process to facilitate childbirth. This delicate balance is essential for ensuring a healthy pregnancy and safe delivery [17,29-31]. Cervical remodelling during pregnancy occurs in four overlapping phases: softening, ripening, dilation, and postpartum repair. Softening increases the cervix's pliability, followed by ripening, which enhances its compliance in preparation for dilation. After childbirth, the cervix undergoes postpartum repair to restore its function for future pregnancies [22,31-33]. Effective cervical remodelling is vital for delivery, as uterine contractions alone cannot facilitate labour if the cervix remains rigid [17,23,26,33-37].

Cervical softening is the initial phase of this transformation, marked by changes in tissue properties, such as reduced stiffness and increased compliance, while maintaining tissue integrity [18,25]. Early signs of softening, driven by cellular proliferation and matrix remodelling involving fibroblasts, myofibroblasts, and epithelial cells, can appear as early as one month after conception [17,18,27,32]. Recent research has explored several biomarkers and imaging methods for detecting early cervical changes linked to preterm birth risk. Biomarkers like foetal fibronectin (fFN), IL-6, and matrix metalloproteinases (MMPs) show promise as early indicators due to their association with cervical remodelling and inflammation [19,38,39].

For imaging, transvaginal ultrasound remains the primary non-invasive tool for assessing cervical length (CL), a key predictor of preterm birth. Also, elastography has emerged as a promising technique for assessing cervical stiffness, detecting early softening before significant shortening occurs [40,41].

At this stage, architectural changes in the ECM are characterized by increased collagen turnover and reduced cross-linking. As pregnancy progresses and matures, cross-linked collagen is gradually replaced by less cross-linked forms, facilitating cervical softening while maintaining structural integrity [20,25-27]. During this process, collagen type I fibres evolve from long, thin structures to thicker, curved forms, though the overall content of collagen type I remains stable [20,21,25-27,29,30]. Additionally, cervical epithelial cells play a critical role in providing immunoprotection and maintaining the barrier to safeguard the stroma during ECM remodelling [21].

Following softening, cervical ripening is critical for preparing the cervix for dilation. This phase involves structural changes such as an elevated Bishop Score and a reduced ability of the cervix to maintain closure as a load-bearing organ [18,42-44]. ECM changes during ripening include altered collagen cross-linking, increased fibre diameter, and shifting from straight to wavy fibres [20,21]. GAGs, particularly hyaluronic acid (HA), play critical roles in collagen packing and tissue hydration, with HA levels rising due to oestrogen-driven upregulation of hyaluronan synthase (Has) 2 [17,21,32,42,45,46]. This increase in HA solubilizes collagen, triggering an inflammatory response that is crucial for ripening. Elevated proteoglycans also regulate ECM organization, influencing cellular adhesion and proliferation [17,47].

In the 1980s, Mont Liggins proposed that immune cell infiltration during cervical ripening leads to ECM degradation and remodelling. However, this idea has since been challenged, as it was based on studies using postpartum biopsy samples, which more likely reflect tissue repair mechanisms after childbirth rather than the pre-labour ripening process [21,29,42,48,49]. Recent research suggests that inflammatory cells and

matrix metalloproteinases (MMPs) primarily aid in active dilation and postpartum repair rather than initiating ripening [17,21,29,42,47,48,50-53]. Current evidence indicates that cervical ripening is mainly driven by collagen reorganization through increased GAG synthesis rather than an inflammatory response [17].

Epithelial cells also contribute to ripening by providing barrier protection and facilitating enzymatic activity [54,55]. This process involves controlled adjustments in barrier properties, hydration levels, and intercellular communication [56-58]. Reversible epithelial-mesenchymal transition (EMT) and its counterpart, mesenchymal-to-epithelial transition (MET), regulate cellular remodelling in both the amnion and cervix, driven by steroid hormones during gestation [21,31]. Research suggests that oestrogen promotes EMT, cellular migration, and cervical inflammation, while progesterone supports tissue repair and counteracts infection or inflammation in the cervix [59-64].

The final stage, dilation, is often difficult to distinguish from ripening, as the two phases overlap [17,18,21,32,42]. At term, increased macrophage and neutrophil densities and higher levels of proinflammatory cytokines are associated with cervical dilation [16,17,29,32,65]. Structural changes such as collagen degradation and altered cell density further characterize this phase [29].

Premature cervical remodelling as a contributing factor to preterm birth

Recent research by Romero et al. has highlighted cervical ripening as a critical component of what is termed "premature parturition syndrome." They noted that PTB frequently involves early changes in gestational tissues occurring weeks before labour onset. Specifically, signs of cervical ripening-such as cervical shortening and internal os patency-can be detected through vaginal examinations and ultrasound imaging weeks before a spontaneous preterm labour [17,33,34,37,66-76]. Understanding early cervical changes provides a crucial opportunity for preventing preterm birth by enabling the identification of high-risk patients through biomarkers or advanced imaging techniques. Early detection allows clinicians to intervene before the cervix reaches a critical stage of ripening, offering the chance for timely interventions, such as progesterone or cervical cerclage. This knowledge also highlights a sequence of physiological events that could be targeted for screening and intervention, potentially improving pregnancy outcomes [15,33].

Initially described as "precocious cervical ripening" by Papiernik et al. in 1986, the phenomenon has evolved into the concept now recognized as "premature cervical remodeling." This term encompasses a range of processes, including softening, shortening, and dilation of the cervix [34,77]. Research demonstrates an inverse relationship between CL and gestational age at birth, which challenges the traditional view of cervical incompetence (CI) as a binary condition [34,37,67-72,78-80]. Recognizing and monitoring these early cervical changes can provide a crucial window for screening and identifying women at increased risk of preterm labour. However, to devise effective interventions, further exploration into the underlying pathophysiology of premature cervical remodelling and the implications of a shortened cervix is essential [15,81].

A multitude of factors have been associated with a short cervix, including the cessation of progesterone action, congenital cervical hypoplasia, history of previous cervical surgeries, and infections. Despite these associations, the precise mechanisms contributing to cervical "failure" and the resulting development of a sonographically short cervix remain poorly understood [80,82-86]. Key challenges in elucidating cervical "failure" include the lack of consistent definitions, the scarcity of robust human data, and methodological discrepancies in research. These discrepancies may arise from variations in biopsy locations, timing, and the presence of inadequate control groups [22,87]. This review aims to address these critical issues by synthesizing and examining evidence from both animal models and human studies. Through this approach, we hope to clarify the known mechanisms underlying premature cervical remodelling while identifying gaps in knowledge. This will enable us to design studies that enhance our understanding and explore novel screening methods and treatment strategies that could be implemented to improve outcomes for at-risk populations.

Molecular mechanisms linked to premature cervical remodelling: insights from animal studies

Much of our understanding of labour, both physiological and premature, has been derived from animal models due to the challenges in obtaining human cervical samples during pregnancy. Research utilizing these models has provided valuable insights into the mechanisms of labour, bridging theoretical knowledge with practical applications in human obstetrics. However, while animal models offer valuable insights into the biological mechanisms underlying cervical changes, their findings may not fully translate to human physiology. For example, in mice, labour is linked to a decline in progesterone levels, a phenomenon that is not observed in humans. It is essential to recognize the significant differences in reproductive anatomy, hormonal regulation, and gestational timelines. Therefore, caution is warranted when extrapolating findings from animal models to human contexts. A thorough understanding of both the similarities and differences between species is crucial before drawing direct correlations (Table 1) [66,88-93].

Animal model	Advantages	Limitations
Rodents	Cost-effective, can study large sample sizes, <i>short gestation period</i> , with rapid data collection and genetic manipulation tools available.	Significant anatomical and hormonal differences from humans
Sheep	Similar cervical structure and reproductive physiology to human, larger size facilitates surgical interventions and tissue collection.	<i>Higher cost, complex management</i> , and fewer tools for genetic modifications.
Non-human Primates	Closest to human reproductive biology	High cost of maintenance and ethical considerations; fewer animals available for studies.
Guinea pigs	Similar cervical changes to humans	Longer gestation periods than rodents; different reproductive physiology
Rabbits	Accessible and cost-effective	Differences in cervical structure and remodelling

TABLE 1: Advantages and limitations of animal models in cervical remodelling and preterm birth research

ECM changes of the cervix can manifest through various processes, depending on the triggers that lead to ripening. For instance, RU486 (mifepristone) models show accelerated cervical ripening, with pronounced matrix disorganization and partially activating pro-inflammatory and repair processes akin to postpartum changes through mechanisms distinct from term ripening [21,94,95]. In contrast, lipopolysaccharide (LPS) treatment induces cervical changes and labour within hours of exposure with architectural alterations of the collagen matrix that resemble those seen in term ripening [21,95]. Additionally, the expression of matrix synthases differs under these conditions, with HAS two genes increasing near term, while Has1 expression rises in preterm scenarios [17,32,95].

Prostaglandin expression plays a critical role in cervical remodelling, as they reduce cervical resistance by reorganizing collagen fibrils [96]. In models of premature parturition, the expression of prostaglandin-cyclooxygenase-endoperoxide synthase (Ptxs) is upregulated, with Ptxs1 levels heightened in RU486-induced ripening and Ptxs2 activated in LPS models [95]. Non-steroidal anti-inflammatory drugs, which inhibit prostaglandin production, have been shown to effectively reduce both term and preterm labour in animal studies. Research indicates that cyclooxygenase (COX) 1 activity is essential for timely term labour in mice, whereas COX 2 expression is induced during inflammation-mediated preterm labour [97,98].

Cellular responses vary significantly depending on the trigger that induces labour. In the context of term cervical ripening, neutrophil depletion does not affect the timing or success of parturition, and general inflammatory responses typically begin only after labour has commenced [29,50]. In a transgenic mouse model, cervical ripening did not occur despite the infiltration of neutrophils and macrophages, suggesting that neutrophil activation is not essential for term cervical ripening and that merely having inflammatory cells may not be sufficient to initiate this process [99]. In contrast, RU486-induced premature cervical ripening is associated with a marked increase in tissue monocytes and eosinophils compared to term conditions, while LPS-induced ripening is characterized by elevated neutrophil levels without corresponding changes in monocyte or eosinophil counts [95]. The precise role of eosinophils remains unclear; however, in rodents, an increase in eotaxin-1 transcripts is followed by a rise in eosinophils on the day of birth, indicating that eosinophils may facilitate the differentiation of monocytes into alternatively activated macrophages during postpartum repair [21,42].

Cytokine expression also varies between term and preterm births. In terms of labour, cytokines tend to increase postpartum rather than during labour itself [58]. Conversely, in preterm models, there is a significant rise in the gene expression of neutrophil chemoattractant Cxcl2 and other pro-inflammatory cytokines, with LPS-induced inflammation showing a greater increase than that induced by RU486 [95,100].

Complement activation also seems to play a significant role in labour. Two mouse models of PTB have highlighted the importance of complement activation in CR and the onset of PTB. Findings from these models demonstrated increased deposition of cervical complement component (C) 3 and elevated levels of C5a in PTB cases compared to gestational age-matched controls. Furthermore, C5a receptor (C5aR)-deficient mice failed to exhibit cervical remodelling or experience PTB in response to treatments with LPS or mifepristone. This strongly suggests that C5aR is essential for the cervical remodelling process that precedes preterm birth [44,101].

Hormonal influence plays a pivotal role in the processes associated with labour and cervical remodelling. In

rodent models, the onset of labour is characterized by a significant decline in maternal progesterone levels, indicating its crucial role in parturition [102-104]. Conversely, multiple animals and in vitro studies have underscored the potential of oestrogen in facilitating cervical ripening, labour, and delivery [105,106]. Increased expression levels of oestrogen receptor α in the cervix have been associated with spontaneous preterm labour and delivery, highlighting oestrogen influence in this context [105-109].

Epithelial cell lesions and disruptions in barrier protection are also associated with premature cervical remodelling. A study utilizing a mouse model demonstrated that the combination of vaginal bacteria and mild cervical injury from a spermicide, which causes cellular damage, led to higher rates of preterm birth compared to exposure to bacteria alone. In contrast, the administration of vaginal progesterone modified the junctional proteins of the cervical epithelium and significantly reduced LPS-induced preterm birth [110-112]. This highlights the critical role of the cervical epithelium in maintaining barrier function against infections and underscores its importance in preventing preterm birth.

Neuromodulation and sensory nerve activity significantly influence reproductive tissue dynamics and cervical remodelling. When stimulated by microorganisms or toxins, sensory nerves in rats release neurotransmitters, initiating local inflammatory responses known as neurogenic inflammation [113,114]. Moreover, oestrogen can stimulate the production of neuropeptides from cervical sensory neurones, which are increased in the cervix during pregnancy, highlighting the intricate interaction between the endocrine and nervous systems [115].

Mechanical forces have recently emerged as a crucial factor in the intricate process of cervical remodelling. Research highlights a strong correlation between mechanical stimuli and biochemical signals, which can lead to significant changes in cellular behaviour and the extracellular matrix. Utilizing proteome-wide technology, studies have investigated the mechano-related signalling molecules present in the mouse cervix, providing valuable insights into how physical forces influence cervical modifications during pregnancy. These investigations have identified an increase in various cytoskeletal components and signalling molecules that correlate with the escalating gravitational forces exerted by the growing foetus on the cervix. This finding underscores the notion that foetal size and weight are not merely passive characteristics; rather, they may play a pivotal role in cervical remodelling through mechano-transduction processes [31].

Molecular mechanism linked to premature cervical remodelling: what we know?

The process of labour is inherently complex and multifactorial, involving a myriad of physiological and biochemical interactions. Given this complexity, it is not surprising that multiple molecular processes may lead to premature CR and subsequently contribute to the risk of PTB. Understanding the molecular mechanisms behind premature CR is crucial for addressing this critical risk factor. Disruptions at both cellular and molecular levels can compromise cervical integrity and trigger preterm labour, influenced by factors such as cellular dysfunction, inflammation, environmental influences, and genetic predispositions.

Cervical epithelial dysfunction compromises the essential barrier that prevents microorganisms from ascending from the vaginal canal to the uterine cavity. Maintaining this barrier is vital during CR, as any compromise can significantly elevate the risk of PTB. Various factors, including cervical surgeries, infections, inflammation, genetic predispositions, and chemical exposure, disrupt this protective barrier, ultimately leading to PTB [16,66,81,110,111].

Reversible EMT, along with its counterpart, MET, orchestrates cellular remodelling in both the amnion and cervix, contributing to the dynamic changes observed during gestation [62,116]. This cyclic remodelling process is intricately regulated by steroid hormones: oestrogen promotes EMT and cervical inflammation, while progesterone supports tissue repair and mitigates adverse effects [59-62]. Disruption of this cycle, triggered by infections or other stressors, can lead to a non-reversible state of EMT, resulting in chronic inflammation and degradation of the cervical matrix. This, in turn, compromises cervical integrity and accelerates premature remodelling [16,29,31,45,47,62-64,116]. These findings underscore the importance of EMT and epithelial functions in maintaining barrier properties and immune surveillance [42,81,116].

Inflammation at the maternal-foetal interface is a critical and common factor associated with PTB. Prolonged cervical inflammation can lead to significant tissue damage, resulting in cervical insufficiency and shortening, which frequently culminates in PTB [110,117-119]. Women diagnosed with cervical insufficiency often exhibit elevated levels of interleukin (IL)-6 in the amniotic fluid, suggesting a strong inflammatory component in these cases [120]. Inflammation can be triggered by both infectious and non-infectious stimuli. Approximately 40% of PTBs are linked to intrauterine infections, which typically originate from ascending bacteria; however, this review does not explore the specifics of these infections in detail [91,110,111,121]. On the other hand, non-infectious stimuli, such as toxic exposure, can also induce a sterile inflammatory response that leads to cervical extracellular matrix remodelling [110,117,122]. Inflammatory processes triggered by infections or non-infectious factors play a crucial role in prematurely remodelling cervical tissues.

Numerous endogenous and exogenous factors contribute to the complex phenomenon of premature CR, yet our understanding of these processes remains incomplete. One significant factor is oxidative stress, which can arise from various sources, including environmental pollutants such as cigarette smoke. A delicate balance between reactive oxygen species (ROS) and antioxidants is essential for maintaining tissue integrity and ensuring optimal maternal-foetal health. When this balance is disrupted, it can lead to premature cellular senescence, chronic inflammation, and an elevated risk of PTB. The specific role of oxidative stress in conditions such as cervical incompetence and short cervix remains somewhat inconsistent, warranting further investigation [117,122]. Cervical trauma and anatomical malformations have also been implicated in the risk of preterm birth [85,123–126]. Endocrine factors, particularly the balance between progesterone and oestrogen, also affect the cervix function [21,31,62]. Furthermore, mechanical factors, including foetal weight and the presence of mechano-sensitive molecules, contribute to the processes of premature cervical remodelling [31]. Understanding how these diverse factors interact and the respective pathways leading to premature cervical remodelling is crucial for developing effective prevention strategies against preterm birth.

Genetic predisposition is another critical element in cervical remodelling. While cervical trauma and inflammation contribute to CI, genetic predispositions explain why only some women with these exposures develop CI and subsequent PTB [123]. Specific polymorphisms in genes involved in connective tissue metabolism and inflammatory responses, such as collagen 1A1, transforming growth factor beta, and the interleukin-10 gene, have been associated with an increased risk of PTB due to their role in disrupting connective tissue regulation [21,78,123,127]. Furthermore, genetic anomalies that affect collagen and elastin synthesis, as observed in conditions like Ehlers-Danlos syndrome and Marfan syndrome, further elevate susceptibility to CI [21,128].

Biomarkers reflect physiological changes and offer valuable insights into the pathways leading to preterm birth [19]. Extensive research has been conducted on various biological fluids to identify effective indicators. Extracellular matrix degradation-related biomarkers, such as fFN, have shown particular promise. The presence of fFN in cervicovaginal fluid after the 24th week of pregnancy indicates matrix disruption and an increased risk of spontaneous preterm birth (sPTB). In clinical practice, fFN is recognized for its high negative predictive value (NPV), making it a valuable tool in predicting preterm labour [129,130]. Inflammatory markers also play a significant role in cervical changes and can serve as potential screening tools for PTB. For instance, IL-6 is associated with cervical remodelling, while other markers like IL-8 and C-reactive protein (CRP) have demonstrated promise in predicting PTB [19,131,132]. Despite the various biomarkers studied, their relevance to early cervical changes necessitates further investigation.

The interplay between endogenous and exogenous factors such as infections, chemical exposures, mechanical damage, oxidative stress, and endocrine disruptions intersects genetic predispositions to influence premature cervical remodeling. This multifactorial nature of PTB emphasizes that CR involves various pathways influenced by different triggers. Notably, the molecular mechanisms governing CR vary significantly between preterm and term births, as well as among different causes of PTB (Table 2). Understanding both normal and abnormal CR processes is crucial for identifying individuals at risk and developing targeted interventions to mitigate preterm birth linked to cervical insufficiency.

Molecular mechanism	Impact on premature cervical remodelling
Cervical epithelial dysfunction	Compromises the cervix's barrier function, increasing susceptibility to infections and inflammation.
Inflammation	Involves immune response activation and cytokine release. Chronic inflammation accelerates CR, causing cervical shortening and insufficient cervix.
Oxidative stress	The imbalance between reactive oxygen species and antioxidants contributes to premature CR by inducing cellular senescence and sterile inflammation.
Hormonal disruption	Hormonal imbalances can precipitate the disruption of normal softening and ripening processes. Oestrogen enhances EMT, cellular migration, and cervical inflammation.
Genetic predisposition	Genetic variations in genes related to connective tissue metabolism (e.g., TGF- β , IL-10) increase sensibility to environmental factors.
Matrix metalloproteinases	Altered MMP activity can lead to abnormal matrix remodelling and premature CR.
Glycosaminoglycan	Changes in GAG composition and metabolism affect matrix hydration and collagen organization and CR.
Epithelial-mesenchymal transition	Persistent EMT can disrupt cervical barrier function and stimulate inflammations, promoting early remodelling
Complement activation	Increased complement activation enhances inflammatory responses and matrix remodelling and can accelerate CR.
Prostaglandins (PGs)	Upregulation of prostaglandins can enhance cervical matrix remodelling and inflammation, leading to premature CR.
Mechanical forces	Mechanical stress can induce CR by activating mechano-sensing pathways in cervical tissues.

TABLE 2: Molecular mechanisms linked to premature cervical remodelling.

CR: cervical remodelling; EMT: epithelial-mesenchymal transition; MMP: matrix metalloproteinases; GAG: glycosaminoglycan.

Clinical implications and future directions

Recent animal studies have highlighted key differences in cervical remodelling between term and preterm births. Term remodelling is primarily characterized by collagen disorganization, while preterm remodelling involves an influx of inflammatory cells, such as neutrophils and mononuclear cells. This distinction is critical, as it suggests that different molecular and cellular pathways may be at play depending on the timing and triggers of labour [29,32,95]. However, significant gaps remain in our understanding of the biological mechanisms of labour, particularly due to the scarcity of human cervical tissue evaluations in affected women, with much of the available data based on animal models [22,32]. Additionally, many studies rely on cervical biopsies obtained postpartum, focusing more on the tissue repair phase than the active remodelling phase. Furthermore, these studies often lack adequately matched control groups in terms of gestational age or use samples from non-pregnant women, limiting the applicability of their findings.

Identifying markers for premature cervical remodelling is crucial for developing effective screening methods. Future research should focus on elucidating the molecular and cellular mechanisms underlying cervical changes, including EMT, inflammation triggers beyond infection, the effects of endocrine imbalance, the influence of toxic and exogenous factors, and the role of genetic polymorphisms. Integrating clinical and molecular insights will significantly enhance our understanding of cervical remodelling, ultimately leading to more targeted and personalized interventions for women at risk of preterm birth.

Advancements in non-invasive imaging technologies and biomarker identification present promising avenues for improving screening and monitoring capabilities. However, there is a pressing need for increased research utilizing human cervical tissue to gain deeper insights into the mechanisms of cervical remodelling and develop targeted therapies. For example, molecular studies on the behaviour of inflammatory cells, such as neutrophils and mononuclear cells, in triggering labour could help clarify how inflammation leads to preterm birth and inform the development of targeted interventions. Moreover, tracking cervical changes in high-risk populations throughout pregnancy will help identify early biomarkers that can predict premature cervical remodelling and preterm birth. It is also vital to investigate the impact

of environmental exposures, lifestyle factors, and maternal health on cervical remodelling.

The main methodological limitation of this study is the use of a single research database. Additionally, the reliance on animal models and the scarcity of robust human data may limit the generalizability of the findings to human populations.

Finally, integrating the fields of obstetrics, molecular biology, genetics, and bioengineering will facilitate innovative research and clinical applications, making this research fundamental for advancing our knowledge and improving outcomes for at-risk pregnancies.

Conclusions

The cervix plays a critical role in maintaining a healthy pregnancy, undergoing intricate remodelling processes that are essential for successful delivery. Throughout pregnancy, the cervix adapts structurally and functionally, with distinct differences observed between term and preterm birth scenarios. This remodelling is influenced by a multitude of factors, including hormonal changes, mechanical stress, and cellular interactions, all of which contribute to the cervix's ability to function effectively during labour.

Despite the progress made in understanding cervical remodelling, significant gaps persist in our knowledge, particularly regarding the specific mechanisms that govern these processes and their direct implications for preterm birth. Addressing these gaps is crucial for developing targeted interventions to prevent preterm labour. By deepening our understanding of cervical dynamics, we can enhance screening and treatment options, ultimately improving outcomes for women at risk of preterm birth.

Appendices

Search strategy

Objective

To explore cervical function in both normal and premature labour and its role in triggering PTB

Databases Searched

PubMed

Search Terms:

The following keywords and phrases were used to conduct the literature search:

"role of cervix in spontaneous preterm birth"

"cervix in labour"

"cervical biopsies in labour"

"cervical biopsies in preterm labour"

Inclusion Criteria

Study Types

Peer-reviewed original research articles

Review articles

Clinical trials

Meta-analyses

Animal studies

Population

Pregnant women at risk of preterm birth

Animal models relevant to cervical remodelling and preterm birth

Language

English language publications

Publication Date

Studies published in the last 50 years to ensure current relevance, with particular emphasis on recent findings.

Exclusion Criteria

Multiple pregnancies

Studies focusing on non-pregnant populations or studies primarily aimed at detecting cervical lesions.

Studies investigating preterm birth with known causes (e.g., infection, hydramnios, foetal malformation

Research lacking a focus on cervical remodelling or preterm birth mechanisms.

Data Extraction

Abstracts and full-text articles were reviewed to extract relevant data concerning the molecular mechanisms, hormonal influences, inflammatory processes, and genetic predispositions linked to premature cervical remodelling.

Key findings related to biomarkers and potential intervention strategies were also noted.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

Concept and design: Joana Félix, Alexandra Matias, Carla Bartosch

Acquisition, analysis, or interpretation of data: Joana Félix, Alexandra Matias, Carla Bartosch

Drafting of the manuscript: Joana Félix

Critical review of the manuscript for important intellectual content: Joana Félix, Alexandra Matias, Carla Bartosch

Disclosures

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III. ARTICLE

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Cellular dynamics of cervical remodelling: insights from preterm and term labour

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Abstract

Introduction Preterm birth remains a global health challenge with significant perinatal morbidity and mortality rates. Despite extensive research, the underlying mechanisms triggering preterm birth remain elusive, needing a deeper understanding of cervical cellular remodelling processes.

Purpose This study aims to elucidate the cellular mechanisms underlying cervical remodelling in spontaneous preterm labour (PTL) compared to term labour (TL), focusing on the roles of inflammatory cells and fibroblasts.

Methods We conducted a prospective observational study from October 2018 to March 2021, at two hospitals. Participants were categorized into two groups: PTL ($n = 14$), TL ($n = 14$). A small set ($n = 6$) of preterm not in labour (PTnotL) was also included. Cervical biopsies were obtained and analysed for the density of fibroblasts and inflammatory cells (neutrophils and mononuclear cells) using digital image analysis. Statistical analysis was performed employing Mann–Whitney and Kruskal–Wallis tests.

Results There was no significant overall difference in global inflammatory cell density between PTL and TL ($p = 0.154$). However, a detailed analysis revealed significantly higher inflammation in the exocervix of PTL compared to TL, particularly involving neutrophils ($p = 0.021$) and mononuclear cells ($p = 0.028$). Neutrophils ($p = 0.035$), but not mononuclear cells ($p = 0.111$), were significantly decreased in PTnotL exocervix compared to PTL. No differences were found in inflammatory cell density in the endocervix. Fibroblast densities were similar across groups.

Conclusions This study highlights the distinct cellular profiles in the cervix during preterm and term labour, with neutrophils playing a pivotal role in preterm labour. These findings may guide the development of targeted interventions to prevent preterm birth.

Keywords Preterm labour · Term labour · Labour physiology · Cervical remodelling · Inflammatory cells · Fibroblast

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What does this study add to the clinical work

This study identifies distinct inflammatory patterns in the cervix during spontaneous preterm and term labour, highlighting higher neutrophils numbers in preterm cases, particularly in the exocervix. Neutrophils may play a significant role in premature cervical remodelling, suggesting potential targets for interventions aimed at preventing preterm birth.

Introduction

Preterm birth (PTB), as defined by the World Health Organization (WHO), refers to birth before completing 37 weeks of gestation or fewer than 259 days from the first day of a woman's last menstrual period [1].

Determining the precise prevalence of preterm birth poses a challenge; however, recent data from 2020 suggest a global prevalence of 9.9%, translating in approximately 13.4 million preterm live births globally [2]. Preterm birth stands as a substantial contributor to the Global Burden of Disease, emerging as the primary cause of neonatal morbidity/mortality and carrying extensive implications for health, economics, and society at large [3]. Its significance is further highlighted by its incorporation into the United Nations Sustainable Development Goals [4].

Clinically, prematurity can be classified into three distinct categories: spontaneous preterm birth (sPTB) accounting for 50%, preterm premature rupture of membranes (PPROM) representing 25%, and medically indicated preterm birth constituting the remaining 25% [2, 5].

Both term and preterm birth share a common pathway involving three essential elements: stimulation of myometrial contractility, cervical ripening, and activation of the decidua/membrane interface, although their activation occurs through distinct processes [6]. For decades, research has predominantly focused on myometrial activities and potential contraction inhibitors, influenced by the Danforth paradigm, which portrayed the human cervix as a passive structure responsive to uterine contractions [7]. However, evolving insights have significantly reshaped our understanding of PTB, revealing an overestimation of uterine activity's role in prematurity and an underestimation of the cervix's behaviour [8–10].

The cervix plays a pivotal role throughout pregnancy and childbirth, with any conditions compromising its integrity, such as cervical trauma, malformations or surgical interventions, significantly increasing the risk of premature birth [11–13].

In the 1990s, premature cervical remodelling emerged as a critical precursor state of PTB, with research revealing a significant correlation between cervical length and the risk of PTB [8, 14–16]. Romero et al. characterized cervical remodelling as a defining feature of the "pre-mature parturition syndrome," underscoring the importance of cervical changes leading up to preterm birth [9]. Furthermore, early signs of cervical remodelling can be detected several weeks prior to the onset of sPTB [15, 17, 18], emphasizing its pivotal role as the primary molecular event in preterm birth and suggesting its potential as a target for both screening and intervention [17].

Recent animal studies indicate differences in the process of cervical remodelling between term and preterm birth. Term remodelling is characterized by collagen disorganization, while premature remodelling involves inflammatory cells, like neutrophils and mononuclear cells [11, 19, 20]. While neutrophils are essential for initiating the inflammatory response and managing infection, mononuclear cells, such as macrophages, are crucial for sustaining the inflammatory response and promoting tissue repair [11, 21].

Significant gaps remain in our understanding of the underlying biological mechanisms of labour, primarily due to the scarcity of human cervical tissue evaluations in affected women, with most information relying on animal models [19, 22]. Furthermore, the majority of studies obtain cervical biopsies postpartum, typically focusing on the repair stage rather than the remodelling stage. These studies often lack adequately matched control groups based on gestational age or may include non-pregnant women, which can limit the accuracy and applicability of their findings.

The objectives of this study are to comprehensively investigate the cellular mechanisms underlying cervical remodelling during spontaneous preterm and term labour. Specifically, the study aims to assess and compare the density of inflammatory cells, including neutrophils and mononuclear cells, as well as fibroblasts, in cervical tissue samples obtained from pregnant women in preterm and term labour. By examining these cellular profiles, the study aims to clarify whether distinct patterns of inflammation and fibroblasts correlate with the timing and nature of labour onset, providing insights into the pathophysiology of preterm birth.

Materials and methods**Study design**

We conducted a prospective observational study spanning from October 2018 to March 2021, with a 12-month hiatus due to the COVID-19 pandemic, at two Obstetric and

Gynaecology wards in Porto: Pedro Hispano Hospital and University Hospital Center of São João.

Participants

Pregnant women were recruited and categorized into two groups: preterm spontaneous labour (PTL), term spontaneous labour (TL). A small set of preterm not in labour (PTnotL) was also included. Gestational age was determined by crown–rump length measurement in the first trimester. Preterm birth (PTB) was defined as delivery occurring between ≥ 24 weeks and < 37 weeks of gestation. The term group comprised pregnant women with a gestational age of > 39 weeks to exclude early term deliveries that might share similar triggers with late preterm labours. For the labour groups, we included pregnant women in the early stage of labour, defined as cervical ripening < 4 cm, as evidence suggests that the labour process is comparable in term and preterm deliveries, though the initial trigger may differ. The PTnotL group encompassed women with medically indicated prematurity and unripe cervixes (defined as Bishop Score < 5), with cervical biopsy performed prior to caesarean section or labour induction. Exclusion criteria included multiple pregnancies, signs of infection, or cervical insufficiency and presence of cervical dysplasia in the histologic examination.

Procedures

Eligible participants underwent a random transvaginal cervical biopsy using appropriate forceps (approximately $5 \times 5 \times 5$ mm). No complications arose from the procedures.

Biopsy specimens were fixed in 4% formaldehyde for up to 24 h before embedding in paraffin, sectioned, and stained with haematoxylin and eosin for histopathological analysis. The histopathological examination focused on evaluating the density of inflammatory and fibroblast lineage cells.

Cellular analysis was performed using QuPath[®], a digital image analysis software. For each sample, two regions of interest (ROIs) measuring $750 \times 1500 \mu\text{m}$ (1.125 mm^2) were selected to encompass representative areas of the endocervix and/or exocervix, including both epithelial and connective tissue components. Within these areas, the cell detection function was applied to identify all cells. The 'Train Object Classifier' feature was then used to classify cells as neutrophils, lymphocytes, plasma cells, and fibroblasts. Other cells, including vascular cells and those that cannot be classified based on morphology, were grouped under the category 'other'. For the purposes of analysis lymphocytes and plasma cells were considered together as mononuclear cells. Representative cells were manually identified by the authors to set the classifier that enabled the program to automatically recognize each type of cell in every ROI.

To ensure objectivity, the authors were blinded to sample identities during the review and refinement of the software's output.

Subsequently, the classifications made by the software were reviewed by the authors, who identified and corrected any misclassifications through additional cell identification, thus improving the classifier. This iterative process was repeated for ROIs in all samples to ensure accurate and consistent cellular identification across the data set (Fig. 1).

Statistical analysis

Continuous variables were described as median and range (minimum, maximum) given the small number of samples and the asymmetric distribution of the inflammatory infiltrate.

Statistical analysis was performed using SPSS[®] software. Kruskal–Wallis test was employed to determine statistical significance between women groups for different cell distribution patterns. It was also employed to evaluate differences in demographic and clinical variables between the groups. Mann–Whitney test and Wilcoxon signed-rank test were used to evaluate differences in median inflammatory cell counts between endocervix and exocervix, using independent and paired analysis, respectively. A *p* value of < 0.05 was considered statistically significant.

Ethical considerations

This study was conducted after obtaining approval from the local Ethics Committee (Ref. No. 81-17), and each participant provided informed consent. All procedures were conducted in accordance with ethical standards and guidelines.

Results

A total of 34 singleton pregnant women participated in the study, distributed into three groups: 14 in PTL, 14 in TL, and 6 in PTnotL. Reasons for early termination in the PTnotL group included severe cardiac disease, uterine dehiscence, and severe growth restriction.

Demographic and clinical characteristics of the study groups are summarized in Table 1. No differences were noted between groups regarding mother's age, parity and body mass index. None had a prior history of preterm birth. Throughout both the parturition and postpartum periods, no signs of infection were observed in any of the participants.

The cervical samples were collected during a phase of cervical effacement and dilation, which made it difficult to visually identify the transformation zone. As a result, there was variability in whether the biopsy included endocervical

or exocervical tissue, depending on the sample. Out of the 34 samples analysed, 26 contained endocervix, 30 had exocervix, and 23 included both endocervix and exocervix. In general, all samples had some degree of inflammatory infiltrate, with intensity ranging from mild to moderate. Quantitatively, there was a median of 380 inflammatory cells/mm² per sample, ranging from a minimum count of 33 inflammatory cells/mm² to a maximum of 1226 inflammatory cells/mm². It predominantly consisted in chronic inflammation (median mononuclear cells/mm² = 295 (33, 793) vs. median neutrophils/mm² = 80 (0, 997)).

Inflammation was more pronounced in the endocervix, with a median of 813 (159, 1898) inflammatory cells/mm² per sample, compared to the exocervix, which had a median of 175 (32, 1115) cells/mm² ($p < 0.001$). This observation persists when considering only the 12 samples with both endocervix and exocervix using a paired analysis ($p < 0.001$). These differences are also significant when analysing neutrophils and mononuclear inflammation separately. The fibroblast lineage had a median of 105 cells/mm² per sample and were significant more frequent in the exocervix ($p < 0.001$).

Next, we compared cells' density between PTL and TL. When considering inflammation in general including both mononuclear cells and neutrophils, irrespective of endocervix and exocervix, there was a tendency for a higher number of inflammatory cells/mm² in PTL (median = 454 cells/mm², range: 104, 1226) compared to TL (median = 326 cells/mm², range: 33, 879), but this was not significant ($p = 0.154$) (Fig. 2). A more detailed analysis focusing on the exocervix, highlighted that inflammation in this location was significantly higher in PTL compared to TL (PTL: median 283 cells/mm² vs. TL: 113 cells/mm², $p = 0.027$). This was true both for neutrophil ($p = 0.021$) and mononuclear cells ($p = 0.028$) (Figs. 3 and 4). In contrast, in the endocervix there was no significant differences in any kind of inflammation between PTL and TL.

To further support that preterm labour associated with inflammation in the exocervix, we analysed a group of preterm pregnancies not in labour. When comparing the

preterm groups, neutrophil inflammation was significantly decreased in the PTnotL exocervix (median 10 neutrophils/mm², range 3, 95) compared to PTL (median 66 neutrophils/mm², range: 26, 147) ($p = 0.035$). In contrast, there was no significant difference in mononuclear cell density (PTnotL: median 99 cells/mm², range 56, 160 vs. PTL: 185 cells/mm², range 20, 1041, $p = 0.111$) (Fig. 5).

Regarding fibroblasts, no significant difference was observed between PTnotL and PTL groups. Globally, PTnotL inflammatory cells density was similar to PT samples.

Discussion

Understanding the pathology of premature cervical remodelling requires an appreciation of the normal physiology of term remodelling. Liggins proposed in 1978 that cervical ripening resembles an inflammatory reaction, with immune cell migration into the cervix promoting collagenase and elastase activities, thereby mediating parturition's ripening process [23]. However, this classic inflammatory model of labour relied on postpartum samples, capturing alterations related to parturition rather than events preparing the cervix for labour onset [11, 24].

Recent evidence indicates that in term births, cervical remodelling is primarily driven by changes in collagen organization, like the reduction in cross-linked collagen and matricellular proteins, which increases tissue compliance while maintaining structural integrity [24–26]. Studies in animal models further suggest that inflammatory cells, particularly neutrophils, may not play a central role in initiating cervical ripening at term [21, 27, 28]; rather, these cells primarily contribute to active dilation and postpartum repair [11, 24, 27]. In contrast, preterm cervical remodelling involves significant participation of inflammatory cells and markers [29–38]. This evidence indicates distinct mechanisms underlying cervical remodelling in term vs. preterm births [20, 39].

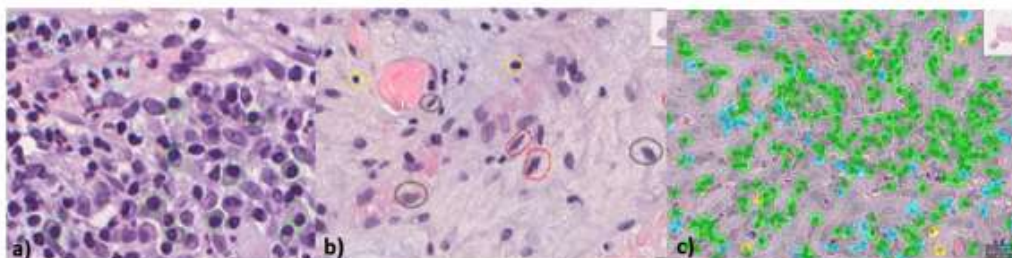


Fig. 1 QuPath® Cell Classification. **a, b** Manual identification of the cells; **c** automatic identification of the cells. Blue for neutrophils, Green for plasma cells, Yellow for lymphocytes, Red for fibroblast lineage, and Grey for other cells

Table 1 Demographic and clinical characteristics

Parameters	PTL (<i>n</i> = 14)	TL (<i>n</i> = 14)	PTnotL (<i>n</i> = 6)	<i>p</i> value
Age (years), median (range)	31 (19, 42)	33 (24, 44)	32 (21, 39)	<i>p</i> = 0.767
Body mass index, median (range)	21 (17, 38)	23 (17, 26)	26 (21, 35)	<i>p</i> = 0.146
Education, <i>n</i>	8	13	5	<i>p</i> = 0.167
< 9th grade	1	4	2	
10–12th grade	3	3	1	
≥ Graduation	4	6	2	
Parity, median (range)	0 (0, 1)	1 (0, 2)	1 (0, 3)	<i>p</i> = 0.271
Nullipara, <i>n</i>	8	6	3	
1, <i>n</i>	5	5	1	
2, <i>n</i>	0	3	1	
3, <i>n</i>	0	0	1	
Prior preterm birth, <i>n</i>	0	0	0	
Weeks of gestation, median (range)	33 (26, 36)	39 (39, 40)	35 (34, 36)	<i>p</i> < 0.001
New born weight, g, median	2540 (980–3340)	3420 (2990–3645)	2400 (1600–2650)	<i>p</i> < 0.001
Sampled mucosa				<i>p</i> = 0.934
Endocervix	2	2	0	
Exocervix	3	4	1	
Both	9	8	5	

PTL preterm spontaneous labour, TL term spontaneous labour, PTnotL preterm not in labour

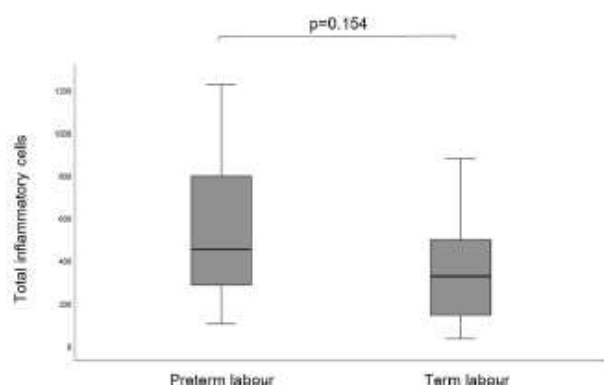


Fig. 2 Box plot depicting inflammatory cell counts in cervical mucosa from preterm labour (PTL) and term labour (TL) groups. The graph shows a slightly higher, but statistically insignificant, inflammatory cell density in the PTL group compared to the TL group, as assessed by the Mann–Whitney test. The analysis included a total of 28 biopsy samples, consisting of 14 samples from the PTL group and 14 samples from the TL group. Of these, 17 samples contained both endocervical and exocervical tissue

One of the objectives of this study was to assess the cellular density of inflammation in cervical tissue samples obtained from pregnant women in preterm and term labour to understand their role in normal and premature remodelling.

We found no significant difference in the inflammatory infiltrate between preterm birth (PTB) and term birth

(TL), but a detailed analysis of different areas of the cervix revealed that while the endocervix did not show significant differences, the exocervix had more pronounced inflammation in PTB. This could be due to the generally higher inflammation in the endocervix that is characteristic of this location, coupled with a reduced sample size, leading to insufficient statistical power to detect differences.

Evaluating the exocervix, neutrophils were increased in spontaneous PTB compared to TL, supporting the evidence that inflammation may have a role in prematurity, but not in term delivery. When comparing spontaneous PTL with PTnotL (medically induced), we aimed to discern whether the differences between PTL and TL were due to the trigger leading to premature labour or merely a consequence of prematurity. We observed that neutrophils were significantly higher in PTL compared to PTnotL. Even though we only had a small set of samples, this suggests that neutrophils are involved in the premature labour process itself and not only a consequence of prematurity. These findings reinforce the notion that neutrophils are essential to the cervical remodelling process leading to preterm labour, supported by studies linking inflammation—including sterile inflammation—to preterm birth [21, 38].

Mononuclear inflammation was more pronounced than neutrophil in all three groups. Their role as barrier and first line in defence may justify their abundance in the cervix. These cells were increased in PTL compared to TL. Conversely, there was no significant difference in mononuclear cells between the premature groups. The absence of

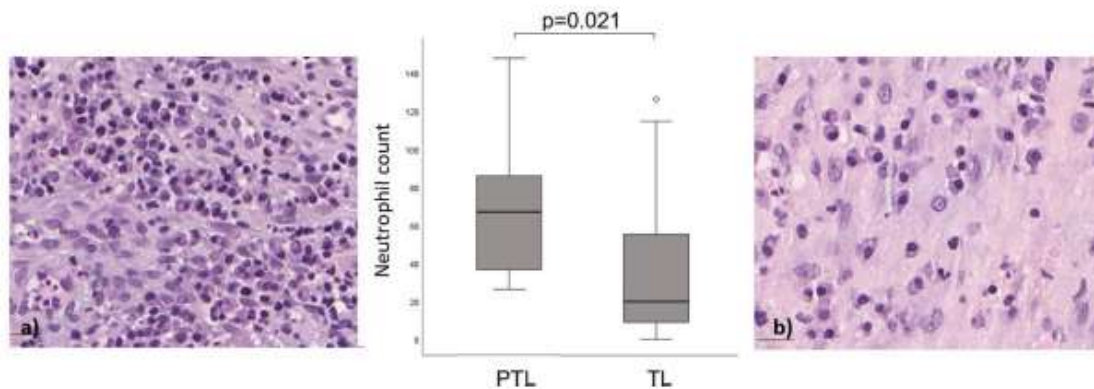


Fig. 3 Neutrophil count quantification in the exocervix. **a** Histological representative image of preterm sample with abundant inflammatory cells including frequent neutrophils; **b** histological representative image of term sample with lower numbers of inflammatory cells, including neutrophils. Box-plot graph comparing the quantitative lev-

els of neutrophil counts between preterm spontaneous labour (PTL) and term spontaneous labour (TL) (Mann–Whitney test). The analysis included a total of 24 biopsy samples, with 12 samples from the PTL group and 12 samples from the TL group

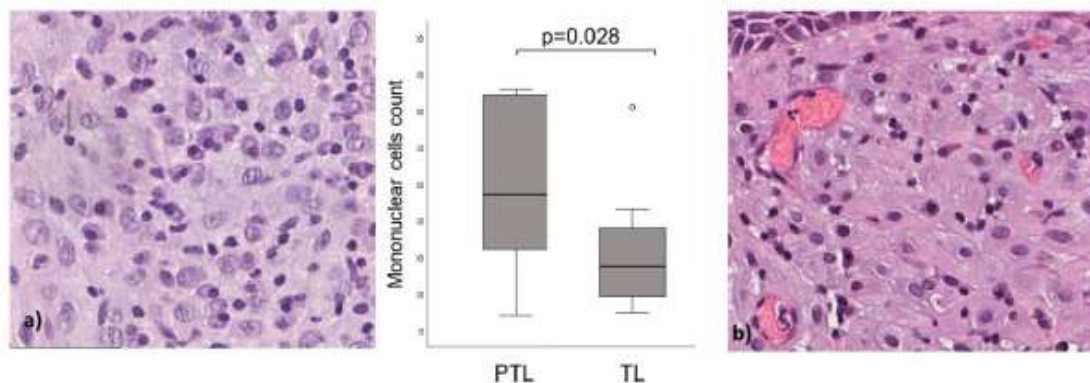


Fig. 4 Mononuclear cell count quantification in the exocervix. **a** Histological representative image of preterm sample with abundant mononuclear cells (lymphocytes and plasmacytes); **b** histological representative image of term sample showing lower numbers of mononuclear cells. Box-plot graph comparing the quantitative levels

of neutrophil counts between preterm spontaneous labour (PTL) and term labour (TL) (Mann–Whitney test). The analysis included a total of 24 biopsy samples, with 12 samples from the PTL group and 12 samples from the TL group

significant differences in mononuclear cell density between the premature groups suggests that while these cells contribute to cervical inflammation, they may not be as critical in triggering labour as neutrophils. The role of mononuclear cells appears to be more pronounced in postpartum repair than in the remodelling process leading to labour initiation. Their abundance in the cervix is consistent with literature indicating that while mononuclear cells play a significant role in the immune response and their presence alone may not be sufficient to initiate cervical remodelling or dilation [39].

As remodelling process is widely recognized as a complex interplay, it involves various cell types and not only inflammatory cells. Fibroblasts, given their pivotal role in

matrix production, are essential contributors to pathophysiological extracellular matrix remodelling [40, 41]. A study analysing postpartum samples concluded that fibroblasts are activated or recruited during the ripening process, playing a crucial role in the final cervical remodelling that occurs mere hours before birth. They are implicated in both tissue degradation/remodelling and the attraction of neutrophils [40]. Despite their significance, prior research has largely overlooked the changes fibroblasts undergo during labour. Our analysis indicated a more pronounced presence of fibroblasts in the exocervix, consistent with the higher abundance of stroma in this region. However, there were no significant differences between TL and PTL, implying that fibroblasts do not trigger labour. This observation aligns with evidence

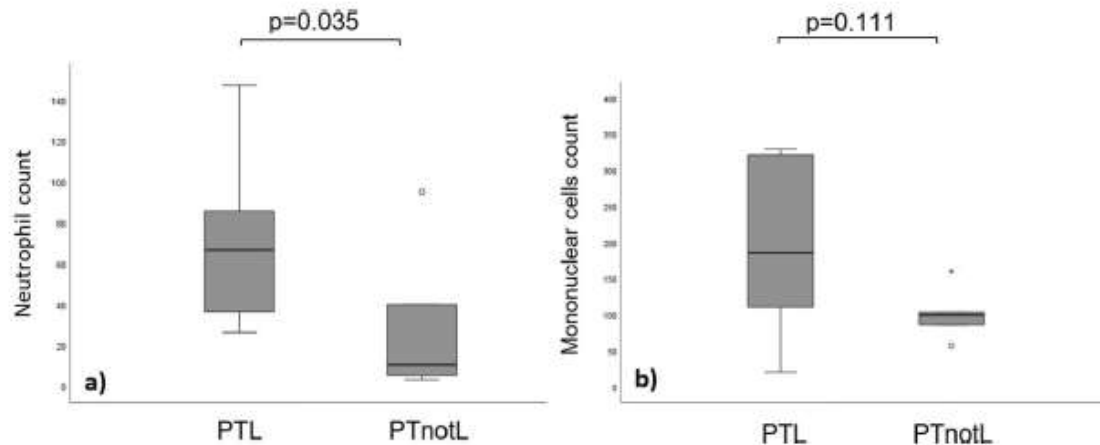


Fig. 5 Inflammatory cell Quantification in the exocervix, comparing preterm spontaneous labour (PTL) and preterm not in labour (PTnotL). **a** Neutrophil count and **b** mononuclear cell count (Mann–

Whitney test). The analysis included a total of 18 biopsy samples, with 12 samples from the PTL group and 6 samples from the PTnotL group

suggesting that remodelling at term is primarily due to changes in collagen crosslinking rather than alterations in matrix production [24, 25]. Further research is needed to fully understand the specific mechanisms governing fibroblast dynamics during remodelling [24, 25].

In summary, our findings reinforce the notion that the mechanisms of cervical remodelling differ fundamentally between term and preterm births, aligning with previous hypotheses. However, several limitations of this study should be noted. The small sample size, limits the statistical power and restricts our ability to perform more comprehensive comparisons across groups. In addition, our analysis was limited to morphological assessments and cell density measurements, which do not capture the functional states or activation levels of the observed cells. Future research is needed to explore the functional roles of inflammatory and fibroblast cells in cervical remodelling, potentially incorporating techniques like immunohistochemistry to assess cell activation and the underlying molecular pathways involved.

Conclusion

This study provides valuable insights into the cellular mechanisms underlying cervical remodelling in both spontaneous preterm and term labour. Our findings highlight distinct inflammatory profiles between preterm and term births, particularly in the exocervix, underscoring the pivotal role of neutrophils in premature cervical remodelling. Importantly, these observations suggest that inflammatory processes may play a critical role in initiating preterm labour, while fibroblasts appear to primarily contribute to matrix maintenance rather than labour initiation. Moving forward, further research is warranted to validate these

findings in larger cohorts and explore the feasibility of targeted interventions aimed at modulating cervical inflammation to improve maternal and neonatal outcomes.

Author contributions J. Félix: protocol/project development, data collection or management, data analysis, and manuscript writing/editing. A. Matias: Félix: protocol/project development and manuscript writing/editing. C. Bartosch: Félix: protocol/project development, data analysis, and manuscript writing/editing.

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Declarations

Conflict of interest The authors declare that they have no financial or personal relationships that could potentially influence or bias the content of this work.

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IV. POSTER

Félix J, Bartosch C, Matias A. Cellular dynamics of cervical remodelling: insights from preterm and term labour. Poster presented at: 21st World Congress in Fetal Medicine; 2024 Jun 23-27; Lisbon, Portugal.

Cellular dynamics of cervical remodelling: insights from preterm and term labour

Joana Félix, Carla Bartosch and Alexandra Matias

Preterm birth (PTB) remains a global health challenge with significant neonatal morbidity and mortality rates. Despite extensive research, the underlying mechanisms triggering labour remain elusive.

Objective: The aim of this study was to investigate the role of inflammatory cells in cervical remodelling during preterm and term labour.

Material and methods: The study employed a prospective observational design conducted from October 2018 to March 2021 at two Obstetrics and Gynaecology wards in Oporto. Participants were categorized into three groups: preterm labour (PTL), term labour (TL), and preterm not in labour (PTnotL). Cervical tissue samples were obtained from eligible participants through random transvaginal biopsies and subjected to histopathological analysis, using Qupath®, to evaluate the density of inflammatory cells.

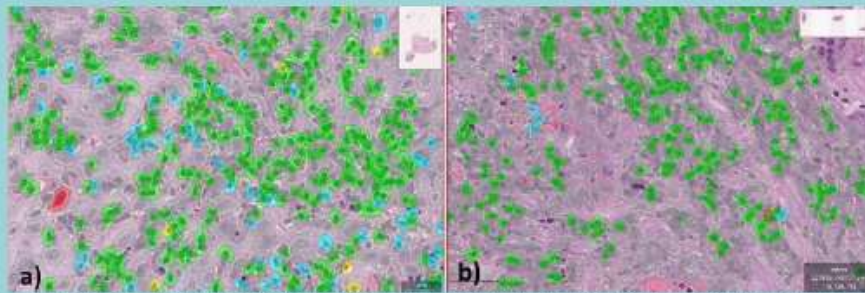


Figure 1: Qupath® analysis of an endocervical sample: a) from preterm labour and b) from term labour. Cell Classification Key – Blue: neutrophils; Green: plasma cells; Yellow: lymphocytes; Red: fibroblast lineage; Grey: other cells.

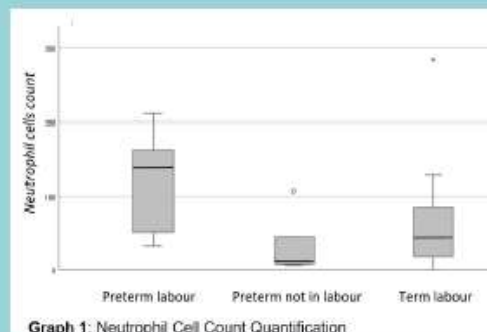
Results: A total of 34 singleton pregnant women participated in the study, with 14 in the PTL group, 14 in the TL group, and 6 in the PTnotL group.

Reasons for early termination in the PTnotL group included severe cardiac disease, uterine dehiscence, and severe growth restriction.

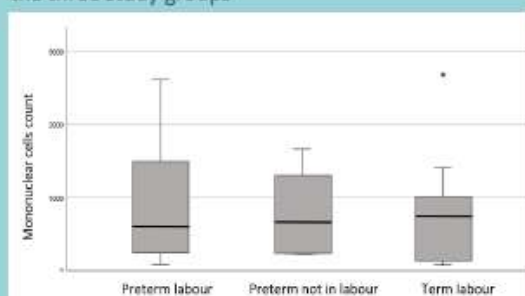
The histopathological examination present with a moderated inflammatory infiltrate, consisting in neutrophils and mononuclear cells (plasma cells plus lymphocytes).

Neutrophil count was found to be elevated in the labour groups compared to PTnotL. But, the PTL group exhibited a higher neutrophil count compared to the TL group.

In contrast, mononuclear inflammation did not display any significant differences in cell count among the three study groups



Graph 1: Neutrophil Cell Count Quantification



Graph 2: Mononuclear cell count quantification

Clinical implications: Understanding the mechanisms triggering premature cervical remodelling can aid in identifying targets for prevention, particularly as universal screening for short cervix gains momentum

Conclusions: The significant elevation in neutrophil count observed in spontaneous preterm birth underscores their potential as key mediators in the pathophysiology of preterm birth.

The evidence suggests that cervical remodelling differs between term and preterm birth. In term births, remodelling is primarily attributed to changes in collagen organization, with inflammatory cells playing a crucial role in dilation and postpartum repair. In contrast, inflammatory cells are significantly involved in the initial stages of remodelling in preterm births.

We observed an increase in neutrophil count in the labour groups (PTL and TL) compared to the PTnotL group. This suggests that the onset of labour is linked to neutrophil chemotaxis, emphasizing their role in the labour process. Notably, there was a pronounced elevation in neutrophil inflammation in the sPTB group compared to the TL group ($p < 0.05$). This underscores that neutrophils may play a role in premature cervical remodelling, whereas in term births, their presence may not be essential to initiating remodelling.

Mononuclear cells shape the immune response in the cervix but they do not actively participate in initial cervical remodelling as they do not differ in the different groups.

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This is to certify that the abstract

***Cellular dynamics of cervical remodelling: insights
from preterm and term labour***

by Félix J, Bartosch C, Matias A

*was accepted and delivered as a poster
presentation during the*

21st World Congress in Fetal Medicine

23-27 June 2024

Lisbon, Portugal



Director: Kypros Nicolaides

V. POSTER AND ABSTRACT PUBLICATION

Félix J, Dória M, Varejão A, Bartosch C, Matias A. Preterm birth screening using routine transvaginal ultrasound: a retrospective study. Poster presented at: ISUOG 30th Virtual World Congress; 2020 Oct 16-18;

Félix J, Dória M, Varejão A, Bartosch C, Matias A. Preterm birth screening using routine transvaginal ultrasound: a retrospective study. *Ultrasound Obstet Gynecol.* 2020 Oct;56(S1):303. doi: 10.1002/uog.23247. Abstract.

Preterm birth screening using routine transvaginal ultrasound: a retrospective study

Joana Félix, Mariana Dória, Ana Varejão, Carla Bartosch, Alexandra Matias

Introduction

Preterm birth (PTB), delivery before 37 weeks of gestation, is the single most important clinical challenge to both obstetricians and pediatricians. Approximately 11% of infants worldwide are born too soon and despite all investigation, its prevalence has not decreased.

In the last decades, many risk scores have been proposed to predict PTB, but 2nd Trimester (2ndT) transvaginal ultrasound cervical length (TUCL) is the most powerful screening tool.

Objective

Determination of 2nd trimester TUCL usefulness and application for the prediction of PTB.

Methods

Retrospective cross-sectional cohort study including all singleton pregnant women who performed 2nd T ultrasound in Hospital São João from January 2013 to October 2017.

Inclusion criteria: TUCL according Fetal Medicine Foundations rules.

Exclusion criteria: delivery before 24th weeks of gestation, medical induced PTB and cervical cerclage prior to ultrasound.



Results/Discussion

Our cohort included 4481 women, with 4% prevalence of spontaneous PTB.

TUCL mean was 33.8mm and range from 3.0 to 53.0mm.

Centiles 3, 5 and 10 were 25.0, 27.0 and 29.0mm.

CL measurement present a high specificity, but very low sensitivity to predict PTB, with an AUC close to 0.5. (table1)

Multivariable logistic regression analysis to evaluate the impact of TUCL in PTB prediction showed a significant negative association, as expected (table 2). Multivariable model, including maternal age > 40 years, history of PTB and cervical surgery, showed an AUC of 0.65, allowing us to concluded that this type of model presents a better screening ability, when compared to TUCL alone.

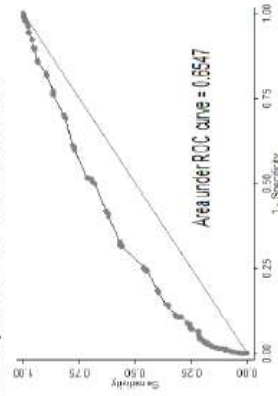


Fig. 2 Graphic representations of sensitivity, specificity and AUC of the multivariable model

Cut-off	Sensitivity	Specificity	Area Under Curve
15mm	2.8	99.7	0.51
20mm	6.2	99.5	0.53
25mm	10.6	97.3	0.54
30mm	30.7	80.1	0.55

Table 1: Cervical length measurement sensitivity and specificity to predict preterm birth.

Outcome	Odds Ratio	95% confidence Interval	P Value
Cervical length	0.925	0.90 0.95	0.000
Maternal age	2.265	1.32 3.90	0.003
Previous PTB	6.754	3.72 12.16	0.000
Cervical surgery	0.178	0.81 0.40	0.000
Constant	14.557	2.61 81.31	0.002

Table 2: Multivariable logistic regression analysis evaluation of TUCL as a predictor of PTB

Conclusion: Preterm birth represents a major health problem and strategies to prevent it are very important. Our study supports the usefulness of TUCL for predicting PTB and emphasized that a multivariable model can improve the screening algorithm.

cerclage of 0.15% in the subsequent pregnancy, which is similar to the baseline rate in the 1st pregnancy (table 1).

Conclusions: Women with a prior preterm birth at 34–36 weeks have very low risk of cerclage in the subsequent pregnancy compared to women who delivered at <34 weeks. Although current guidelines recommend serial transvaginal ultrasounds in all women with a prior preterm birth, this group may need less surveillance.

VP52.29

Using celecoxib for the suppression of preterm labour instead of magnesium sulfate

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Objectives: We aimed to use celecoxib to suppress preterm labour instead magnesium sulfate (MgSO₄) to prevent preterm labour.

Methods: It was a randomised clinical trial study, which was done on 600 pregnant women. All subjects were divided into two groups by simple random sampling. One group was given 4 grams of MgSO₄ intravenously and second group was given 100 mg celecoxib orally every 12 hours for at least 2 days. The data were entered and analysed using SPSS 11 and performed using t-test and chi-square test.

Results: The finding of this study has shown that preterm labour may be prevented in 75.7% of subjects who had received celecoxib and there were no significant difference between two groups in frequency of history of preterm labour ($p=1$), frequencies of nulliparity ($p=0.99$), duration of drug use and arrest contraction ($p=29$), delivery before 48 hours ($p=20$), and mean gestational age in lack of response to treatment ($p=24$).

Conclusions: Result has shown that celecoxib was similar to MgSO₄ as a medication to prevent preterm labour; it was recommended to be prescribe to prevent preterm labour, because it was cheaper than magnesium sulfate.

VP52.30

Preterm birth screening using routine transvaginal ultrasound: a retrospective study

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Objectives: Determination of second trimester TUCI usefulness and application for the prediction of PTB in a cohort of Portuguese pregnant women.

Methods: Retrospective cross-sectional cohort study that included all singleton pregnant women who performed second trimester ultrasound (18 to 22+6 weeks) in Centro Hospitalar Universitário São João, between January of 2013 and October of 2017.

Results: Our cohort included 4,481 women, with 4% spontaneous PTB. Within premature labours, 0.7% occur before the 34th week of gestation. TUCI mean was 33.8mm and TUCIs of 25.0mm, 27.0mm and 29.0mm represented percentiles 3, 5 and 10, respectively. Confounders, like maternal age, previous PTB and cervical surgery were included in a multiple logistic regression analysis that showed a significant association between shorter TUCI and PTB. The best cut-off for PTB prediction in this cohort of pregnant women was 20mm and not the most used value of 25mm.

Conclusions: Our study supports the usefulness of TUCI for predicting PTB and emphasised that the cut-off to define short cervix can differ according to the population.

VP52.31

The relationship between amniotic fluid sludge and proteomic profile in a cohort of pregnant women with acute cervical insufficiency

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Objectives: Emergency cervical cerclage is contraindicated in the presence of intra-amniotic infection, and traditional methods (amniocentesis with Gram stain and culture) to rule out infection prior to cerclage have low sensitivity. Visualisation of intra-amniotic sludge (IAS) may be a non-invasive marker of inflammation/infection in women with cervical insufficiency. We aim to determine the relationship between IAS and the amniotic fluid proteome amongst women with acute cervical insufficiency.

Methods: This was a secondary analysis of data collected from a small cohort of women presenting with acute cervical insufficiency. Amniotic fluid was prospectively analysed by Gram stain, culture and proteomics. Post-processing review of stored, perioperative ultrasound images was performed by 2 blinded observers to evaluate for presence/absence of IAS. Proteomic profiles, Gram stains and cultures were compared to ultrasound findings to determine the difference between groups.

Results: 10 participants were included for analysis (4 in the 'IAS Group' and 6 in the 'Non-IAS Group'). 75% of participants with IAS had negative Gram stain and culture by amniocentesis compared to 34% in those without. The IAS group had higher amounts of keratinizing proteins while higher amounts of inflammatory proteins were seen in the non-IAS group.

Conclusions: These preliminary findings suggest that proteomic profiles of amniotic fluid with and without IAS tend to differ: IAS tends to be associated with an abundance of keratinizing proteins as opposed to the inflammatory proteins seen without IAS. Given the small sample size, these results must be interpreted with caution and large prospective studies are still needed to determine the utility of IAS and proteomics in diagnosing intrauterine infection.

VP53: STILLBIRTH

VP53.01

Reduction of stillbirths in England according to uptake of the Growth Assessment Protocol 2008–2017

O. Hugh, S. Turner, M. Williams, J. Gardosi

Perinatal Institute, Birmingham, United Kingdom

Objectives: Antenatal detection of SGA can significantly reduce the risk of stillbirths. The Growth Assessment Protocol (GAP) was developed to address the problem that most SGA fetuses are missed. We set out to analyse the effect that the GAP program had on stillbirth rates in England.

VI. PORTUGUESE SOCIETY OF MATERNAL-FETAL MEDICINE COLABORATION

Félix J. Participation in the project of the Portuguese Society of Maternal-Fetal Medicine aimed at updating obstetricians' knowledge, through the suggestion, summary and discussion of the relevance of the article: "The introduction of a universal transvaginal cervical length screening program is associated with a reduced preterm birth rate." [Internet]. 2024 [cited 2024 Jan 17]. Available from: https://www.spommf.pt/sugestao-da-semana-2023/#Joana_Felix



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Sociedade Portuguesa
de Obstetria e
Medicina Materno-Fetal

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Assistente convidada da Faculdade de
Medicina da Universidade do Porto



19 de Maio 2023

The introduction of a universal transvaginal
cervical length screening program is associated
with a reduced preterm birth rate

Aude Figarella, Cécile Chau, Anderson Loundou, Claude d'Ercole, Florence Bretelle



Am J Obstet Gynecol. 2023; 228(2):219.e1-219.e14

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Por Joana Félix | ASSISTENTE HOSPITALAR DE GINECOLOGIA E OBSTETRÍCIA DO HOSPITAL PEDRO HISPANO; ASSISTENTE CONVIDADA DA FACULDADE DE MEDICINA DA UNIVERSIDADE DO PORTO.

O parto pré-termo (PPT) é a principal causa de morbi-mortalidade perinatal. Pelo seu elevado impacto socioeconómico, a redução da prevalência da prematuridade é um Millennium Development Goals das Nações Unidas.

O estudo apresentado é um estudo coorte prospetivo, com o objetivo de avaliar o impacto da implementação do rastreio do comprimento cervical na prevalência de PPT. Foi, assim, dividido em duas fases: antes da promoção do rastreio do comprimento cervical (coorte A) e após promoção do rastreio (coorte B). O outcome primário do estudo foi comparar a prevalência de PPT nas duas coortes.

Foram incluídas 336603 gestações unifetais, cujo parto ocorreu após as 24 semanas em 17 maternidades Francesas. A coorte A incluiu 171079 grávidas (parto entre 2012 e 2014) e a coorte B incluiu 165524 grávidas (parto entre 1 de maio de 2015 a 30 de abril de 2018). Entre as duas fases do estudo, foram desenvolvidas ações de promoção e sensibilização para a medição do comprimento cervical na ecografia do 2º trimestre, sendo que a realização deste rastreio aumentou de 28.9% na coorte A para 52.9% na coorte B. A prevalência de grávidas com colo curto a quem foi realizada intervenção (progesterona vaginal ou Cerclage) também aumentou entre o período A (11.5%) e o período B (18%). Na coorte A ocorreram 9903 PPT (5.79%) vs. 9275 (5.6%) na coorte B, traduzindo uma redução estatisticamente significativa de 0.19% na taxa de prematuros. Estatisticamente, seria necessário rastrear 1250 grávidas para prevenir 1 prematuro.

Os resultados deste estudo demonstram que o rastreio do comprimento cervical se associa a uma redução de PPT, sendo que uma adesão superior à realização do rastreio poderia ter maior impacto na prevenção dos prematuros. Vários trabalhos têm obtido resultados semelhantes e estudos de custo-eficácia concluem que mesmo uma pequena redução da prevalência de PPT é custo-efetiva.