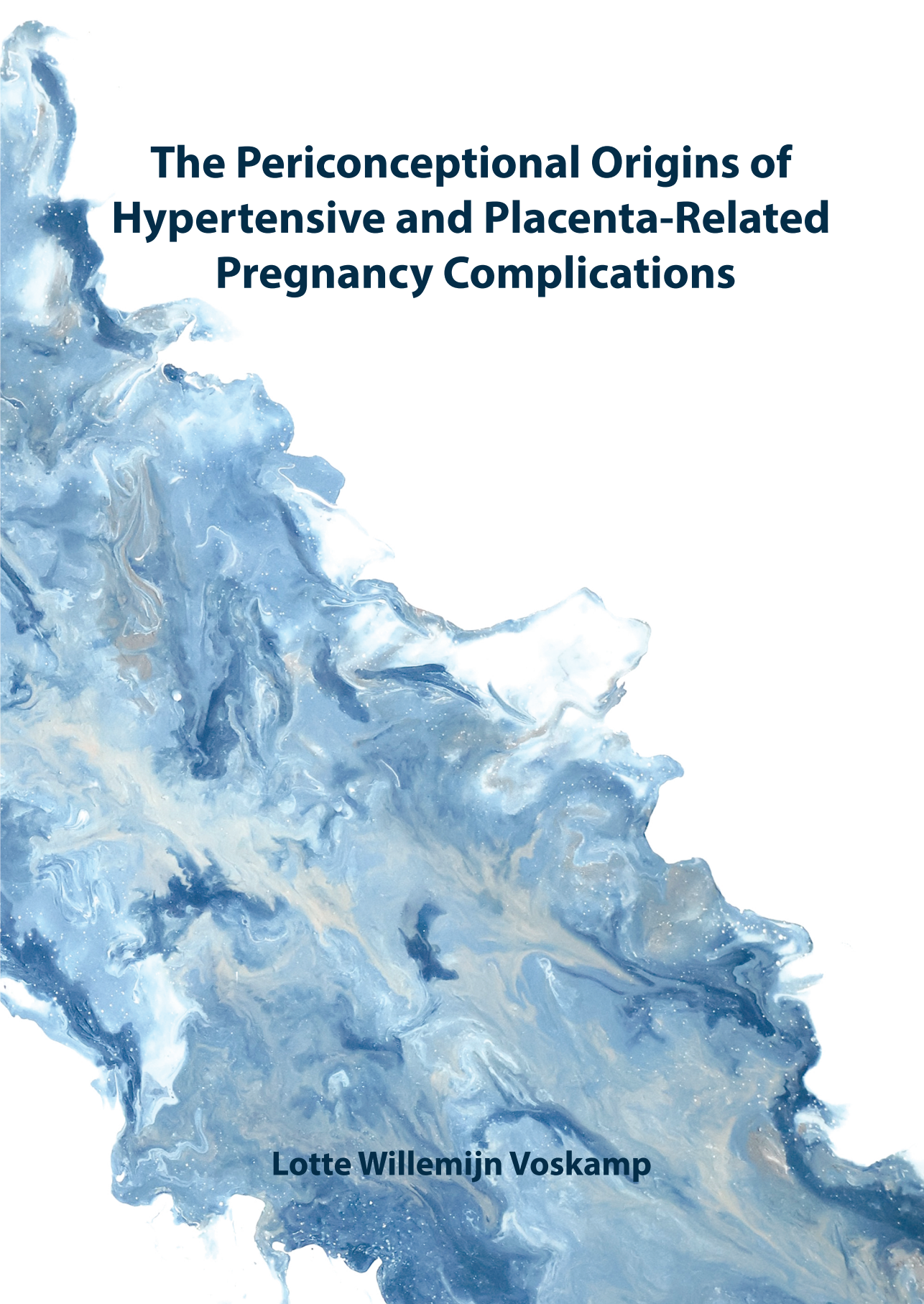
The Periconceptional Origins of Hypertensive and Placenta-Related Pregnancy Complications



Lotte Willemijn Voskamp

PROPOSITIONS

1. A shift toward a less anti-inflammatory immunoglobulin glycosylation profile in placenta-related pregnancy complications suggests that dysregulated humoral immune modulation contributes to placental dysfunction | This thesis
2. Natural cycle frozen embryo transfer should be the default protocol over artificial cycle frozen embryo transfer in eligible women | This thesis
3. Uterine artery Doppler measurements are essential for assessing maternal circulatory adaptation and should be performed at least once during each trimester of pregnancy | This thesis
4. Women with preeclampsia requiring antihypertensive treatment before 24 weeks of gestation, or with a persistently rising systolic blood pressure trajectory during pregnancy, should receive intensified postpartum hypertension monitoring | This thesis
5. Postpartum hypertension risk models after preeclampsia should incorporate pregnancy-specific factors, such as blood pressure trajectory, recurrence, and gestational age at onset, alongside classical cardiovascular risk factors | This thesis
6. A woman's health before, during, and after pregnancy is inseparable – healthcare should reflect this continuum.
7. Routine care data, collected within the existing therapeutic relationship between patient and care provider, should be accessible for clinical research and quality improvement without the full consent burden of formal research protocols.
8. Obstetric Medicine should be formally recognized as a medical subspecialty in the Netherlands, with the option for both internal medicine specialists and obstetricians to specialize in this field.
9. Evidence-based medicine cannot fulfill its promise as long as women—and pregnant women in particular—remain underrepresented in clinical research.
10. The elimination of unnecessary care is as much a marker of scientific progress in medicine as the introduction of new diagnostics and treatments.
11. Inzichten en ontdekkingen ontstaan zelden vanuit controle, maar in de stilte van aandachtige aanwezigheid.

The Periconceptional Origins of Hypertensive and Placenta-Related Pregnancy Complications

Lotte Willemijn Voskamp

ISBN: 978-94-6534-618-2
Printing: Optima Grafische Communicatie
Cover: original artwork by Sophie van der Vet; editing by Lotte Voskamp
Layout: Lotte Voskamp
Financial support for the production of this thesis was kindly provided by:
- The Department of Obstetrics & Gynaecology, Erasmus MC Rotterdam
- Erasmus University Rotterdam
- ChipSoft



Copyright © 2026 Lotte Voskamp
All rights reserved. No part of this thesis may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, without written permission from the author or, when appropriate, from the publisher.

The Periconceptional Origins of
Hypertensive and Placenta-Related Pregnancy Complications

Periconceptionele oorzaken van
hypertensieve en placenta-gerelateerde zwangerschapscomplicaties

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof.dr.ir. A.J. Schuit

en volgens besluit van het College voor Promoties.
De openbare verdediging zal plaatsvinden op

woensdag 23 september 2026 om 15.30 uur

door

Lotte Willemijn Voskamp
geboren te Vlaardingen.

Erasmus University Rotterdam



PROMOTIECOMMISSIE:

Promotoren:	Prof.dr. R.P.M. Steegers-Theunissen
	Prof.dr. A.H.J. Danser
Copromotoren:	Dr. M. Rousian
	Dr. K. Verdonk
Beoordelingscommissie:	Prof.dr. M. Kavousi
	Prof.dr. R.J.E.M. Dolhain
	Dr. J.W. Ganzevoort

CONTENTS

Chapter 1 General introduction 9

Part I - Immune modulation, hormonal signaling and vascular adaptation in pregnancy

Chapter 2 Longitudinal profiling of IgG and IgA glycosylation in pregnancy reveals early associations with BMI, primigravidity and conception mode: The Rotterdam Periconception Cohort 21
Submitted

Chapter 3 Longitudinal maternal IgG and IgA glycosylation profiles in pregnancy reveal early immune alterations in placenta-related complications: The Rotterdam Periconception Cohort 61
Hypertension. 2026;83:e26531.

Chapter 4 Corpus luteum number and circulatory adaptation from early pregnancy onwards: The Rotterdam Periconception Cohort 93
Hum Reprod. 2025 Nov 1;40(11):2078-2087

Chapter 5 Impact of corpus luteum number on maternal pregnancy and birth outcomes: The Rotterdam Periconception Cohort 123
Fertil Steril. 2024 Dec 6:S0015-0282(24)02431-2.

Part II - Clinical markers associated with adverse pregnancy outcomes and maternal cardiovascular health

Chapter 6 The ratio of mean arterial pressure and uterine artery pulsatility index is associated with embryonic growth and birthweight: The Rotterdam Periconception Cohort 151
Submitted

Chapter 7 Risk factors for chronic hypertension 5 years after a pregnancy complicated by preeclampsia: a systematic review and meta-analysis 173
J Hypertens. 2025 Jun 1;43(6):939-948.

Chapter 8 Blood pressure patterns during pregnancy and early postpartum in preeclampsia with severe features: predictors of chronic hypertension 197
Pregnancy Hypertens. 2025 Aug 1;41:101240.

Part III - Discussion and appendices

Chapter 9 General discussion 219

Chapter 10 Summary (English) 233
Samenvatting (Dutch)

Addendum Authors and affiliations 243
List of abbreviations 244
List of publications 247
PhD portfolio 249
About the author 251
Dankwoord 252

1

General introduction

GENERAL INTRODUCTION

A healthy pregnancy has far-reaching implications for both maternal and offspring health during the life course. Complications during pregnancy can be considered as early indicators of an underlying maternal health condition that is likely to be associated with increased risks of chronic diseases later in life [1]. Preeclampsia, a hypertensive disorder defined by elevated blood pressure and presence of proteinuria, organ dysfunction, and/or fetal growth restriction, is a key example [1, 2]. While advances in understanding the pathophysiology and prevention of preeclampsia have improved maternal and perinatal outcomes, accurate prediction and risk stratification for both short- and long-term health complications remain challenging [3, 4]. Addressing this requires both clinical risk assessment and translational research into the underlying mechanisms of pregnancy complications, with the goal of identifying biomarkers, risk factors, and therapeutic and preventative targets to support evidence-based prenatal and postpartum care. In this thesis, we combine clinical and translational approaches to enhance our understanding of cardiovascular adaptation to pregnancy, hypertensive disorders, including preeclampsia, and their associated complications during and after pregnancy.

Hypertensive disorders are among the most common and serious complications of pregnancy, contributing significantly to maternal and fetal morbidity and mortality worldwide [5]. Of these disorders, preeclampsia is the most severe form as it might even be life-threatening. It occurs in 2-8% of pregnancies worldwide, and 1-3% in the Netherlands [6-8]. Despite decades of research, its exact pathophysiology is still not fully understood, largely due to its heterogeneous nature and broad clinical spectrum [2, 5]. The underlying mechanisms of preeclampsia are thought to involve a combination of impaired placentation, a mismatch between the blood supply to the uteroplacental circulation and fetal demand, immunological disturbances, and maternal predisposition [8]. This mismatch induces syncytiotrophoblast stress, leading to the release of placental debris, proinflammatory cytokines, and antiangiogenic factors into the maternal circulation. These circulating factors cause systemic endothelial dysfunction and inflammation, which, in susceptible women, together progress to the clinical manifestations of preeclampsia [8]. This model suggests that the origin of at least some forms of preeclampsia lies within the periconceptional period, when early placental and maternal adaptations are established.

The periconceptional period is of particular interest for translational research. It spans from at least three months before conception up to and until three months thereafter, and encompasses several critical biological processes, including gametogenesis, conception, implantation, decidualization, and the establishment of the maternal-fetal interface [9]. Formed after ovulation, the corpus luteum serves as the primary source of reproductive hormones including estrogen, progesterone, relaxin, and prorenin, during the first trimester and until placental endocrine function is established [10, 11]. Compared to the physi-

ological situation in spontaneously conceived pregnancies where typically one corpus luteum is present, certain assisted reproductive technology (ART) protocols may result in either the absence of a corpus luteum or the presence of multiple corpora lutea. Previous studies have shown that the incidence of pregnancy complications varies between ART protocols, which may be partly explained by the altered hormonal environment. For instance, pregnancies conceived in the absence of a corpus luteum exhibit undetectable circulating levels of relaxin, a hormone known to promote vasorelaxation, and reduced activation of the renin-angiotensin-aldosterone system (RAAS), as evidenced by lower levels of prorenin and renin activity in early pregnancy [10, 12, 13]. These hormonal differences, driven by variations in corpus luteum number, may influence maternal cardiovascular adaptation and placental development [13-15].

Another key factor in the adaptation to pregnancy, particularly during implantation and placentation, is the maternal immune system [16, 17]. Because 50% of the fetus and placenta is derived from paternal genetic material, the maternal immune system must establish a degree of immunotolerance to prevent rejection of embryonic and placental tissues [16]. Placental development begins shortly after implantation, involving trophoblast invasion, remodeling of maternal spiral arteries, and the establishment of uteroplacental circulation [18]. These processes are tightly regulated by immune and endocrine signals, and it is known that disruptions in early placental development are associated with pregnancy complications such as preeclampsia and fetal growth restriction [18]. Two components of the adaptive immune system are immunoglobulin G (IgG) and immunoglobulin A (IgA). Because only IgG can cross the placental barrier, it is essential for transferring passive immunity from mother to the fetus [19]. The function of immunoglobulins is influenced by glycosylation, the biochemical process by which sugar molecules (glycans) are attached to proteins [20]. Indeed, the type and abundance of glycans attached to immunoglobulins affect their structural stability and receptor-binding affinity (particularly to Fc receptors on immune cells) [20]. Changes in glycosylation may therefore affect maternal immunotolerance, with downstream effects on placental development. Translational research into these mechanisms may help to better understand placenta-related pregnancy complications and indicate possible directions for future prevention or interventions.

Women with a history of preeclampsia have an increased risk of developing chronic hypertension shortly after pregnancy compared to pregnancies without hypertensive disorders [21]. It has been reported that up to one third of women have hypertension within ten years following a pregnancy complicated by preeclampsia [22]. Hypertension is a major risk factor for cardiovascular disease, and it is therefore not surprising that women with hypertensive disorders of pregnancy are also at increased risk of long-term cardiovascular complications, including stroke and ischemic heart disease [1]. This increased risk may be due to shared predisposing factors, such as obesity, diabetes and renal disease, or may result from vascular damage caused by the disorder itself [1, 3, 23]. Consequently,

both international and national guidelines, including those from the European Society of Cardiology, the American Heart Association and the Dutch Society of Obstetrics and Gynecology, recommend considering hypertensive disorders of pregnancy as cardiovascular risk factors [24-26]. Early interventions to improve cardiovascular health, whether behavioral or pharmacological, can reduce the overall risk of cardiovascular disease [27, 28]. However, implementing universal follow-up with routine clinical monitoring of cardiovascular health and lifestyle interventions for all women with preeclampsia would place a significant burden on healthcare systems and may lead to overtreatment, especially since the absolute risk of cardiovascular events remains relatively low for many. To ensure efficient and personalized care, early risk stratification is essential to identify those most likely to benefit from long-term monitoring and intervention.

Aims and outline of this thesis

Understanding maternal hypertensive and placenta-related hypertensive disorders of pregnancy and their complications requires an integrated approach that examines both the underlying mechanisms and their implications for prediction and management. This thesis addresses these aspects in two parts.

In **Part I**, we aim to investigate possible pathophysiological mechanisms that contribute to the development of maternal hypertensive and placenta-related hypertensive disorders. In **Chapter 2,** we explore changes in glycosylation patterns of IgG and IgA immunoglobulins throughout pregnancy and their association with maternal and pregnancy characteristics. Subsequently, in **Chapter 3,** we investigate whether these glycosylation patterns of IgG and IgA differ between pregnancies with and without placenta-related complications. **Chapter 4** investigates the physiological adaptations influenced by corpus luteum number, and **Chapter 5** the subsequent impacts on maternal and birth outcomes.

Building on the mechanistic insights from Part I, **Part II** aims to identify risk factors and predictors for maternal hypertensive and placenta-related disorders and their long-term consequences. In **Chapter 6**, we present a ratio of mean arterial pressure and uterine artery doppler indices and investigate its associations with embryonic growth, fetal growth, and birthweight. In **Chapter 7**, we summarize risk factors for the development of maternal chronic hypertension at least 5 years after a pregnancy complicated by preeclampsia. Focusing exclusively on women with preeclampsia with severe features in **Chapter 8,** we assess whether blood pressure patterns in pregnancy are associated with the prevalence of hypertension one to three years after pregnancy.

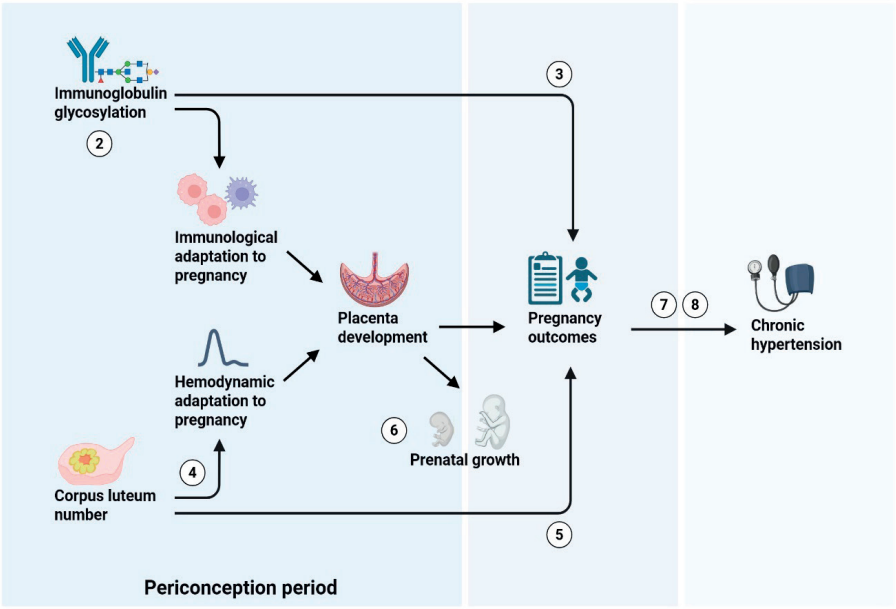


Figure 1. Overview of the exposures, intermediates, and outcomes addressed in this thesis. The arrows represent the hypothesized associations and mechanisms, showing how each chapter (indicated by number) contributes to understanding hypertensive and placenta-related pregnancy complications.

General study design

The research in this thesis was conducted within the Rotterdam Periconception Cohort (Predict Study), an ongoing prospective cohort study that was initiated after a pilot in 2010 [29, 30]. In this study, women and their partners aged 18 years or older are included preconceptionally or before the 10th week of pregnancy. At baseline and follow-up visits, questionnaires are used to obtain data on maternal demographics, medical and obstetric history, and lifestyle factors such as folic acid supplement use, nutrition, exercise, smoking, alcohol and sleep. Where possible, this information is validated against electronic medical records. Women attend 4-5 visits throughout pregnancy, with at least two within the first trimester (7, 9 or 11 weeks). Since 2017, additional visits have been scheduled in the second and third trimesters. At each visit, ultrasonography is performed to assess markers of prenatal growth and uteroplacental circulation. During the first trimester visits, crown-rump length, embryonic volume and placenta volume are measured by 3D ultrasound and processed offline by Virtual Reality and Artificial Intelligence. During the second and third trimester visits, fetal biometry is obtained by 2D ultrasound. At all visits, uterine artery pulsatility and resistance indices are measured using Doppler ultrasound,

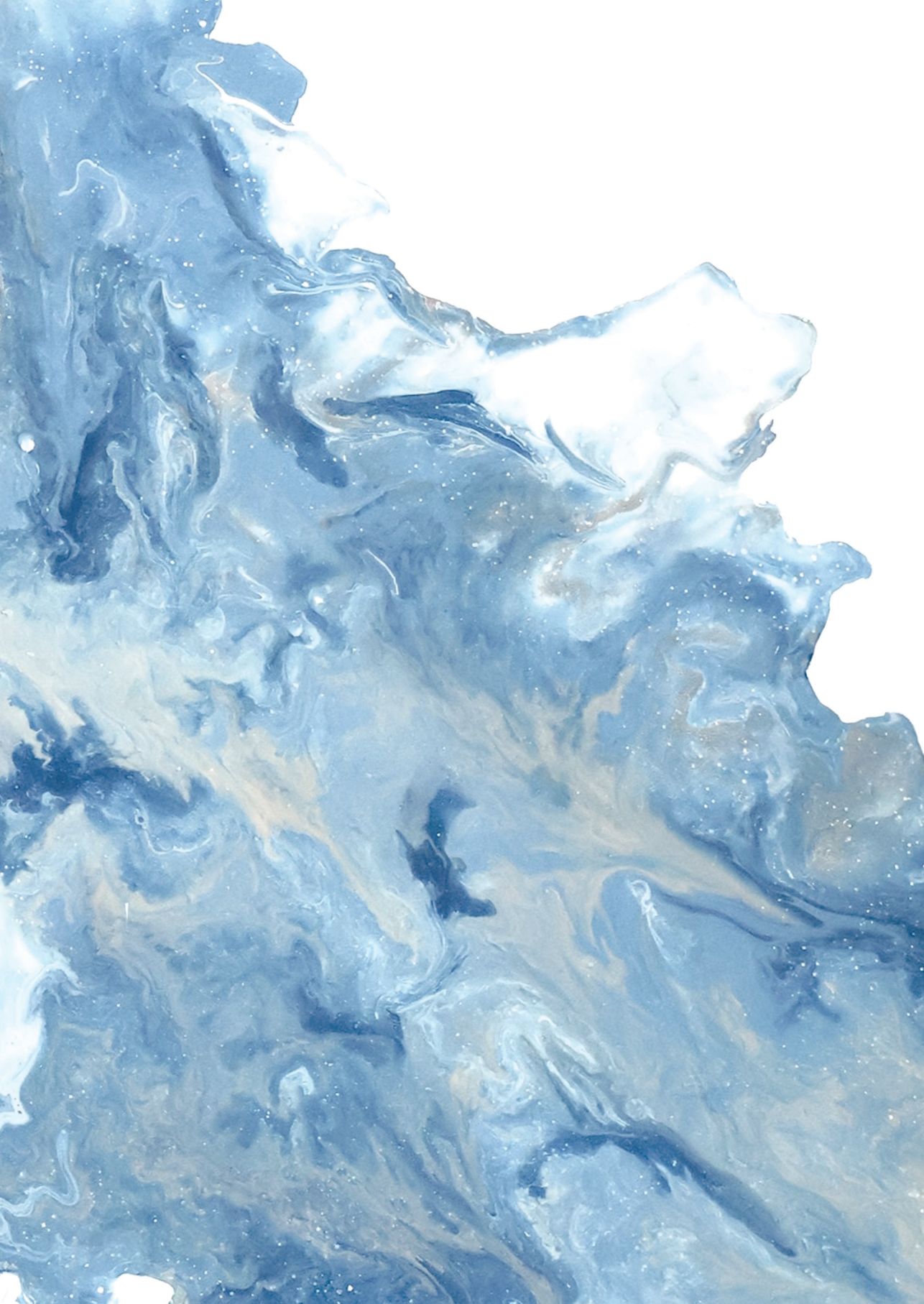
together with fetal umbilical and middle cerebral artery indices in the second and third trimester.

We combined data from the Rotterdam Periconception Cohort with another prospective study conducted at Erasmus MC, the Follow-up After Preeclampsia Cohort (FUPEC study) [31]. This cohort follows women with a history of preeclampsia from pregnancy up to the age of 50, with the aim of monitoring and improving long-term cardiovascular and metabolic health. By linking these two cohorts, we created a unique dataset that captures patient information from the periconception period through pregnancy and up to three years postpartum.

REFERENCES

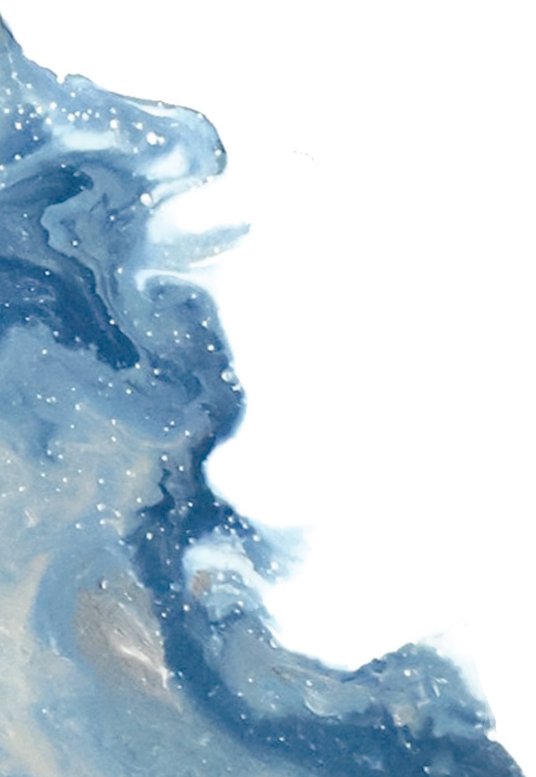
1. Bellamy L, Casas JP, Hingorani AD, Williams DJ. Pre-eclampsia and risk of cardiovascular disease and cancer in later life: systematic review and meta-analysis. BMJ. 2007 Nov 10;335(7627):974.
2. Magee LA, Brown MA, Hall DR, Gupte S, Hennessy A, Karumanchi SA, et al. The 2021 International Society for the Study of Hypertension in Pregnancy classification, diagnosis & management recommendations for international practice. Pregnancy hypertension. 2022 2022/3//;27:148-69.
3. Bartsch E, Medcalf KE, Park AL, Ray JG, High Risk of Pre-eclampsia Identification G. Clinical risk factors for pre-eclampsia determined in early pregnancy: systematic review and meta-analysis of large cohort studies. BMJ. 2016 Apr 19;353:i1753.
4. Rolnik DL, Wright D, Poon LC, O'Gorman N, Syngelaki A, de Paco Matallana C, et al. Aspirin versus Placebo in Pregnancies at High Risk for Preterm Preeclampsia. N Engl J Med. 2017 Aug 17;377(7):613-22.
5. Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. Lancet. 2010 Aug 21;376(9741):631-44.
6. Abalos E, Cuesta C, Grosso AL, Chou D, Say L. Global and regional estimates of preeclampsia and eclampsia: a systematic review. Eur J Obstet Gynecol Reprod Biol. 2013 Sep;170(1):1-7.
7. Post Uiterweer ED, Veerbeek JH, Franx A. [Pre-eclampsia] Pre-eclampsie. Ned Tijdschr Tandheelkd. 2015 Feb;122(2):79-83.
8. Magee LA, Nicolaides KH, von Dadelszen P. Preeclampsia. N Engl J Med. 2022 May 12;386(19):1817-32.
9. Steegers-Theunissen RP, Twigt J, Pestinger V, Sinclair KD. The periconceptional period, reproduction and long-term health of offspring: the importance of one-carbon metabolism. Hum Reprod Update. 2013 Nov-Dec;19(6):640-55.
10. Wiegel RE, Jan Danser AH, Steegers-Theunissen RPM, Laven JSE, Willemsen SP, Baker VL, et al. Determinants of Maternal Renin-Angiotensin-Aldosterone-System Activation in Early Pregnancy: Insights From 2 Cohorts. J Clin Endocrinol Metab. 2020 Nov 1;105(11):3505-17.
11. Conrad KP, Baker VL. Corpus luteal contribution to maternal pregnancy physiology and outcomes in assisted reproductive technologies. American journal of physiology Regulatory, integrative and comparative physiology. 2013 2013/1//;304(2):R69-72.
12. Wiegel RE, von Versen-Hoynck F, Steegers-Theunissen RPM, Steegers EAP, Danser AHJ. Prorenin periconceptionally and in pregnancy: Does it have a physiological role? Mol Cell Endocrinol. 2021 Feb 15;522:111118.
13. Conrad KP, Graham GM, Chi YY, Zhai X, Li M, Williams RS, et al. Potential influence of the corpus luteum on circulating reproductive and volume regulatory hormones, angiogenic and immunoregulatory factors in pregnant women. Am J Physiol Endocrinol Metab. 2019 Oct 1;317(4):E677-E85.
14. Conrad KP, von Versen-Hoynck F, Baker VL. Potential role of the corpus luteum in maternal cardiovascular adaptation to pregnancy and preeclampsia risk. Am J Obstet Gynecol. 2022 May;226(5):683-99.
15. Conrad KP, von Versen-Hoynck F, Baker VL. Pathologic maternal and neonatal outcomes associated with programmed embryo transfer. J Assist Reprod Genet. 2024 Apr;41(4):821-42.
16. Erlebacher A. Immunology of the maternal-fetal interface. Annu Rev Immunol. 2013;31:387-411.
17. Mor G, Aldo P, Alvero AB. The unique immunological and microbial aspects of pregnancy. Nat Rev Immunol. 2017 Aug;17(8):469-82.
18. Jauniaux E, Poston L, Burton GJ. Placental-related diseases of pregnancy: In-

volvement of oxidative stress and implications in human evolution. Hum Reprod Update. 2006 Nov-Dec;12(6):747-55.
19. Jauniaux E, Jurkovic D, Gulbis B, Liesnard C, Lees C, Campbell S. Materno-fetal immunoglobulin transfer and passive immunity during the first trimester of human pregnancy. Hum Reprod. 1995 Dec;10(12):3297-300.
20. Arnold JN, Wormald MR, Sim RB, Rudd PM, Dwek RA. The impact of glycosylation on the biological function and structure of human immunoglobulins. Annu Rev Immunol. 2007;25:21-50.
21. Giorgione V, Ridder A, Kalafat E, Khalil A, Thilaganathan B. Incidence of postpartum hypertension within 2 years of a pregnancy complicated by pre-eclampsia: a systematic review and meta-analysis. BJOG. 2021 Feb;128(3):495-503.
22. Behrens I, Basit S, Melbye M, Lykke JA, Wohlfahrt J, Bundgaard H, et al. Risk of post-pregnancy hypertension in women with a history of hypertensive disorders of pregnancy: nationwide cohort study. BMJ. 2017 Jul 12;358:j3078.
23. Yang C, Baker PN, Granger JP, Davidge ST, Tong C. Long-Term Impacts of Preeclampsia on the Cardiovascular System of Mother and Offspring. Hypertension. 2023 Sep;80(9):1821-33.
24. Brown HL, Warner JJ, Gianos E, Gulati M, Hill AJ, Hollier LM, et al. Promoting Risk Identification and Reduction of Cardiovascular Disease in Women Through Collaboration With Obstetricians and Gynecologists: A Presidential Advisory From the American Heart Association and the American College of Obstetricians and Gynecologists. Circulation. 2018 Jun 12;137(24):e843-e52.
25. (NVOG) NVvOeG. CVRM na een reproductieve aandoening. 2023 [cited 2025 August 26]; Available from: https://richtlijnendatabase.nl/richtlijn/cvrm_na_een_reproductieve_aandoening/cvrm_na_reproductieve_aandoening_-_startpagina.html.
26. De Backer J, Haugaa KH, Hasselberg NE, de Hosson M, Brida M, Castelletti S, et al. 2025 ESC Guidelines for the management of cardiovascular disease and pregnancy. Eur Heart J. 2025 Aug 29.
27. Chen H, Li R, Liu S, Zhao S, Guo T, Tian S, et al. Life's Essential 8 and cardiovascular disease in women with a history of adverse pregnancy outcomes. Eur J Prev Cardiol. 2025 Jan 31.
28. Hojeij B, Schoenmakers S, Van Mullem I, Willemsen SP, Dinnyes A, Sinclair KD, et al. Smarter Pregnancy Coaching and Maternal Blood Pressure. Am J Prev Med. 2025 Mar;68(3):455-64.
29. Steegers-Theunissen RP, Verheijden-Paulissen JJ, van Uitert EM, Wildhagen MF, Exalto N, Koning AH, et al. Cohort Profile: The Rotterdam Periconceptional Cohort (Predict Study). Int J Epidemiol. 2016 Apr;45(2):374-81.
30. Rousian M, Schoenmakers S, Eggink AJ, Gootjes DV, Koning AHJ, Koster MPH, et al. Cohort Profile Update: the Rotterdam Periconceptional Cohort and embryonic and fetal measurements using 3D ultrasound and virtual reality techniques. Int J Epidemiol. 2021 Nov 10;50(5):1426-7 .
31. van der Bijl MF, Al-Hassany L, Wijtzes AI, Verdonk K, Duvekot JJ, Roeters van Lennep J. FUPEC study, a prospective open-cohort on severe pre-eclampsia and cardiovascular risk factors based in the Netherlands. BMJ Open. 2024 Nov 12;14(11):e093423.



Part I

Immune modulation, hormonal signaling and vascular adaptation in pregnancy



2

Longitudinal profiling of IgG and IgA glycosylation in pregnancy reveals early associations with BMI, primigravidity and conception mode: The Rotterdam Periconception Cohort

Lotte W. Voskamp, Anna Daniels, Melek Rousian, Mandy van Hoek,
Manfred Wuhrer, Régine P.M. Steegers-Theunissen, A.H. Jan Danser, Koen Verdonk

Submitted

ABSTRACT

Study question: How do IgG and IgA glycosylation patterns change across pregnancy, particularly in the first trimester, and which maternal and pregnancy-related factors are associated with these changes?

Summary answer: IgG and IgA glycosylation patterns shift towards an anti-inflammatory profile as early as the first trimester and are influenced by maternal body-mass index, gravidity, and use of assisted reproductive technologies.

What is known already: During early pregnancy, the maternal immune system undergoes a recalibration to tolerate the fetus, which expresses paternal genes, while still maintaining protection against pathogens. Immunoglobulin function is modulated by glycosylation, the attachment of glycans to the protein backbone. Previous research has revealed increases in anti-inflammatory glycosylation profiles (increased galactosylation and sialylation) in IgG between the first and third trimesters of pregnancy, and to lesser extent in IgA. However, it remains unknown how IgG and IgA glycosylation profiles change within the periconceptional period, a critical period for implantation and placentation, and how these changes are influenced by maternal and pregnancy characteristics.

Study design, size, duration: This study used data from the ongoing prospective Rotterdam Periconception Cohort that is conducted at the Erasmus MC University Medical Center, a tertiary hospital in the Netherlands. Serial blood samples were collected from 202 women with singleton pregnancies between 2017 and 2018.

Participants/materials, setting, methods: Serum samples were collected at 9, 11, 13, 22, and 32 weeks of gestation and from cord blood. IgA and IgG glycopeptides were analyzed by liquid chromatography–mass spectrometry and summarized into glycosylation traits: galactosylation, sialylation, fucosylation, and bisection. Statistical analysis included Spearman correlations between IgG and IgA glycosylation traits, descriptive summaries per timepoint, and linear mixed-effects models to assess longitudinal changes and associations with maternal and pregnancy characteristics, with false discovery rate adjustment for multiple testing.

Main results and the role of chance: Already in the first trimester, a significant increase of galactosylation and sialylation and decrease of bisection were observed for both IgG and IgA. IgG galactosylation and sialylation increased progressively throughout pregnancy, whereas most IgA traits reversed after the first trimester. Higher maternal body-mass index was associated with a more pro-inflammatory profile showing lower galactosylation on IgG and higher fucosylation on IgG and IgA. Assisted reproduction

and primigravidity were associated with anti-inflammatory changes, including higher galactosylation and sialylation.

Limitations, reasons for caution: Although this is a unique and one of the largest pregnancy cohorts with longitudinal IgA and IgG glycosylation profiling in the first trimester onwards, statistical power for subgroup analyses was limited, and multiple testing may have led to chance findings despite correction. In addition, the tertiary hospital setting limits the generalizability of the results.

Wider implications of the findings*:* Our study provides novel insights into trimester-specific shifts in antibody glycosylation, advancing the understanding of maternal immune adaptation in early pregnancy. We demonstrate that the previously reported shift towards anti-inflammatory IgG glycosylation already occurs in the first trimester, while IgA shows a transition towards a more pro-inflammatory profile after 13 weeks of gestation. These findings raise new questions about the role of glycosylation at the cellular level in processes such as trophoblast invasion and placental development. Moreover, the observed associations with maternal BMI, gravidity, and mode of conception suggest that glycosylation alterations may represent a common biological pathway through which these diverse maternal factors, each with distinct effects on pregnancy, influence clinical outcomes.

Study funding/competing interest(s): This research was funded by the departments of Obstetrics & Gynecology, and Internal Medicine of the Erasmus Medical Center, Rotterdam, the Netherlands. The authors declare no conflicts of interest.

Trial registration number: This study is registered at the Dutch Trial Register (NTR6854).

INTRODUCTION

The complex relationship between the maternal immune system and fetal development is fundamental to achieving a successful pregnancy. During pregnancy, the maternal immune system undergoes significant modifications to accommodate and protect the developing fetus, which is genetically distinct from the mother, while also maintaining the maternal protection against pathogens [1, 2]. Most of these modifications occur during the periconceptional period, which spans from three months before conception up to the end of the first trimester of pregnancy [3]. Within this period, critical processes such as implantation, embryogenesis, placentation, and the formation of the maternal-fetal interface are initiated [3]. The maternal immune system plays a crucial role in mediating these processes [2].

Immunoglobulins are important components of the immune system. Of the various classes of immunoglobulins, immunoglobulin G (IgG) is the only one capable of crossing the placental barrier, which makes IgG vital for providing the fetus with passive immunity during pregnancy [4, 5]. Besides IgG, immunoglobulin A (IgA) is the second most abundant immunoglobulin in serum and plays a key role in non-inflammatory immune defense and pathogen neutralization, yet its function in pregnancy remains largely unexplored [6, 7]. Both IgG and IgA are composed of two heavy and two light chains, and their function is modulated by glycosylation, the process by which carbohydrate chains (glycans) are enzymatically attached to proteins [8]. The attached glycans can be modified through galactosylation (addition of galactose), sialylation (addition of sialic acid to galactose), fucosylation (addition of a fucose), and bisection (addition of a bisecting N-acetylglucosamine, contributing to the overall glycosylation profile (Figure 1) [8]. A recent comprehensive review summarized the diverse roles of glycosylation in human reproduction, including trophoblast invasion, placental development, and maternal–fetal immune regulation [9]. Notably, immunoglobulin glycosylation was highlighted as an important component of maternal immune adaptation, as it influences antibody structure and function, particularly by modulating their affinity for Fc receptors [8-11]. In general, increased galactosylation and sialylation are associated with anti-inflammatory activity, while increased bisection may have pro-inflammatory effects [8, 11-13]. Glycosylation profiles are affected by different conditions, including a more pro-inflammatory pattern observed with aging, obesity, diabetes or auto-immune disease [10, 11, 14, 15].

Previous studies observed that in uncomplicated pregnancies, galactosylation and sialylation of Fc glycans of IgG increase from the first trimester to the second or third trimester of pregnancy [16, 17]. In contrast, changes in serum IgA glycosylation during pregnancy have been examined in only a few studies, highlighting a significant gap in current knowledge [18-20]. Moreover, both IgG and IgA glycosylation patterns have not yet been studied within the first trimester.

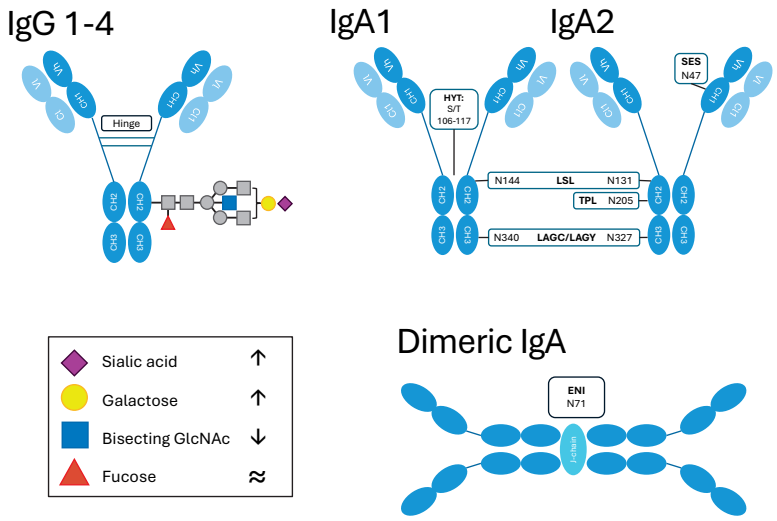


Figure 1. Schematic overview of IgG and IgA subtypes with glycosylation sites and summary of observed pregnancy-associated changes in IgG glycosylation. The analyzed IgA glycopeptides are defined in Supplementary Table 2

We hypothesized that pregnancy induces specific shifts in antibody glycosylation early in gestation, reflecting a transition toward a more complex, anti-inflammatory profile. These changes may be influenced by maternal and pregnancy-related factors known to affect immune adaptation. Nulliparous women have a higher risk of immune-mediated complications such as pre-eclampsia and may exhibit a more pro-inflammatory profile [21]. Assisted reproductive technologies (ART) are used in cases of subfertility and involve exogenous administration of estrogen and progesterone, which may influence immune adaptation [22]. The objective of this study was to characterize both IgG and IgA glycosylation profiles throughout pregnancy, with a focus on the first trimester, and assess associations with maternal age, body-mass index, conception mode and gravidity.

METHODS

Study design

This study used data from the VIRTUAL Placenta study, a subcohort of the Rotterdam Periconception Cohort (Predict study), an ongoing longitudinal prospective cohort study that has been including women periconceptionally between 2017 and 2018 at the Erasmus University Medical Center, Rotterdam, the Netherlands [23, 24]. Eligibility criteria for inclusion were women with a singleton pregnancy above 18 years old, with a gestational age (GA) of less than ten weeks. Pregnancies ending in miscarriage or stillbirth were excluded due to incomplete

follow-up, while perinatal deaths were excluded to avoid bias from severe complications. Informed consent was obtained from all participants in accordance with the Declaration of Helsinki, and this study was approved by the local ethics committee (MEC-2004-227).

Data collection

At inclusion, participants were asked to complete a questionnaire on their general health and medical and obstetrical history. At least two study visits were scheduled in the first trimester at 7-, 9-, 11- and 13 weeks GA. During the intake, weight and height were measured to calculate body mass index (BMI). For natural pregnancies with regular cycles between 25 and 35 days, GA was calculated from the first day of the last menstrual period (LMP). If the LMP was unknown or cycles were irregular, GA was determined using Crown-Rump-Length (CRL). If there was a discrepancy of more than six days between the two methods, the GA based on CRL was considered accurate. For pregnancies conceived through in vitro fertilization (IVF) with or without intra-cytoplasmic sperm injection (ICSI), GA was calculated from the day of oocyte retrieval plus fourteen days. In cases of cryo-preserved embryo transfer, GA was calculated by adding 17 or 19 days to the embryo transfer date for day 3 and day 5 embryos, respectively. After birth, medical records were used to obtain information on pregnancy outcomes, including GA at birth, birthweight and occurrence of maternal pregnancy complications including pregnancy induced hypertension, preeclampsia and gestational diabetes.

Glycosylation measurements

Venous serum samples were collected during study visits at approximately 9, 11, 13, 22, and 32 weeks GA. Blood samples were collected in 6 ml SST tubes, allowed to clot for at least 60 minutes, and centrifuged at 2000 g for 10 minutes at 4 °C. Serum was aliquoted (4 × 0.5 ml) and immediately stored at −80 °C. IgG and IgA glycosylation analysis was performed at the glycopeptide level following a previously validated protocol [25]. Briefly, IgG and IgA were affinity-captured, subjected to reduction, alkylation, and proteolytic cleavage with trypsin, followed by nano-scale Liquid Chromatography-Mass Spectrometry (LC-MS) analysis. Data were processed using the proprietary software LaCyTools, as described previously [26]. Peptide- and site-specific glycosylation was normalized to 1.0 for the sum of all glycosylation variants of a specific peptide. For each of the peptides, glycosylation traits were calculated reflecting specific glycosylation motifs (see Supplementary Table 1). Laboratory personnel conducting LC–MS analysis were blinded to participant clinical characteristics.

Statistical analysis

Baseline characteristics were reported as mean and standard deviations, median and interquartile range for non-normally distributed variables, or number and percentage for categorical variables. To assess relationships between glycosylation traits, we calculated pairwise correlation coefficients using the Spearman method for each trimester sepa-

Table 1. Maternal demographic and pregnancy characteristics.

	N = 202	N missing
Patient characteristics		
Maternal age, mean (SD)	32.1 (4.4)	
Maternal BMI, median [IQR]	25.0 [22.2, 28.8]	
Country of origin, n (%)		6 (3.0)
Dutch	170 (84.2)	
Western, not Dutch	6 (3.0)	
Non-Western	20 (9.9)	
Primigravidity, n (%)	80 (39.6)	1 (0.5)
Smoking periconceptional, n (%)	25 (12.3)	9 (4.5)
Pre-existing hypertension, n (%)	5 (2.5)	2 (1.0)
Pre-existing diabetes mellitus, n (%)		
Type I	3 (1.5)	
Type II	0 (0.0)	
Pregnancy characteristics		
Mode of conception, n (%)		
Spontaneous	103 (51.0)	
Ovulation induction	9 (4.5)	
Intra-uterine insemination	7 (3.5)	
IVF	33 (16.3)	
ICSI	50 (24.8)	
Gestational age at birth in days, median [IQR]	273 [265, 278]	
Neonatal birthweight percentile, median [IQR]	46 [21, 72]	
Pregnancy complication, n (%)		2 (1.0)
Preeclampsia	7 (3.5)	
with onset <34 weeks	3 (1.5)	
Pregnancy induced hypertension	15 (7.4)	
Gestational diabetes	31 (15.3)	

BMI = body-mass index; ICSI = intra-cytoplasmatic sperm injection; IQR = interquartile range; IVF = in vitro fertilization; SD = standard deviation.

rately. For all glycosylation traits, we calculated the mean and standard deviation at each time point. To assess changes between time points, linear mixed-effects models were fitted for each trait, with timepoint as a fixed effect and a random intercept for each subject to account for repeated measurements. Estimated marginal means were then compared using pairwise t-tests, and p-values were adjusted for multiple testing using the False Discovery Rate (FDR). P-values <0.05 were considered statistically significant. Mixed linear regression models were used to assess associations between glycosylation traits and maternal and pregnancy characteristics, including age and BMI at conception, primigravidity and mode of conception. Primigravidity was defined as no prior pregnancies, including no previous miscarriages. Mode of conception was classified as natural if pregnancy occurred spontaneously or with the aid of ovulation induction or intrauterine insemination. Assisted reproductive technology (ART) included pregnancies conceived using IVF or ICSI. For each model, we tested for significant interactions between GA and the exposure variable. Models including pregnancy characteristics were adjusted for maternal age and BMI, with BMI interactions included where applicable. We performed a post-hoc sensitivity analysis to explore whether the associations observed for primigravidity were confounded by mode of conception (spontaneous vs. IVF/ICSI). The analysis was limited to women in their first pregnancy, and pregnancies conceived via ovulation induction or intrauterine insemination were excluded due to limited numbers and heterogeneous treatment protocols. Statistical analyses were performed using R (version 4.3.2).

RESULTS

Study population and baseline characteristics

A total of 202 ongoing pregnancies were included in the current study. Baseline characteristics are depicted in Table 1. Mean age at conception was 32 years, and median BMI was 25.0 kg/m2. Around 12% of women smoked before pregnancy and/or during the first trimester. The majority of women (n=156) had glycosylation measurements available for four gestational time points. A smaller number had five (n=4), three (n=34), two (n=7), or one measurement (n=1).

Correlation of glycosylation traits

To investigate the relationships between different glycosylation traits, we computed correlation matrices for each trimester. The analysis revealed several key patterns, primarily defined by the type of glycan modification (such as O- versus N-glycosylation, bisection, galactosylation), antibody (sub-)class and glycosylation site. Overall, correlations between glycosylation traits were found to be comparable between the three trimesters (Figure 2; Supplementary Figure 2).

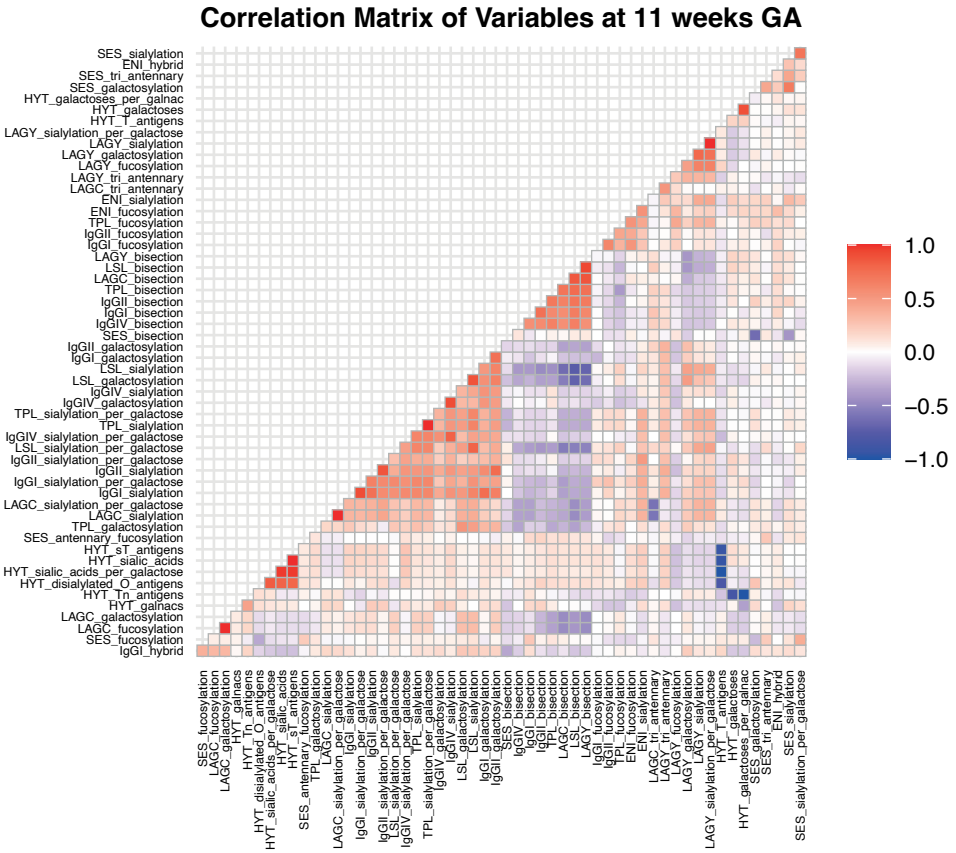


Figure 2. Correlation matrix of glycosylation traits measured at 11 weeks gestational age.

Patterns of bisected glycans

Bisected glycans were found to be positively correlated across different immunoglobulins and glycosylation sites but tended to be negatively correlated with other modifications like sialylation and galactosylation. Specifically, strong positive correlations (r between 0.72 and 0.94) were found for the bisection levels of the four IgA N-glycosylation sites LAGY, LAGC, LSL, and TPL. Bisection of these four IgA N-glycosylation sites also showed moderate-to-strong correlations with IgG Fc N-glycosylation for the various subclasses (r 0.57 to 0.70).

Bisection showed predominantly negative correlations with sialylation and galactosylation. The strength of this inverse relationship varied, with the strongest negative correlations (r=−0.6 to −0.7) found between bisected LAGC, LAGY, and LSL glycans and

the sialylation and galactosylation of LSL. Bisected IgG glycans displayed weaker negative correlations (r≈–0.3) with galactosylation and sialylation of LSL (IgA). Interestingly, for IgG1 bisection showed weak negative correlation with galactosylation (r=–0.17) and sialylation (r=–0.26), while such a negative correlation was not apparent for IgG4.

Hierarchical correlations in galactosylation and sialylation

For terminal modifications like galactosylation and sialylation, the correlations followed a logical hierarchy. The strongest correlations were found within antibody classes, while correlations between IgG and IgA were weaker and less consistent. Within the IgG class, IgG1 and IgG2 galactosylation were strongly correlated (r=0.73), while the correlations of IgG1 and IgG2 galactosylation with IgG4 galactosylation were more moderate (r=0.51 and 0.46, respectively). Similarly, IgG1 and IgG2 sialylation showed strong correlation (r=0.91). Again, sialylation of IgG1 and IgG2 showed moderate correlation with IgG4 sialylation (r=0.53 and 0.48, respectively).

Between antibody classes, correlations were less consistent. For instance, IgG1 and IgG2 galactosylation were positively correlated with LSL galactosylation (r=0.55 and 0.64, respectively). Similarly, for IgG1 and IgG2 sialylation, correlations with LSL sialylation were observed (r=0.62 and 0.48, respectively). Of note, these glycosylation sites on IgG and IgA are structurally homologous, implying that the structural similarity of the glycosylation sites may play a role in steering galactosylation in a conserved way across antibody classes. In contrast, IgG1 Fc galactosylation showed only low (r=0.23) or no (r=0.02) correlation with LAGC and LAGY galactosylation, respectively. Interestingly, LAGC and LAGY glycopeptides which only differ in the absence or presence of the terminal tyrosine, showed virtually no correlation with respect to galactosylation levels (0.09) while their sialylation levels showed moderate correlation (r=0.37).

Correlations in fucosylation

Core fucosylation on IgG1, IgG2, ENI, and TPL was moderately correlated (r=0.35 to 0.6), while fucosylation at other sites, such as SES and LAGC, seemed to be independently regulated. Of note, LAGC and LAGY fucosylation showed no correlation.

IgA2 N-glycosylation

The TPL and SES glycosylation sites of IgA2 are not shared with IgA1 and therefore provide insights into IgA2-specific N-glycosylation. TPL glycosylation traits correlated with various other immunoglobulin N-glycosylation traits. Interestingly, TPL fucosylation showed the strongest correlation with ENI fucosylation (r=0.52), followed by LAGY fucosylation (r=0.41) whilst there was hardly any correlation with LAGC fucosylation (r=0.12). These data point towards shared fucosylation programs of dimeric IgA2 as reflected in joining chain glycosylation (ENI) and glycosylation of the tyrosine-terminating LAGY glycopeptide. In contrast, SES glycosylation showed no strong correlations with other glycosyl-

ation sites, possibly reflecting the distant location of the SES glycosylation site in the CH1 domain of the IgA2 heavy chain.

IgA1 hinge region O-glycosylation

The O-glycosylation traits of the HYT hinge-region O-glycopeptide of IgA1 largely showed correlation with other traits of the HYT cluster, whilst no strong correlations with N-glycosylation features were observed.

Changes in glycosylation across pregnancy

N-glycosylation of IgG

For all subtypes of IgG, we observed a significant increase in galactosylation as well as sialylation within the first and second trimesters. Notably, sialylation showed a more prominent increase than galactosylation, leading to an increase in sialylation per galactose. This effect was strongest for IgG2-III (Figure 3 A, B). In contrast, bisection significantly decreased in IgG1 during the first trimester, from 15.88 (±2.86) at week 9 to 15.13 (±2.58) at week 11 (p=0.008), and further to 14.79 (±2.63) at week 13 (p=0.019). Bisection in IgG2

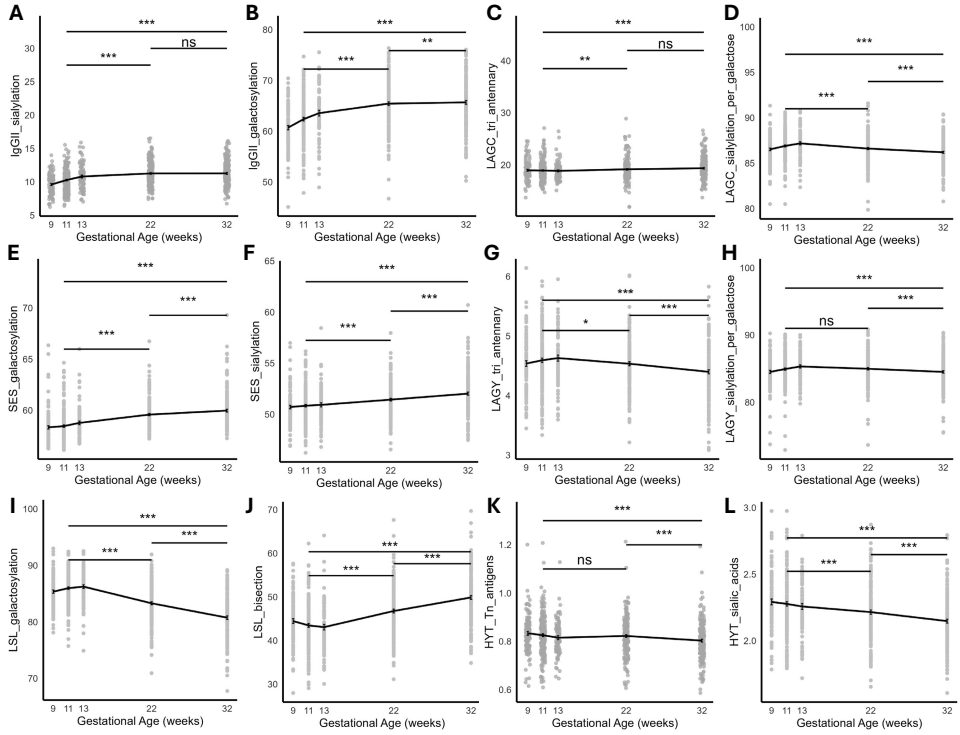


Figure 3. Relative abundance of glycosylation (selection).

and IgG4 showed slight, but non-significant declines. After 13 weeks, bisection levels in all IgG subclasses increased. At 11 weeks, the mean percentage of fucosylation was 91.8% for IgG1 and 99.3% for IgG2 and this remained unchanged during the full course of gestation. All measured means of glycosylation traits are reported in Supplementary Table 3 and depicted in Supplementary Figure 1.

N-glycosylation of IgA

Different patterns were observed for IgA glycosylation traits. For LSL, TPL, LAGC and LAGY, bisection showed a decreasing trend between 9 and 13 weeks gestation, while sialylation and galactosylation increased. After 13 weeks, the directions change, with a significant increase in bisection and decrease in galactosylation and sialylation towards the second and third trimester for LSL, TPL and LAGY (Figure 3 I and J, Supplementary Table 3, Supplementary Figure 1). In contrast, galactosylation in LAGC remained stable after the first trimester (99.34%-99.36%). On both LAGC and LAGY sites, sialylation per galactose increases during the first trimester, and then decreases towards the third (Figure 3 D, H). However, while tri-antennary LAGC increased between the first and third trimester (18.91 to 19.35, $p<0.001$), tri-antennary LAGY decreased (4.63 to 4.40, $p<0.001$) (Figure 3 C, G).

In contrast to the IgA glycosylation sites described above, bisection at the SES site remained stable in the first trimester and slightly decreased towards the third trimester. SES fucosylation increased only between the first and second trimesters, while both galactosylation and sialylation showed a continuous increase across all trimesters (Figure 3 E, F, Supplementary Table 3). At the monitored glycosylation site of the joining chain (ENI), sialylation significantly decreased between the first, second, and third trimesters, while the abundance of hybrid-type glycan structures increased.

O-Glycosylation of IgA (HYT region)

Tn antigens showed a slight decrease over pregnancy, from 0.83 relative abundance at week 9 to 0.80 at week 32, with a significant decline between the second and third trimester ($p<0.001$, Figure 3 K). Galactoses of the HYT hinge region glycopeptide slightly increased in pregnancy, from 3.66 at 11 weeks to 3.69 at 32 weeks ($p<0.001$), as did GalNAc levels and galactoses per GalNAc. Sialylation in the HYT region gradually decreased throughout pregnancy, as indicated by a decline in total sialic acids (2.29 at week 9 to 2.15 at week 32), disialylated O-antigens (3.05 to 2.75), and sialic acids per galactose (0.63 to 0.58).

Associations with clinical parameters

Table 2 presents the significant main associations between the exposure variables and IgG glycosylation as derived from the linear mixed regression models. Only variables that showed a significant overall difference throughout pregnancy (main effect) are included in this summary. For some glycosylation traits, an interaction effect with GA was observed,

indicating differences in trajectory over pregnancy rather than a consistent increase or decrease. These interactions are not included in the table but are described in detail in the text and visualized in Figure 4.

Table 2. Significant associations between maternal characteristics and IgG and IgA glycosylation across pregnancy

Variable	Age [1]	BMI [2]	Primigravidity [3]	IVF/ICSI pregnancy [3]
IgG1	x	Fucosylation ↑	X	Galactosylation ↑ Sialylation ↑ Hybrid ↑
IgG2	x	X	X	x
IgG4	x	Galactosylation ↓ Sialylation ↓	Galactosylation ↑ Sialylation ↑	X
LAGC	x	X	X	Fucosylation ↑ Galactosylation ↑
LAGY	x	X	Sialylation per galactose ↑ Sialylation ↑	X
LSL	x	X	x	X
TPL	x	Fucosylation ↑	Sialylation ↑ Sialylation per galactose ↑	Sialylation ↑ Sialylation per galactose ↑
SES	x	Sialylation Tri-antennary ↑	X	Fucosylation ↑ Sialylation ↑ Tri-antennary ↑
ENI	x	Fucosylation ↑	X	X
HYT	x	Galactoses ↓ Galactoses per GalNac ↑ Tn antigens ↓	Galactoses ↓ Galactoses per GalNAc ↓ Tn antigens ↑	X

[1] Adjusted for maternal BMI; [2] Adjusted for maternal age; [3] Adjusted for maternal age and BMI. X: No significant main effect of exposure on glycosylation.

Age

In a crude model, maternal age was associated with lower fucosylation at IgG1 and ENI sites, and increased bisection at IgG4 during the first half of pregnancy. After adjusting for body mass index, no significant associations were observed for any of the investigated glycosylation traits with maternal age at conception.

Body mass index

Women with a higher BMI exhibited significant differences in glycosylation patterns across both IgG and IgA. For IgG, differences were observed in the IgG4 subclass, where women with a higher BMI have significantly lower levels of galactosylation and sialylation throughout pregnancy. Additionally, the IgG4 sialylation per galactose increases less in women with a higher BMI (Figure 4). Women with a high BMI showed increased core fucosylation of IgG1, as well as at IgA N-glycosylation sites TPL and the joining chain ENI. Moreover, sialylation and sialylation per galactoses were slightly higher in the first trimester, but comparable at 22 and 32 weeks. In the SES region, higher sialylation and tri-

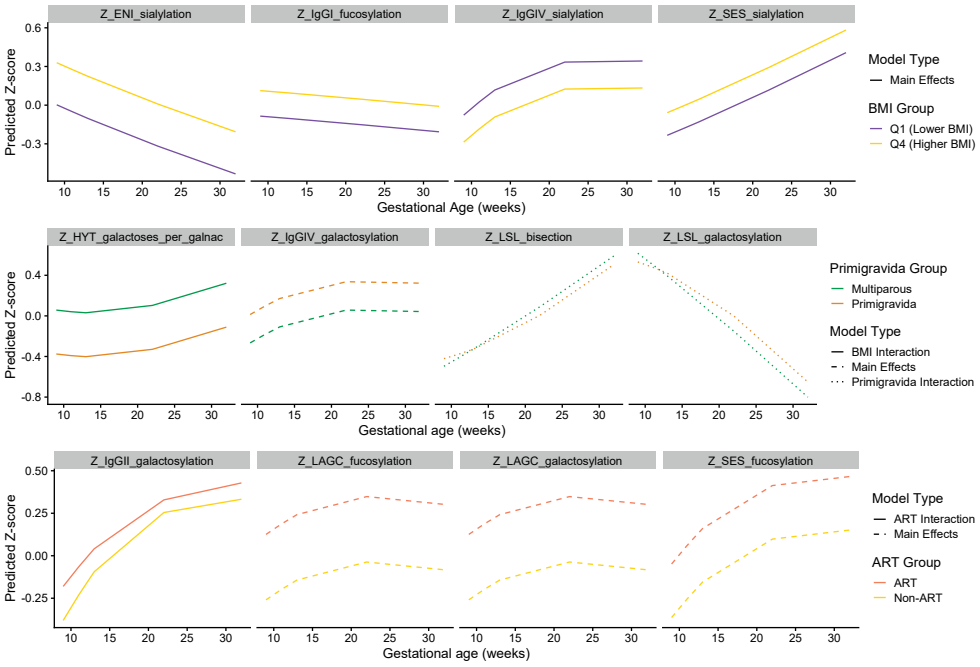


Figure 4. Trajectories of glycosylation traits based on linear mixed regression models. Differences for body-mass index (BMI), primigravidity, assisted reproduction technologies (ART), and pregnancy complications.

antennary structures were observed for women with higher BMI. In the HYT region, lower levels of galactoses and Tn antigens were observed, alongside higher levels of galactoses per GalNAc in women with higher BMI.

Mode of conception

We compared glycosylation patterns between pregnancies conceived spontaneously and with ART. Galactosylation levels were significantly higher in IgG1, IgG2, and LAGC. In IgG2, the trajectory differed by conception mode, with ART pregnancies showing higher levels in the first trimester, followed by a less steep increase thereafter (Figure 4). Sialylation was increased in IgG2, LAGC, SES, and TPL, and fucosylation was increased in LAGC and SES. Moreover, in the SES region, tri-antennary structures increased in both groups, but ART pregnancies consistently exhibited higher levels throughout gestation.

Sensitivity analysis

Since primigravidity was more common in the ART group (51.8% vs. 25.2%), and both ART and primigravidity were individually associated with differences in glycosylation patterns, we conducted a sensitivity analysis to disentangle potential overlapping effects. In this analysis, we stratified by mode of conception (spontaneous vs. IVF/ICSI), focusing on first pregnancies. Ovulation induction and intrauterine insemination were excluded due to small sample sizes and differing protocols. Details on baseline characteristics for this sensitivity analysis are depicted in Supplementary Table 5 and results of the linear regression models in Supplementary Table 6 and Supplementary Figure 4. In summary, the glycosylation differences observed in the TPL and HYT regions among all first pregnancies were only replicated within the IVF/ICSI group. In contrast, the increases in IgG4 and LAGY sialylation and galactosylation were no longer significant as main effects after stratification, possibly due to reduced statistical power. However, these changes remained significant as interactions with GA in the spontaneously conceived group (Figure S3).

Neonatal cord blood

Cord blood samples were available for 78 neonates. The median GA at delivery was 271 days (range 212-294). IgG and SES glycosylation traits could be measured and their mean values with 95% confidence intervals over the three trimesters and in cord blood are depicted in Figure 5 and Supplementary Table 4. For IgG glycosylation traits, both galactosylation and sialylation were significantly higher in CB compared to the third trimester in subtypes IgG2 and IgG4, and a similar trend was observed for subtypes IgG1. Only for IgG2, sialylation per galactose was significantly higher. For SES, galactosylation, sialylation, bisection, and fucosylation were all significantly lower in cord blood compared to maternal blood in the third trimester. In contrast, fucosylation of SES antennary glycans was higher. Other IgA glycosylation traits could not be detected in cord blood samples.

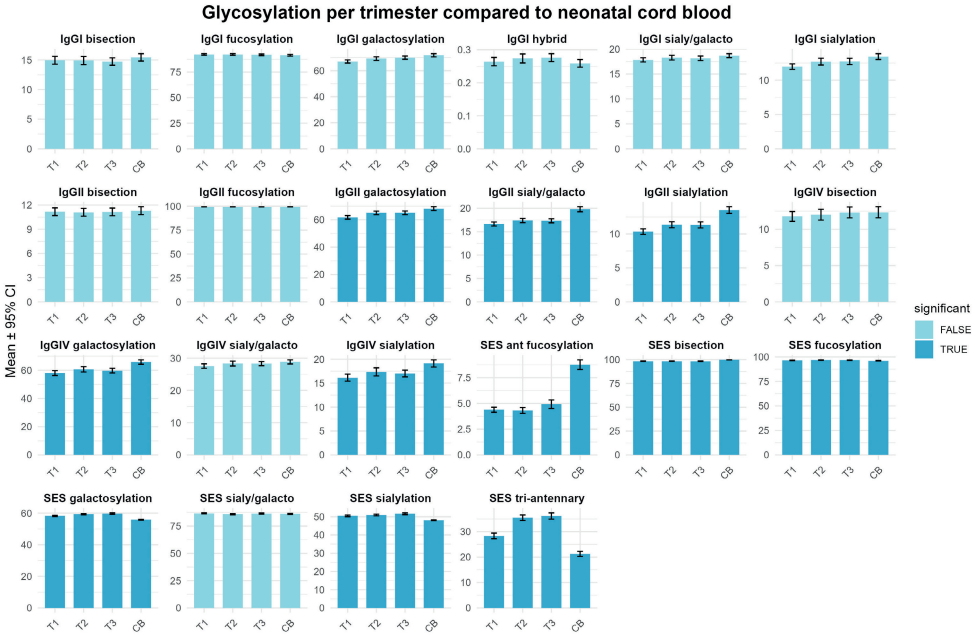


Figure 5. Means and 95% confidence intervals of glycosylation traits measured in neonatal cord blood after delivery (CB) compared to measurements in maternal blood in the first, second, and third trimester. CI = confidence interval; sialy/galacto = sialylation per galactose; SES ant fucosylation = SES antennary fucosylation.

Correlations with clinical outcomes

Among periconceptional factors, maternal smoking was weakly associated with increased sialylation and galactosylation of IgG1 and IgG4 in cord blood (r=0.22 and 0.29, respectively), but not with IgG2. Antibody glycosylation was further evaluated in relation to pregnancy outcomes. GA at birth was positively correlated with sialylation, galactosylation, and tri-antennary glycans in the SES region, likely reflecting increased post-translational modification of IgA, which is produced by the fetus mainly in the late third trimester. Some sex differences between male and female fetuses were observed, e.g., male fetal sex was weakly correlated with lower IgG4 bisection (r=0.21) and higher IgG1 hybrid glycans (r=0.25). Moreover, a higher birthweight percentile correlated with higher abundance of IgG2 sialylated and IgG1 hybrid glycans (r=0.23 and 0.24, respectively). All correlations between glycosylation patterns in cord blood and clinical parameters are depicted in Supplementary Figure 3.

DISCUSSION

Principle findings

In this cohort of 202 pregnant women, we observed that immunoglobulin glycosylation undergoes significant changes already within the first trimester. For IgG, galactosylation and sialylation increased throughout pregnancy. Bisection decreased between week 9 and 13 for IgG1 and IgG2, but then increased toward the third trimester in IgG1 and IgG4. For most IgA N-glycosylation sites (LSL, TPL, LAGY and LAGC), we observed a distinct switching point at the end of the first trimester. Between 9 and 13 weeks, galactosylation and sialylation tended to increase while bisection decreased, whereas from 13 to 32 weeks, these trends reversed. Pregnancies conceived through IVF/ICSI generally exhibited higher levels of galactosylation, sialylation, and fucosylation on both IgG and IgA glycosylation sites. Moreover, women pregnant for the first time displayed reduced galactosylation of the O-glycans (HYT), as well as increased sialylation in IgG4, TPL, and LAGY sites during the first trimester. Glycosylation traits in neonatal cord blood showed higher levels of IgG galactosylation and sialylation compared to maternal third trimester serum, and weak correlations with maternal smoking and birthweight.

Comparison to current understanding in the field

Our findings regarding the correlations of IgG and IgA glycosylation traits align with previous studies conducted outside of pregnancy [25, 27]. Consistent with Visconti et al. and Momcilovic et al., we observed the strongest correlations between IgG subtypes and the IgA LSL, LAGY, LAGC, and TPL regions, particularly for N-glycan traits such as galactosylation, sialylation, and bisection [25, 27]. Glycosylation traits within the HYT region, reflecting O-glycosylation, did not correlate with other IgA or IgG features, suggesting a distinct regulatory mechanism. Visconti *et al.* attributed these correlations primarily to shared genetic factors, particularly through enzymes like MGAT3 and ST6GAL1 [27]. In addition, the observed correlations may also reflect functional and structural parallels between immunoglobulin classes. In IgG molecules, the conserved N-glycosylation site at asparagine 297 (N297) is located on the heavy chain's Fc region and is important for maintaining structural integrity and modulating effector functions, such as binding to Fc gamma receptors and complement activation [12]. Similarly, the N-glycosylation sites LSL, LAGC, LAGY, and TPL regions are also located on the heavy chain Fc region of IgA molecules [6, 27]. What our study uniquely contributes is the demonstration that these correlation patterns are preserved during pregnancy. Moreover, despite changes in individual glycosylation traits over the course of pregnancy, the underlying correlation structure between traits was preserved across all trimesters, pointing towards preserved regulatory mechanisms.

Several studies have investigated changes in IgG N-glycosylation in pregnancy, and observed comparable patterns, including the increase of galactosylation and sialylation, and a decrease of bisection between the first and third trimester [16, 17]. Generally, an

increase of galactosylation and sialylation reflects an anti-inflammatory, tolerogenic environment [12, 13]. Previous research showed that it leads to more complement dependent cytotoxicity (CDC) outside of pregnancy [12]. Pregnancy is known to increase activity of the complement system [28], and this could be mediated by glycosylation changes of IgG1. Functionally, an increase in sialylation may reduce binding to activating Fcγ-receptors, thereby dampening the inflammatory response. Likewise, DC-SIGN has been suggested to convey anti-inflammatory properties of IgG sialylation [29]. Interestingly, we observed that bisecting GlcNAc levels changed dynamically throughout pregnancy, with a significant decrease during the first trimester followed by an increase after week 13. While bisection may to some extent influence immune effector functions, its mechanistic role in endogenous IgG remains poorly defined. In the production of therapeutic antibodies, bisecting GlcNAc can enhance antibody-dependent cellular cytotoxicity (ADCC) by interfering with core fucosylation, thereby increasing FcγRIIIa binding and promoting a pro-inflammatory response [13, 30, 31]. However, evidence for a similar inverse relationship between bisection and fucosylation in endogenous antibodies is limited, and this mechanism may not translate to physiological settings. Still, changes in bisection have been associated with autoimmune and inflammatory conditions, suggesting a broader immunomodulatory role [32]. Together, high galactosylation and sialylation together with low bisection in early pregnancy may thus contribute to maternal immune tolerance. In later gestation, the gradual rise in bisection may reflect immune recalibration, enhancing maternal defense against infections while preserving fetal tolerance [33]. This immune shift may also serve as preparation for labor, which is recognized as a coordinated inflammatory process involving cytokine signaling and immune cell activation [34].

Regarding IgA, however, the functional implications of its glycosylation remain underexplored, particularly in relation to effector functions. Similar to a previous smaller cohort study (n=29), we observed lowered IgA LAGY and LSL bisection levels in the first trimester, with an increase later in pregnancy. However, by zooming in on the first trimester, we observed that this is preceded by a decrease between 9-13 weeks of gestation, as in IgG1 bisection. Moreover, while we observed a similar increase for LAGC tri-antennary glycans between the first and third trimester, these decreased at the LAGY site. Another key difference was that no significant changes in fucosylation were observed at either site. These differences underscore the added value of our study in expanding the limited knowledge on IgA glycosylation during pregnancy, particularly by capturing dynamic changes already occurring within the first trimester. Furthermore, regarding IgA1 hinge region O-glycosylation we found a decrease of sialic acids per galactose. In combination with a decrease in Tn antigens and an increase of galactoses per GalNAc, these findings suggest a general shift from immature towards more mature, galactosylated structures over the course of pregnancy, thereby supporting the anti-inflammatory state.

Maternal and pregnancy characteristics

The absence of significant associations between maternal age and glycosylation changes may be explained by the narrow age range in our cohort (IQR 28–35 years). Previous studies have shown that IgG glycosylation tends to be more pro-inflammatory at younger and older age extremes, while remaining relatively stable around the early thirties [15].

Outside of pregnancy, higher BMI correlates with reduced IgG galactosylation, reflecting a more pro-inflammatory state [35]. We indeed observed lower galactosylation and sialylation in subtype IgG4, though not for IgG1 or IgG2. In contrast, a recent study found higher A2G2S1 levels in third-trimester IgG from obese women compared to those with normal weight but did not distinguish between IgG subtypes and within a smaller sample size [36]. Our observation of increased fucosylation for IgG1, but not for IgG2, in women with a higher BMI aligns with studies outside of pregnancy [37]. To date, the associations between BMI and IgA N- or O-glycosylation have not been extensively investigated, and no relevant literature on this topic has been identified.

In pregnancies conceived through ART, we observed a generalized increase in glycan complexity, particularly in galactosylation, sialylation, and fucosylation across multiple IgG and IgA sites, reflecting a more anti-inflammatory glycan profile. Differences between naturally conceived pregnancies and those achieved through assisted reproductive technologies may be influenced by confounding factors such as underlying comorbidities causing subfertility. Additionally, the hormonal therapies used in fertility treatments could impact glycosylation patterns, contributing to the observed variations. For example, estrogen has been identified as a modulator of IgG glycosylation. A previous study showed that estradiol promotes anti-inflammatory IgG glycosylation patterns, increasing galactosylation and sialylation while reducing bisection [38]. Fucosylation appeared unaffected by estradiol [38]. For IgA glycosylation, there is no current literature on the influence of estrogen or progesterone.

Women who were pregnant for the first time had lower levels of galactose and galactose per GalNAc, alongside higher levels of Tn antigens in O-glycosylated hinge region of IgA1 compared to those who had been pregnant before. A previous study by Bondt et al (2016) showed that there was a slight increase in galactosylation of this region that persisted postpartum, which would explain the lower galactosylation in women that have not been pregnant before [18]. However, in the sensitivity analysis stratified by conception mode, this association was only seen in IVF/ICSI pregnancies. This could potentially reflect the impact of assisted reproduction technologies or limited statistical power in the spontaneous pregnancy group. Additionally, we observed a more anti-inflammatory N-glycosylation profile in women who were pregnant for the first time, a finding not previously described.

Neonatal cord blood

Previous studies have primarily focused on IgG placental transfer, demonstrating that galactosylated and sialylated Fc glycoforms are preferentially enriched, possibly due to selective binding to the neonatal Fc receptor [39]. Consistent with these findings, our analysis revealed significantly higher levels of IgG galactosylation and sialylation in neonatal cord blood compared with maternal third-trimester serum. Beyond IgG, we identified a novel positive correlation between SES-region IgG sialylation and galactosylation and gestational age. Since IgA is not supposed to be transported across the placenta, these patterns likely reflect endogenous fetal IgA production and maturation, where longer gestation may allow for increased synthesis or more complex glycan processing. Notably, we were only able to quantify SES-region IgA modifications in cord blood, likely due to the low concentration of neonatal IgA. Lastly, while we observed initial associations between glycosylation and fetal sex or birthweight, these findings remain exploratory and should be investigated further in larger studies.

Strengths and limitations

A major strength of this study is that, to our knowledge, it is the first to investigate glycosylation profiles during the first trimester of pregnancy (9, 11 and 13 weeks GA), providing novel insights into early gestational immune modulation. Additionally, we uniquely compare IgG and IgA subtypes across different glycosylation regions, offering a more comprehensive view of antibody glycosylation. Our well-characterized periconceptional cohort, which includes detailed clinical parameters such as mode of conception and pregnancy outcomes, further enhances the clinical relevance of our findings. By adjusting for relevant covariates such as age and/or BMI, we aimed to estimate the independent associations between maternal characteristics and glycosylation changes. However, our study also has limitations. Maternal samples were collected at a maximum of 30 weeks gestation, while cord blood was measured at birth and only in a subset of participants, limiting direct longitudinal comparisons. Furthermore, the statistical power for subgroup analyses is limited. Another important limitation is the issue of multiple testing, given the large number of glycosylation traits and subgroup analyses performed. As a result, some associations may still reflect chance findings. However, we observed consistent and biologically plausible differences across subgroups such as mode of conception and gravidity, and ours is currently the largest pregnancy cohort to examine these associations.

Future perspectives

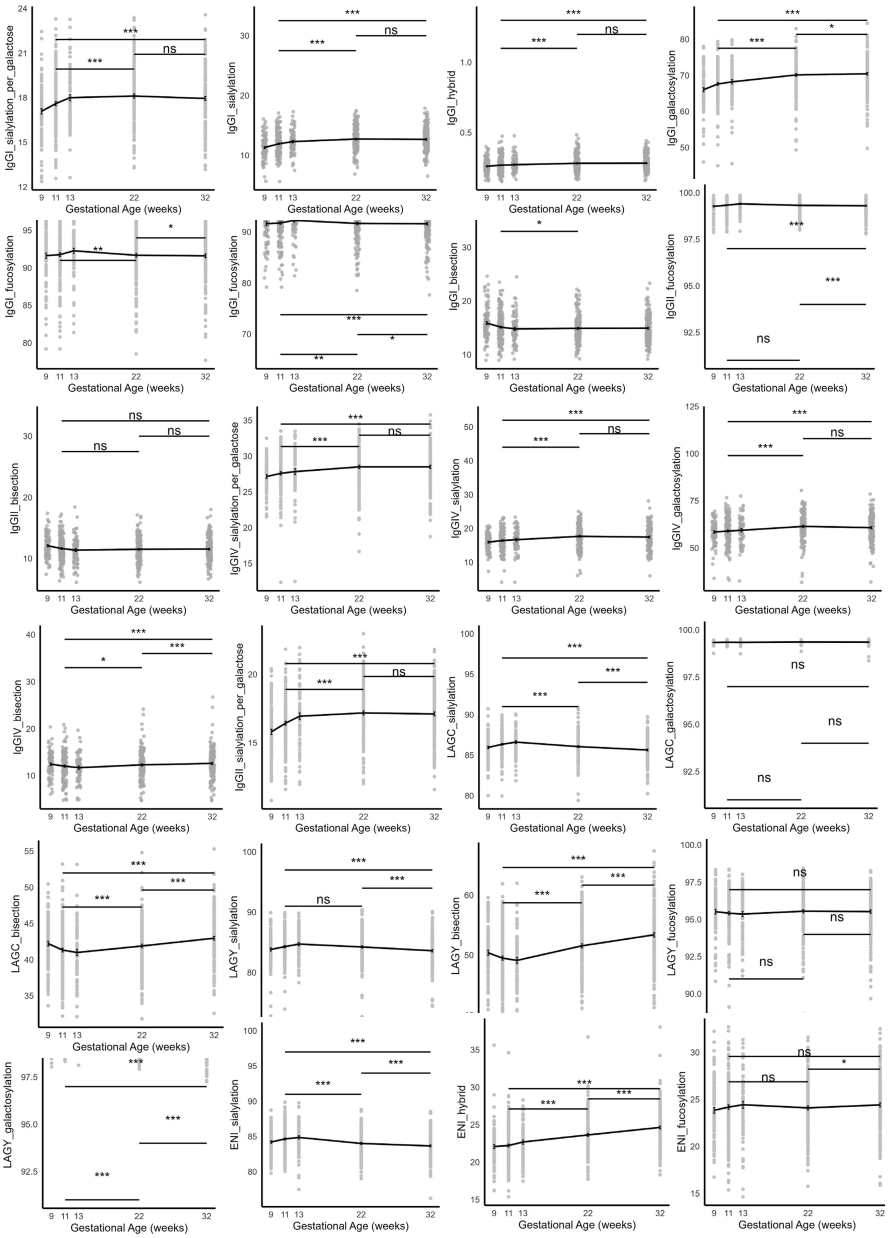
The periconception period is a critical window, and the observed early glycosylation shifts may hold promise as predictive biomarkers. Evidence from other fields supports this potential: in rheumatoid arthritis, reduced galactosylation and sialylation of IgG Fc glycans precede disease onset and correlate with activity and treatment response [40]. Similarly, in metabolic and cardiovascular disease, lower IgG galactosylation is associated with insulin resistance, hypertension, and increased cardiovascular risk [41]. Future studies could focus

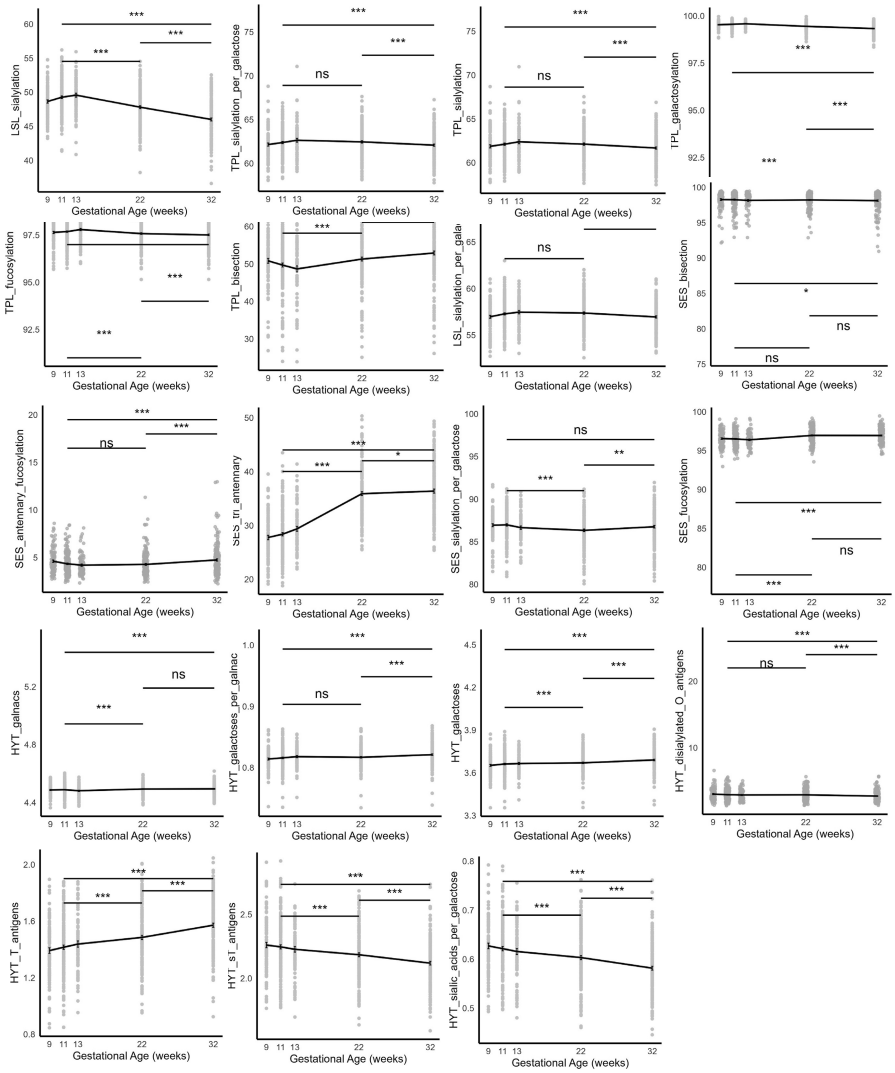
on associations of first trimester glycosylation changes and adverse pregnancy outcomes, and on first trimester placentation [9]. As we observed associations of pregnancy characteristics on glycosylation traits, mechanistic studies should account for the influence of these characteristics, including hormonal regulation, mode of conception, gravidity, and BMI. Lastly, the correlations observed for neonatal cord blood in a relatively small sample size warrant further investigation, ideally including matched maternal samples at the time of delivery to enable more accurate comparisons.

Conclusion

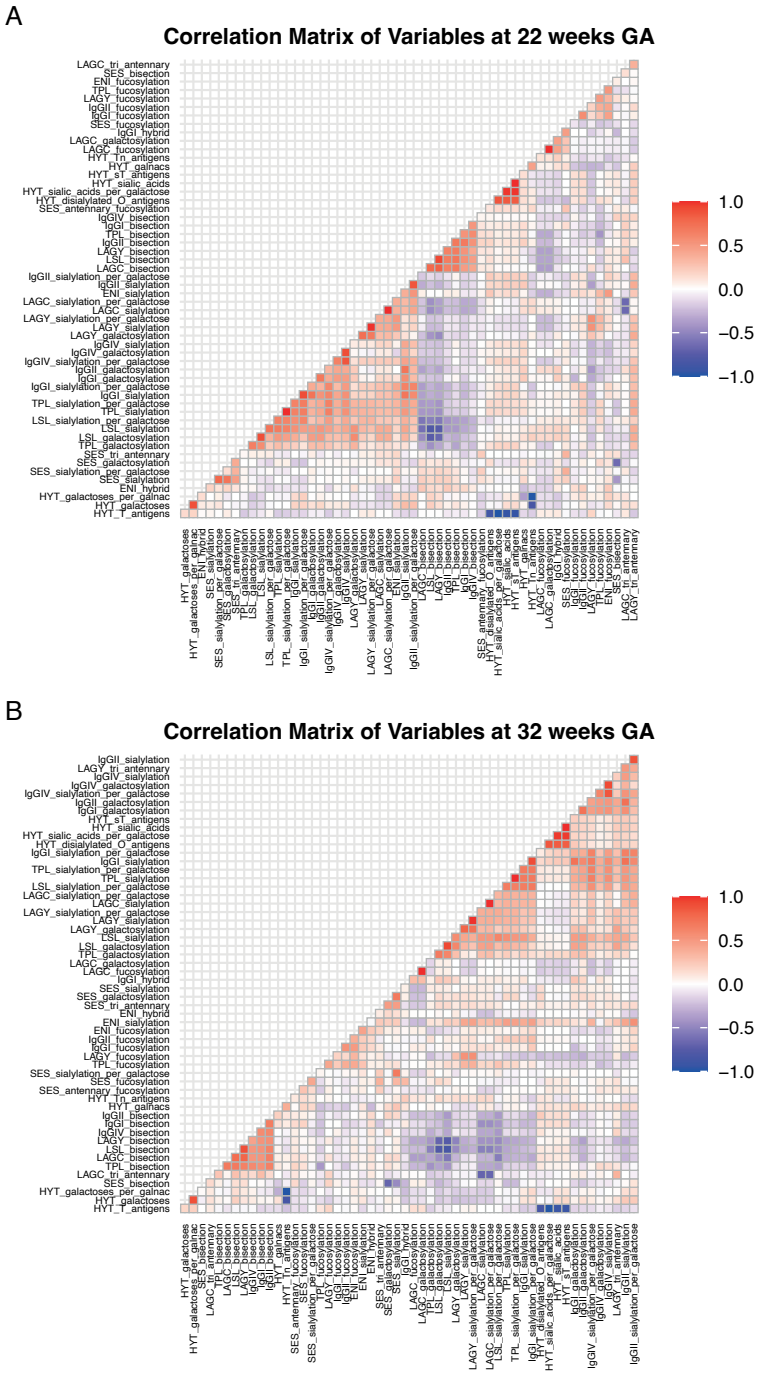
IgG and IgA glycosylation traits are generally correlated and shift toward a more anti-inflammatory profile over the course of the first trimester. Maternal BMI, mode of conception, and gravidity significantly influence glycosylation changes, highlighting the dynamic and adaptable nature of immunoglobulin glycosylation during pregnancy.

SUPPLEMENTARY MATERIAL

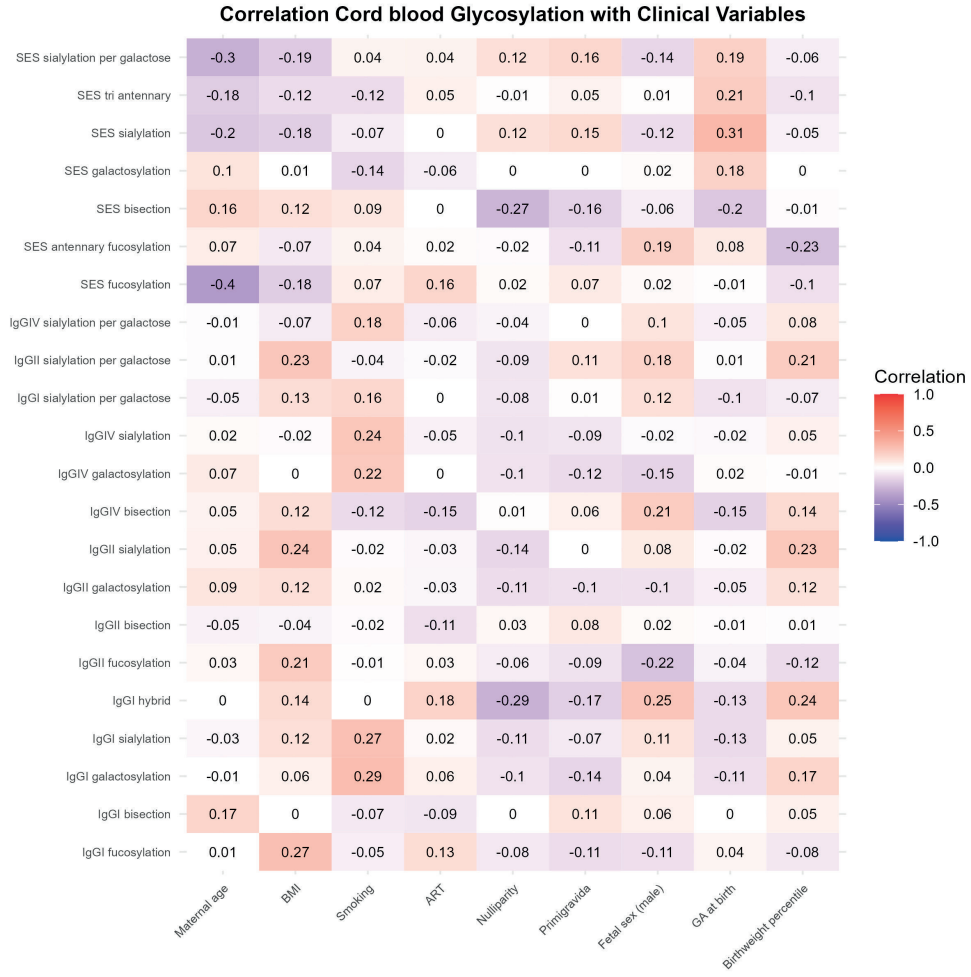




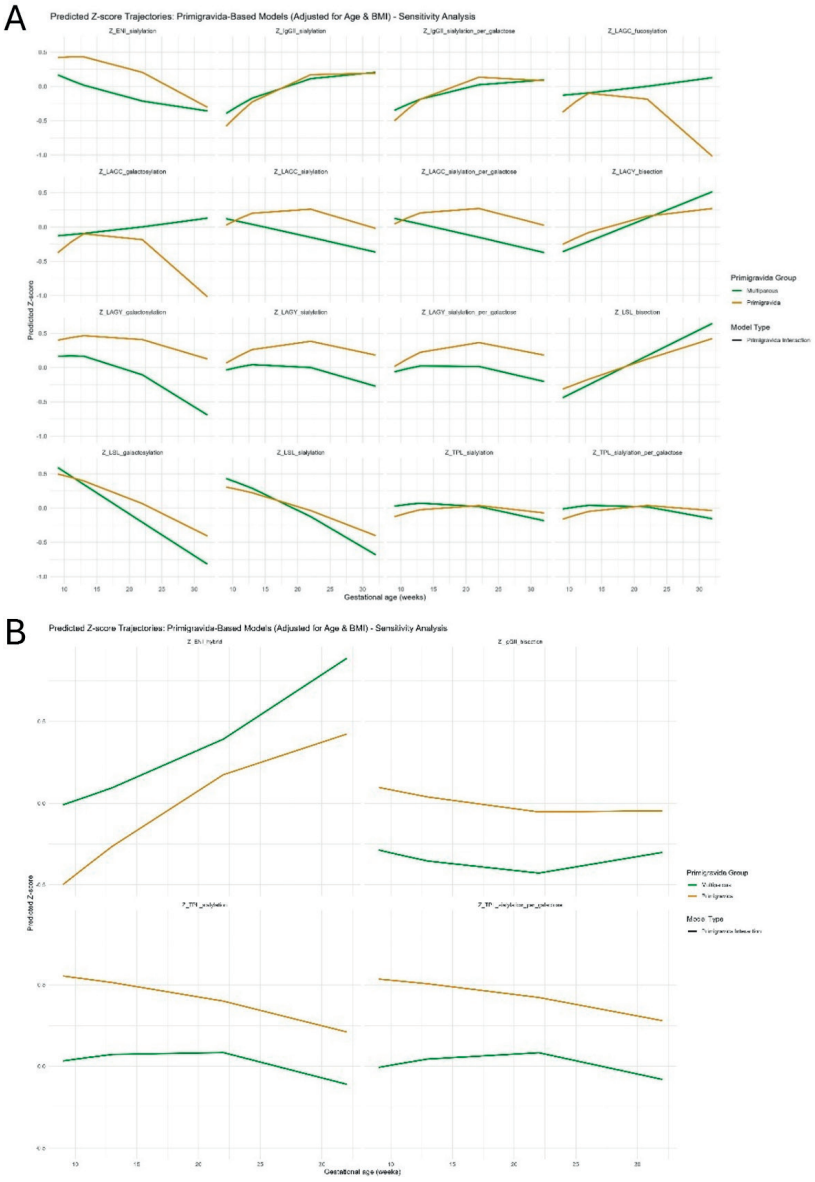
Supplementary Figure 1. Relative abundance of glycosylation traits over pregnancy.



Supplementary Figure 2. Correlation matrix of glycosylation traits measured at 22 weeks GA (A) and 32 weeks (B).



Supplementary Figure 3. Correlation matrix of measured glycosylation traits in neonatal cord blood with periconceptional and pregnancy characteristics.



Supplementary Figure 4 . Significant differences in glycosylation trajectories for spontaneous pregnancies (A) and IVF pregnancies (B) between primigravidae and multiparous women.

Supplementary Table 1. Derived glycosylation traits with corresponding formulas.

Trait	Formula
IgGI_fucosylation	(1 * (IgGI1H3N4F1 + IgGI1H4N4F1 + IgGI1H5N4F1 + IgGI1H4N4F1S1 + IgGI1H5N4F1S1 + IgGI1H5N4F1S2 + IgGI1H3N5F1 + IgGI1H4N5F1 + IgGI1H5N5F1 + IgGI1H5N5F1S1 + IgGI1H4N5F1S1 + IgGI1H5N5F1S1 + IgGI1H4N3F1)) / (IgGI1H3N4F1 + IgGI1H4N4F1 + IgGI1H5N4F1 + IgGI1H4N4F1S1 + IgGI1H5N4F1S1 + IgGI1H5N4F1S2 + IgGI1H3N5F1 + IgGI1H4N5F1 + IgGI1H5N5F1 + IgGI1H4N5F1S1 + IgGI1H5N5F1S1 + IgGI1H3N4 + IgGI1H4N4 + IgGI1H5N4 + IgGI1H5N4S1 + IgGI1H3N5 + IgGI1H4N5 + IgGI1H5N5 + IgGI1H5N5S1 + IgGI1H4N3F1) * 100
IgGI_bisection	(1 * (IgGI1H3N5F1 + IgGI1H4N5F1 + IgGI1H5N5F1 + IgGI1H4N5F1S1 + IgGI1H5N5F1S1 + IgGI1H3N5 + IgGI1H4N5 + IgGI1H5N5 + IgGI1H5N5S1)) / (IgGI1H3N4F1 + IgGI1H4N4F1 + IgGI1H5N4F1 + IgGI1H4N4F1S1 + IgGI1H5N4F1S1 + IgGI1H5N4F1S2 + IgGI1H3N5F1 + IgGI1H4N5F1 + IgGI1H5N5F1 + IgGI1H4N5F1S1 + IgGI1H5N5F1S1 + IgGI1H3N4 + IgGI1H4N4 + IgGI1H5N4 + IgGI1H5N4S1 + IgGI1H3N5 + IgGI1H4N5 + IgGI1H5N5 + IgGI1H5N5S1 + IgGI1H4N3F1) * 100
IgGI_galactosylation	(0.5 * (IgGI1H4N4F1 + IgGI1H4N4F1S1 + IgGI1H4N5F1 + IgGI1H4N5F1S1 + IgGI1H4N4 + IgGI1H4N5) + 1 * (IgGI1H5N4F1 + IgGI1H5N4F1S1 + IgGI1H5N4F1S2 + IgGI1H5N5F1 + IgGI1H5N5F1S1 + IgGI1H5N4 + IgGI1H5N4S1 + IgGI1H5N5 + IgGI1H5N5S1 + IgGI1H4N3F1)) / (IgGI1H3N4F1 + IgGI1H4N4F1 + IgGI1H5N4F1 + IgGI1H4N4F1S1 + IgGI1H5N4F1S1 + IgGI1H5N4F1S2 + IgGI1H3N5F1 + IgGI1H4N5F1 + IgGI1H5N5F1 + IgGI1H4N5F1S1 + IgGI1H5N5F1S1 + IgGI1H3N4 + IgGI1H4N4 + IgGI1H5N4 + IgGI1H5N4S1 + IgGI1H3N5 + IgGI1H4N5 + IgGI1H5N5 + IgGI1H5N5S1 + IgGI1H4N3F1) * 100
IgGI_sialylation	(0.5 * (IgGI1H4N4F1S1 + IgGI1H5N4F1S1 + IgGI1H4N5F1S1 + IgGI1H5N5F1S1 + IgGI1H5N4S1 + IgGI1H5N5S1) + 1 * IgGI1H5N4F1S2) / (IgGI1H3N4F1 + IgGI1H4N4F1 + IgGI1H5N4F1 + IgGI1H4N4F1S1 + IgGI1H5N4F1S1 + IgGI1H5N4F1S2 + IgGI1H3N5F1 + IgGI1H4N5F1 + IgGI1H5N5F1 + IgGI1H4N5F1S1 + IgGI1H5N5F1S1 + IgGI1H3N4 + IgGI1H4N4 + IgGI1H5N4 + IgGI1H5N4S1 + IgGI1H3N5 + IgGI1H4N5 + IgGI1H5N5 + IgGI1H5N5S1 + IgGI1H4N3F1) * 100
IgGI_hybrid	1 * (IgGI1H6N3F1S1 + IgGI1H6N4F1)
IgGII_fucosylation	1 * (IgGII1H3N4F1 + IgGII1H4N4F1 + IgGII1H5N4F1 + IgGII1H4N4F1S1 + IgGII1H5N4F1S1 + IgGII1H3N5F1 + IgGII1H4N5F1 + IgGII1H5N5F1 + IgGII1H5N5F1S1)
IgGII_bisection	1 * (IgGII1H3N5F1 + IgGII1H4N5F1 + IgGII1H5N5F1 + IgGII1H5N5F1S1)
IgGII_galactosylation	0.5 * (IgGII1H4N4F1 + IgGII1H4N4F1S1 + IgGII1H4N5F1 + IgGII1H4N4) + 1 * (IgGII1H5N4F1 + IgGII1H5N4F1S1 + IgGII1H5N5F1 + IgGII1H5N5F1S1)
IgGII_sialylation	0.5 * (IgGII1H4N4F1S1 + IgGII1H5N4F1S1 + IgGII1H5N5F1S1)
IgGIV_bisection	1 * (IgGIV1H3N5F1 + IgGIV1H4N5F1 + IgGIV1H5N5F1)
IgGIV_galactosylation	0.5 * (IgGIV1H4N4F1 + IgGIV1H4N4F1S1 + IgGIV1H4N5F1) + 1 * (IgGIV1H5N4F1 + IgGIV1H5N4F1S1 + IgGIV1H5N5F1)
IgGIV_sialylation	0.5 * (IgGIV1H4N4F1S1 + IgGIV1H5N4F1S1)
IgGI sialylation per galactose	IgGI_sialylation / IgGI_galactosylation * 100
IgGII sialylation per galactose	IgGII_sialylation / IgGII_galactosylation * 100

Supplementary Table 1. Derived glycosylation traits with corresponding formulas. (continued)

Trait	Formula
IgGIV sialylation per galactose	IgGIV_sialylation / IgGIV_galactosylation * 100
SES_fucosylation	1 * (SES1H4N5F1 + SES1H4N5F1S1 + SES1H4N5F2 + SES1H4N5F2S1 + SES1H5N4F1S2 + SES1H5N5F1S1 + SES1H5N5F1S2 + SES1H5N6F1 + SES-1H5N6F2 + SES1H5N6F1S1 + SES1H5N6F1S2 + SES1H5N6F2S2)
SES_antennary_fucosylation	1 * (SES1H4N5F2 + SES1H4N5F2S1 + SES1H5N6F2 + SES1H5N6F2S2)
SES_bisection	1 * (SES1H4N5F1 + SES1H4N5F1S1 + SES1H4N5F2 + SES1H4N5F2S1 + SES1H5N5S1 + SES1H5N5F1S1 + SES1H5N5F1S2 + SES1H5N6F1 + SES-1H5N6F2 + SES1H5N6F1S1 + SES1H5N6F1S2 + SES1H5N6F2S2)
SES_galactosylation	0.5 * (SES1H4N5F1 + SES1H4N5F1S1 + SES1H4N5F2 + SES1H4N5F2S1) + 1 * (SES1H5N4F1S2 + SES1H5N5S1 + SES1H5N5F1S1 + SES1H5N5F1S2) + 0.667 * (SES1H5N6F1 + SES1H5N6F2 + SES1H5N6F1S1 + SES1H5N6F1S2 + SES1H5N6F2S2)
SES_sialylation	0.5 * (SES1H4N5F1S1 + SES1H4N5F2S1 + SES1H5N5S1 + SES1H5N5F1S1) + 1 * (SES1H5N4F1S2 + SES1H5N5F1S2) + 0.333 * SES1H5N6F1S1 + 0.667 * (SES1H5N6F1S2 + SES1H5N6F2S2)
SES_tri_antennary	1 * (SES1H5N6F1 + SES1H5N6F2 + SES1H5N6F1S1 + SES1H5N6F1S2 + SES1H5N6F2S2)
SES_sialylation_per_galactose	SES_sialylation / SES_galactosylation * 100
LSL_bisection	1 * (LSL1H3N5 + LSL1H4N5 + LSL1H4N5S1 + LSL1H5N5S1)
LSL_galactosylation	0.5 * (LSL1H4N5 + LSL1H4N5S1) + 1 * (LSL1H5N4S1 + LSL1H5N4S2 + LSL1H5N5S1)
LSL_sialylation	0.5 * (LSL1H4N5S1 + LSL1H5N4S1 + LSL1H5N5S1) + 1 * LSL1H5N4S2
LSL_sialylation_per_galactose	LSL_sialylation / LSL_galactosylation * 100
TPL_fucosylation	1 * (TPL1H4N5F1S1 + TPL1H5N4F1S1 + TPL1H5N4F1S2 + TPL1H5N5F1S1 + TPL1H5N5F1S2 + TPL1H6N5F1S1)
TPL_bisection	1 * (TPL1H4N5F1S1 + TPL1H5N5S1 + TPL1H5N5F1S1 + TPL1H5N5F1S2)
TPL_galactosylation	0.5 * TPL1H4N5F1S1 + 1 * (TPL1H5N4F1S1 + TPL1H5N4F1S2 + TPL-1H5N5S1 + TPL1H5N5F1S1 + TPL1H5N5F1S2 + TPL1H6N5F1S1)
TPL_sialylation	0.5 * (TPL1H4N5F1S1 + TPL1H5N4F1S1 + TPL1H5N5S1 + TPL1H5N5F1S1) + 1 * (TPL1H5N4F1S2 + TPL1H5N5F1S2) + 0.333 * TPL1H6N5F1S1
TPL_sialylation_per_galactose	TPL_sialylation / TPL_galactosylation * 100
HYT_sialic_acids	(2 * (HYT1H2N3S2 + HYT1H2N4S2 + HYT1H3N3S2 + HYT1H3N4S2 + HYT1H3N5S2 + HYT1H3N6S2 + HYT1H4N4S2 + HYT1H4N5S2 + HYT1H4N6S2 + HYT1H5N5S2 + HYT1H5N6S2) + 1 * (HYT1H2N5S1 + HYT1H3N3S1 + HYT1H3N4S1 + HYT1H3N5S1 + HYT1H3N6S1 + HYT1H4N4S1 + HYT1H4N5S1 + HYT1H5N5S1) + 3 * (HYT1H3N3S3 + HYT1H3N4S3 + HYT1H3N5S3 + HYT1H3N6S3 + HYT1H4N4S3 + HYT1H4N5S3 + HYT1H4N6S3 + HYT1H5N5S3 + HYT1H5N6S3) + 4 * (HYT1H3N3S4 + HYT1H3N4S4 + HYT1H4N4S4 + HYT1H4N5S4 + HYT1H5N5S4 + HYT1H3N5S4) + 5 * HYT1H4N4S5) / 100

Supplementary Table 1. Derived glycosylation traits with corresponding formulas. (continued)

Trait	Formula
HYT_galactoses	(2 * (HYT1H2N3S2 + HYT1H2N4S2 + HYT1H2N5S1) + 3 * (HYT1H3N3S1 + HYT1H3N3S2 + HYT1H3N3S3 + HYT1H3N3S4 + HYT1H3N4 + HYT1H3N4S1 + HYT1H3N4S2 + HYT1H3N4S3 + HYT1H3N4S4 + HYT1H3N5 + HYT1H3N5S1 + HYT1H3N5S2 + HYT1H3N5S3 + HYT1H3N6S1 + HYT1H3N6S2 + HYT1H3N6S3 + HYT1H3N5S4) + 4 * (HYT1H4N4 + HYT1H4N4S1 + HYT1H4N4S2 + HYT1H4N4S3 + HYT1H4N4S4 + HYT1H4N4S5 + HYT1H4N5 + HYT1H4N5S1 + HYT1H4N5S2 + HYT1H4N5S3 + HYT1H4N5S4 + HYT1H4N6S2 + HYT1H4N6S3 + HYT1H4N6) + 5 * (HYT1H5N5 + HYT1H5N5S1 + HYT1H5N5S2 + HYT1H5N5S3 + HYT1H5N5S4 + HYT1H5N6S3 + HYT1H5N6S2)) / 100
HYT_galnacs	(3 * (HYT1H2N3S2 + HYT1H3N3S1 + HYT1H3N3S2 + HYT1H3N3S3 + HYT1H3N3S4) + 4 * (HYT1H2N4S2 + HYT1H3N4 + HYT1H3N4S1 + HYT1H3N4S2 + HYT1H3N4S3 + HYT1H3N4S4 + HYT1H4N4 + HYT1H4N4S1 + HYT1H4N4S2 + HYT1H4N4S3 + HYT1H4N4S4 + HYT1H4N4S5) + 5 * (HYT1H2N5S1 + HYT1H3N5 + HYT1H3N5S1 + HYT1H3N5S2 + HYT1H3N5S3 + HYT1H4N5 + HYT1H4N5S1 + HYT1H4N5S2 + HYT1H4N5S3 + HYT1H4N5S4 + HYT1H5N5 + HYT1H5N5S1 + HYT1H5N5S2 + HYT1H5N5S3 + HYT1H5N5S4 + HYT1H3N5S4) + 6 * (HYT1H3N6S1 + HYT1H3N6S2 + HYT1H3N6S3 + HYT1H4N6S2 + HYT1H4N6S3 + HYT1H5N6S3 + HYT1H4N6 + HYT1H5N6S2)) / 100
HYT_Tn_antigens	(1 * (HYT1H2N3S2 + HYT1H3N4 + HYT1H3N4S1 + HYT1H3N4S2 + HYT1H3N4S3 + HYT1H3N4S4 + HYT1H4N5 + HYT1H4N5S1 + HYT1H4N5S2 + HYT1H4N5S3 + HYT1H4N5S4 + HYT1H5N6S3 + HYT1H5N6S2) + 2 * (HYT1H2N4S2 + HYT1H3N5 + HYT1H3N5S1 + HYT1H3N5S2 + HYT1H3N5S3 + HYT1H4N6S2 + HYT1H4N6S3 + HYT1H4N6 + HYT1H3N5S4) + 3 * (HYT1H2N5S1 + HYT1H3N6S1 + HYT1H3N6S2 + HYT1H3N6S3)) / 100
HYT_T_antigens	(1 * (HYT1H2N5S1 + HYT1H3N3S2 + HYT1H3N4S2 + HYT1H3N5S2 + HYT1H3N6S2 + HYT1H4N4S3 + HYT1H4N5S3 + HYT1H4N6S3 + HYT1H5N5S4) + 2 * (HYT1H3N3S1 + HYT1H3N4S1 + HYT1H3N5S1 + HYT1H3N6S1 + HYT1H4N4S2 + HYT1H4N5S2 + HYT1H4N6S2 + HYT1H5N5S3 + HYT1H5N6S3) + 3 * (HYT1H3N4 + HYT1H3N5 + HYT1H4N4S1 + HYT1H4N5S1 + HYT1H5N5S2 + HYT1H5N6S2) + 4 * (HYT1H4N4 + HYT1H4N5 + HYT1H5N5S1 + HYT1H4N6) + 5 * HYT1H5N5) / 100
HYT_sT_antigens	(2 * (HYT1H2N3S2 + HYT1H2N4S2 + HYT1H3N3S2 + HYT1H3N4S2 + HYT1H3N5S2 + HYT1H3N6S2 + HYT1H4N4S2 + HYT1H4N5S2 + HYT1H4N6S2 + HYT1H5N5S2 + HYT1H5N6S2) + 1 * (HYT1H2N5S1 + HYT1H3N3S1 + HYT1H3N4S1 + HYT1H3N5S1 + HYT1H3N6S1 + HYT1H4N4S1 + HYT1H4N5S1 + HYT1H5N5S1) + 3 * (HYT1H3N3S3 + HYT1H3N3S4 + HYT1H3N4S3 + HYT1H3N4S4 + HYT1H3N5S3 + HYT1H3N6S3 + HYT1H4N4S3 + HYT1H4N5S3 + HYT1H4N6S3 + HYT1H5N5S3 + HYT1H5N6S3 + HYT1H3N5S4) + 4 * (HYT1H4N4S4 + HYT1H4N4S5 + HYT1H4N5S4 + HYT1H5N5S4)) / 100
HYT_disialylated_O_antigens	1 * (HYT1H3N3S4 + HYT1H3N4S4 + HYT1H4N4S5 + HYT1H3N5S4)
HYT_sialic_acids_per_galactose	HYT_sialic_acids / HYT_galactoses
HYT_galactoses_per_galnac	HYT_galactoses / HYT_galnacs

Supplementary Table 1. Derived glycosylation traits with corresponding formulas. (continued)

Trait	Formula
ENI_fucosylation	(1 * (ENI1H5N4F1S1 + ENI1H5N4F1S2)) / (ENI1H5N4F1S1 + ENI1H5N4F1S2 + ENI1H5N4S1 + ENI1H5N4S2 + ENI1H5N5S1) * 100
ENI_sialylation	(0.5 * (ENI1H5N4F1S1 + ENI1H5N4S1 + ENI1H5N5S1) + 1 * (ENI1H5N4F1S2 + ENI1H5N4S2)) / (ENI1H5N4F1S1 + ENI1H5N4F1S2 + ENI1H5N4S1 + ENI-1H5N4S2 + ENI1H5N5S1) * 100
ENI_hybrid	1 * (ENI1H5N3F1S1 + ENI1H5N3S1)
LAGC_fucosylation	1 * (LAGC1H5N4F1S1 + LAGC1H5N4F1S2 + LAGC1H5N5F1S1 + LAGC1H-5N5F1S2 + LAGC1H6N5F1S1 + LAGC1H6N5F1S2 + LAGC1H6N5F1S3 + LAGC1H6N6F1S2 + LAGC1H5N4F1S2Su1 + LAGC1H6N5F1S1Su1)
LAGC_bisection	1 * (LAGC1H5N5F1S1 + LAGC1H5N5F1S2 + LAGC1H5N6S2 + LAGC1H-6N6F1S2)
LAGC_galactosylation	1 * (LAGC1H5N4F1S1 + LAGC1H5N4F1S2 + LAGC1H5N5F1S1 + LAGC1H-5N5F1S2 + LAGC1H6N5F1S1 + LAGC1H6N5F1S2 + LAGC1H6N5F1S3 + LAGC1H6N6F1S2 + LAGC1H5N4F1S2Su1 + LAGC1H6N5F1S1Su1) + 0.667 * LAGC1H5N6S2
LAGC_sialylation	0.5 * (LAGC1H5N4F1S1 + LAGC1H5N5F1S1) + 1 * (LAGC1H5N4F1S2 + LAGC1H5N5F1S2 + LAGC1H6N5F1S3 + LAGC1H5N4F1S2Su1) + 0.333 * (LAGC1H6N5F1S1 + LAGC1H6N5F1S1Su1) + 0.667 * (LAGC1H6N5F1S2 + LAGC1H5N6S2 + LAGC1H6N6F1S2)
LAGC_tri_antennary	1 * (LAGC1H6N5F1S1 + LAGC1H6N5F1S2 + LAGC1H6N5F1S3 + LAGC1H5N6S2 + LAGC1H6N6F1S2 + LAGC1H6N5F1S1Su1)
LAGY_fucosylation	(1 * (LAGY1H4N5F1 + LAGY1H5N4F1S1 + LAGY1H5N4F1S2 + LAGY1H-5N5F1 + LAGY1H5N5F1S1 + LAGY1H5N5F1S2 + LAGY1H6N5F1S1 + LAGY1H6N5F1S2 + LAGY1H5N4F1S2Su1 + LAGY1H5N5F1S2Su1 + LAGY1H6N5F1S2Su1 + LAGY1H6N6F1S2Su1)) / (LAGY1H4N5F1 + LAGY1H5N4S1 + LAGY1H5N4S2 + LAGY1H5N4F1S1 + LAGY1H5N4F1S2 + LAGY1H5N5F1 + LAGY1H5N5F1S1 + LAGY1H5N5F1S2 + LAGY1H6N5F1S1 + LAGY1H6N5F1S2 + LAGY1H5N4F1S2Su1 + LAGY1H5N5F1S2Su1 + LAGY1H6N5F1S2Su1 + LAGY1H6N6F1S2Su1) * 100
LAGY_bisection	(1 * (LAGY1H4N5F1 + LAGY1H5N5F1 + LAGY1H5N5F1S1 + LAGY1H-5N5F1S2 + LAGY1H5N5F1S2Su1 + LAGY1H6N6F1S2Su1)) / (LAGY1H4N5F1 + LAGY1H5N4S1 + LAGY1H5N4S2 + LAGY1H5N4F1S1 + LAGY1H5N4F1S2 + LAGY1H5N5F1 + LAGY1H5N5F1S1 + LAGY1H5N5F1S2 + LAGY1H-6N5F1S1 + LAGY1H6N5F1S2 + LAGY1H5N4F1S2Su1 + LAGY1H5N5F1S-2Su1 + LAGY1H6N5F1S2Su1 + LAGY1H6N6F1S2Su1) * 100
LAGY_galactosylation	(0.5 * LAGY1H4N5F1 + 1 * (LAGY1H5N4S1 + LAGY1H5N4S2 + LAGY1H-5N4F1S1 + LAGY1H5N4F1S2 + LAGY1H5N5F1 + LAGY1H5N5F1S1 + LAGY1H5N5F1S2 + LAGY1H6N5F1S1 + LAGY1H6N5F1S2 + LAGY1H-5N4F1S2Su1 + LAGY1H5N5F1S2Su1 + LAGY1H6N5F1S2Su1 + LAGY1H-6N6F1S2Su1)) / (LAGY1H4N5F1 + LAGY1H5N4S1 + LAGY1H5N4S2 + LAGY1H5N4F1S1 + LAGY1H5N4F1S2 + LAGY1H5N5F1 + LAGY1H5N5F1S1 + LAGY1H5N5F1S2 + LAGY1H6N5F1S1 + LAGY1H6N5F1S2 + LAGY1H-5N4F1S2Su1 + LAGY1H5N5F1S2Su1 + LAGY1H6N5F1S2Su1 + LAGY1H-6N6F1S2Su1) * 100

Supplementary Table 1. Derived glycosylation traits with corresponding formulas. (continued)

Trait	Formula
LAGY_sialylation	(0.5 * (LAGY1H5N4S1 + LAGY1H5N4F1S1 + LAGY1H5N5F1S1) + 1 * (LAGY1H5N4S2 + LAGY1H5N4F1S2 + LAGY1H5N5F1S2 + LAGY1H-5N4F1S2Su1 + LAGY1H5N5F1S2Su1) + 0.333 * LAGY1H6N5F1S1 + 0.667 * (LAGY1H6N5F1S2 + LAGY1H6N5F1S2Su1 + LAGY1H6N6F1S2Su1)) / (LAGY1H4N5F1 + LAGY1H5N4S1 + LAGY1H5N4S2 + LAGY1H5N4F1S1 + LAGY1H5N4F1S2 + LAGY1H5N5F1 + LAGY1H5N5F1S1 + LAGY1H5N5F1S2 + LAGY1H6N5F1S1 + LAGY1H6N5F1S2 + LAGY1H5N4F1S2Su1 + LAGY1H-5N5F1S2Su1 + LAGY1H6N5F1S2Su1 + LAGY1H6N6F1S2Su1) * 100
LAGY_tri_antennary	(1 * (LAGY1H6N5F1S1 + LAGY1H6N5F1S2 + LAGY1H6N5F1S2Su1 + LAGY1H6N6F1S2Su1)) / (LAGY1H4N5F1 + LAGY1H5N4S1 + LAGY1H5N4S2 + LAGY1H5N4F1S1 + LAGY1H5N4F1S2 + LAGY1H5N5F1 + LAGY1H-5N5F1S1 + LAGY1H5N5F1S2 + LAGY1H6N5F1S1 + LAGY1H6N5F1S2 + LAGY1H5N4F1S2Su1 + LAGY1H5N5F1S2Su1 + LAGY1H6N5F1S2Su1 + LAGY1H6N6F1S2Su1) * 100
LAGC sialylation per galactose	LAGC_sialylation / LAGC_galactosylation * 100
LAGY sialylation per galactose	LAGY_sialylation / LAGY_galactosylation * 100

Supplementary Table 2. Overview of IgA glycopeptide clusters, corresponding IgA subclasses, and glycosylation sites indicated by residue number according to the IgA1 or IgA2 heavy chain sequence [18, 42].

Cluster	IgA subtype	Glycosylation site
SES	IgA2	N47
LSL	IgA1/IgA2	N144/N131
TPL	IgA2	N205
LAGC	IgA1/IgA2	N327/N340
LAGY	IgA1/IgA2	N327/N340
HYT	IgA1	T106/T109/S111/S113/T114/T117 (O-glycans)
ENI		Joining Chain N71

Supplementary Table 3. Mean and Standard Deviation (SD) of Measured Glycosylation Traits Across Pregnancy. The 'Significant' column indicates whether any of the predefined pairwise comparisons between time points were statistically significant (FDR-adjusted $p < 0.05$), based on linear mixed-effects models with a random intercept for each subject: (a) 11–22 weeks, (b) 11–32 weeks, (c) 22–32 weeks, (d) 9–11 weeks, and (e) 9–13 weeks.

Variable	9	11	13	22	32	Significant
N	108	192	86	187	78	
IgGI_fucosylation	91.62 (3.65)	91.76 (3.45)	92.28 (3.11)	91.68 (3.48)	91.60 (3.42)	a,b,c
IgGI_bisection	15.88 (2.86)	15.13 (2.58)	14.79 (2.63)	14.89 (2.51)	14.93 (2.36)	a,d,e
IgGI_galactosylation	66.02 (5.80)	67.62 (4.95)	68.21 (5.38)	70.11 (5.14)	70.45 (5.04)	a,b,c,d,e
IgGI_sialylation	11.32 (1.84)	11.94 (1.78)	12.31 (1.83)	12.74 (1.91)	12.69 (1.91)	a,b,d,e
IgGI_hybrid	0.26 (0.05)	0.27 (0.06)	0.27 (0.05)	0.28 (0.06)	0.28 (0.05)	a,b
IgGII_fucosylation	99.27 (0.39)	99.34 (0.35)	99.41 (0.32)	99.32 (0.36)	99.31 (0.37)	b, c
IgGII_bisection	12.09 (1.84)	11.61 (1.94)	11.37 (2.09)	11.51 (1.89)	11.54 (1.90)	
IgGII_galactosylation	60.71 (4.50)	62.38 (4.99)	63.56 (5.23)	65.43 (4.96)	65.68 (5.14)	a,b,c,d,e
IgGII_sialylation	9.61 (1.55)	10.28 (1.71)	10.82 (1.93)	11.28 (1.82)	11.28 (1.86)	a,b,d,e
IgGIV_bisection	12.44 (2.59)	12.04 (2.87)	11.70 (2.92)	12.31 (3.02)	12.61 (3.12)	a,b,c
IgGIV_galactosylation	58.47 (6.24)	58.97 (7.06)	59.39 (7.29)	61.43 (7.31)	60.77 (7.06)	a,b,d,e
IgGIV_sialylation	15.96 (2.64)	16.40 (3.12)	16.67 (3.27)	17.65 (3.42)	17.45 (3.33)	a,b,d,e
IgGI_sialylation_per_galactose	17.09 (1.89)	17.61 (1.86)	18.01 (1.87)	18.12 (1.93)	17.95 (1.92)	a,b,e
IgGII_sialylation_per_galactose	15.80 (1.98)	16.43 (1.95)	16.94 (2.12)	17.18 (2.02)	17.11 (1.97)	a,b,d,e
IgGIV_sialylation_per_galactose	27.16 (2.29)	27.60 (2.87)	27.84 (3.36)	28.50 (2.99)	28.51 (2.87)	a,b
SES_fucosylation	96.57 (1.00)	96.51 (0.92)	96.40 (0.94)	96.97 (0.96)	96.95 (0.89)	a,b
SES_antennary_fucosylation	4.61 (1.27)	4.34 (1.11)	4.20 (1.12)	4.27 (1.26)	4.74 (1.59)	b,c,d,e
SES_bisection	98.28 (1.32)	98.26 (1.16)	98.16 (1.19)	98.22 (1.04)	98.13 (1.33)	b
SES_galactosylation	58.34 (1.73)	58.46 (1.63)	58.78 (1.58)	59.59 (1.48)	59.98 (1.88)	a,b,c,e
SES_sialylation	50.72 (1.78)	50.85 (1.79)	50.94 (1.82)	51.45 (1.85)	52.04 (2.05)	a,b,c
SES_tri_antennary	27.77 (4.27)	28.38 (4.36)	29.37 (4.14)	35.90 (4.92)	36.39 (5.21)	a,b,c,d,e
SES_sialylation_per_galactose	86.94 (1.85)	86.99 (1.86)	86.66 (1.85)	86.33 (2.07)	86.76 (2.08)	a,c,e
LSL_bisection	44.48 (5.80)	43.49 (5.94)	43.09 (5.89)	46.79 (5.94)	49.90 (6.16)	a,b,c
LSL_galactosylation	85.37 (2.99)	85.99 (3.03)	86.26 (3.13)	83.31 (3.72)	80.76 (4.25)	a,b,c
LSL_sialylation	48.65 (2.53)	49.28 (2.65)	49.59 (2.68)	47.82 (2.92)	46.02 (3.05)	a,b,c
LSL_sialylation_per_galactose	56.97 (1.70)	57.29 (1.66)	57.47 (1.67)	57.38 (1.69)	56.95 (1.56)	b,c
TPL_fucosylation	97.65 (0.61)	97.69 (0.56)	97.80 (0.54)	97.58 (0.61)	97.51 (0.60)	a,b,c
TPL_bisection	50.83 (6.75)	49.71 (7.38)	48.68 (7.59)	51.31 (7.08)	52.93 (7.20)	a,b,c
TPL_galactosylation	99.54 (0.18)	99.56 (0.17)	99.59 (0.16)	99.45 (0.22)	99.33 (0.27)	a,b,c
TPL_sialylation	61.87 (1.92)	62.12 (1.82)	62.39 (2.04)	62.12 (1.80)	61.68 (1.79)	b,c,e

Supplementary Table 3. Mean and Standard Deviation (SD) of Measured Glycosylation Traits Across Pregnancy. The 'Significant' column indicates whether any of the predefined pairwise comparisons between time points were statistically significant (FDR-adjusted $p < 0.05$), based on linear mixed-effects models with a random intercept for each subject: (a) 11–22 weeks, (b) 11–32 weeks, (c) 22–32 weeks, (d) 9–11 weeks, and (e) 9–13 weeks. (continued)

Variable	9	11	13	22	32	Signifi-
N	108	192	86	187	78	cant
TPL_sialylation_per_galactose	62.16 (1.91)	62.39 (1.80)	62.65 (2.03)	62.47 (1.78)	62.09 (1.78)	b,c,e
HYT_sialic_acids	2.29 (0.23)	2.28 (0.23)	2.26 (0.21)	2.21 (0.22)	2.15 (0.21)	a,b,c
HYT_galactoses	3.65 (0.08)	3.66 (0.08)	3.67 (0.07)	3.67 (0.07)	3.69 (0.08)	a,b,c
HYT_galnacs	4.49 (0.04)	4.49 (0.04)	4.48 (0.04)	4.49 (0.04)	4.49 (0.04)	a,b
HYT_Tn_antigens	0.83 (0.08)	0.83 (0.08)	0.82 (0.08)	0.82 (0.08)	0.80 (0.08)	b,c
HYT_T_antigens	1.39 (0.21)	1.42 (0.22)	1.44 (0.20)	1.49 (0.21)	1.57 (0.20)	a,b,c
HYT_sT_antigens	2.26 (0.22)	2.25 (0.22)	2.23 (0.20)	2.19 (0.21)	2.12 (0.20)	a,b,c
HYT_disialylated_O_antigens	3.05 (0.88)	2.96 (0.85)	2.92 (0.77)	2.93 (0.83)	2.75 (0.77)	b,c
HYT_sialic_acids_per_galactose	0.63 (0.06)	0.62 (0.06)	0.62 (0.06)	0.60 (0.06)	0.58 (0.05)	a,b,c
HYT_galactoses_per_galnac	0.81 (0.02)	0.82 (0.02)	0.82 (0.02)	0.82 (0.02)	0.82 (0.02)	b,c
ENI_fucosylation	23.84 (3.18)	24.18 (3.24)	24.43 (3.36)	24.10 (3.08)	24.41 (3.03)	c
ENI_sialylation	84.22 (1.74)	84.65 (1.92)	84.87 (2.19)	84.01 (1.90)	83.67 (1.93)	a,b,c
ENI_hybrid	22.08 (2.61)	22.22 (2.65)	22.69 (2.62)	23.63 (2.65)	24.64 (2.67)	a,b,c,e
LAGC_fucosylation	98.02 (0.25)	98.05 (0.17)	98.04 (0.27)	98.09 (0.18)	98.07 (0.31)	
LAGC_bisection	42.22 (3.42)	41.33 (3.71)	40.99 (3.86)	41.89 (3.56)	42.97 (3.52)	a,b,c
LAGC_galactosylation	99.34 (0.08)	99.35 (0.06)	99.35 (0.09)	99.36 (0.06)	99.36 (0.10)	
LAGC_sialylation	85.95 (1.57)	86.34 (1.46)	86.61 (1.58)	86.06 (1.62)	85.64 (1.60)	a,b,c,d,e
LAGC_tri_antennary	18.96 (1.92)	18.91 (1.86)	18.84 (1.73)	19.14 (2.09)	19.35 (2.12)	a,b
LAGY_fucosylation	95.52 (1.64)	95.42 (1.57)	95.36 (1.60)	95.55 (1.55)	95.53 (1.54)	
LAGY_bisection	50.39 (4.64)	49.51 (4.75)	49.13 (4.55)	51.56 (4.78)	53.39 (4.83)	a,b,c
LAGY_galactosylation	99.16 (0.31)	99.21 (0.30)	99.27 (0.31)	99.10 (0.35)	98.90 (0.49)	a,b,c,e
LAGY_sialylation	83.81 (2.65)	84.29 (2.50)	84.72 (2.44)	84.23 (2.56)	83.61 (2.72)	b,c,d,e
LAGY_tri_antennary	4.54 (0.49)	4.59 (0.51)	4.63 (0.47)	4.53 (0.46)	4.40 (0.47)	b,c
LAGC_sialylation_per_galactose	86.52 (1.58)	86.90 (1.47)	87.18 (1.61)	86.61 (1.62)	86.20 (1.62)	a,b,c,d,e
LAGY_sialylation_per_galactose	84.52 (2.50)	84.95 (2.33)	85.34 (2.25)	84.99 (2.37)	84.53 (2.45)	b,c,d,e

Supplementary Table 4. Glycosylation traits in mother-neonate pairs, percentage and 95% CI, in the first (T1), second (T2), and third trimester (T3) and in cord blood (CB). a = t-test between T3 and CB.

	T1	**T2**	**T3**	**CB**		**p-value[a]**
N	73	69	72	78	**p-value[a]**	**FDR adj**
IgGI_fucosylation	92.21 (3.17)	92.20 (3.23)	91.89 (3.40)	91.38 (3.65)	0,386	0,446
IgGI_bisection	14.95 (2.87)	14.91 (2.89)	14.73 (2.66)	15.44 (2.81)	0,117	0,161
IgGI_galactosylation	67.01 (5.14)	69.25 (5.96)	69.97 (5.37)	71.82 (5.41)	**0,038**	0,065
IgGI_sialylation	11.98 (1.75)	12.71 (2.09)	12.74 (1.96)	13.44 (1.90)	**0,029**	0,052
IgGI_hybrid	0.26 (0.05)	0.27 (0.06)	0.28 (0.05)	0.26 (0.05)	**0,043**	0,068
IgGII_fucosylation	99.33 (0.33)	99.33 (0.35)	99.30 (0.35)	99.35 (0.44)	0,464	0,510
IgGII_bisection	11.17 (2.08)	11.10 (2.04)	11.15 (2.08)	11.30 (2.20)	0,660	0,691
IgGII_galactosylation	61.78 (5.67)	65.08 (5.53)	65.13 (5.80)	68.16 (6.28)	**0,003**	**0,006**
IgGII_sialylation	10.34 (1.79)	11.36 (1.86)	11.34 (1.92)	13.53 (2.19)	**<0,001**	**<0,001**
IgGIV_bisection	11.77 (2.96)	12.02 (3.17)	12.32 (3.26)	12.34 (3.45)	0,974	0,974
IgGIV_galactosylation	58.04 (7.53)	60.65 (8.10)	59.76 (7.04)	65.87 (7.06)	**<0,001**	**<0,001**
IgGIV_sialylation	16.14 (3.23)	17.36 (3.60)	17.02 (3.04)	19.13 (3.40)	**<0,001**	**<0,001**
IgGI_sialylation_per_galactose	17.83 (1.80)	18.28 (2.05)	18.16 (1.95)	18.67 (1.81)	0,096	0,141
IgGII_sialylation_per_galactose	16.66 (1.84)	17.40 (1.90)	17.34 (1.90)	19.81 (2.40)	**<0,001**	**<0,001**
IgGIV_sialylation_per_galactose	27.54 (3.03)	28.32 (3.18)	28.31 (2.78)	28.85 (2.90)	0,300	0,388
SES_fucosylation	96.55 (0.96)	96.85 (0.98)	96.77 (0.97)	96.07 (1.27)	**<0,001**	**<0,001**
SES_antennary_fucosylation	4.39 (1.07)	4.32 (1.20)	4.93 (1.77)	8.78 (2.11)	**<0,001**	**<0,001**
SES_bisection	98.33 (1.10)	98.35 (0.92)	98.29 (1.35)	99.75 (0.12)	**<0,001**	**<0,001**
SES_galactosylation	58.26 (1.52)	59.36 (1.44)	59.74 (1.91)	55.85 (1.02)	**<0,001**	**<0,001**
SES_sialylation	50.47 (1.85)	50.97 (1.92)	51.62 (2.11)	48.07 (1.19)	**<0,001**	**<0,001**
SES_tri_antennary	28.34 (4.76)	35.45 (4.68)	36.14 (5.37)	21.27 (4.54)	**<0,001**	**<0,001**
SES_sialylation_per_galactose	86.61 (1.94)	85.86 (2.14)	86.42 (2.28)	86.09 (1.93)	0,367	0,446

Supplementary Table 5. Baseline characteristics, stratified by mode of conception.

N	Spontaneous conception 103	ART 83	p-value
Patient characteristics			
Maternal age, mean (SD)	31.5 (4.4)	32.8 (4.5)	**0.043**
Maternal BMI, median [IQR]	25.5 [22.6, 29.9]	24.1 [21.8, 27.7]	**0.034**
Country of origin, n (%)			0.138
Dutch	84 (81.6)	72 (86.7)	
Western, not Dutch	2 (1.9)	3 (3.6)	
Non-Western	11 (10.7)	8 (9.6)	
Missing	*6 (5.0)*	*0 (0.0)*	
Smoking periconceptional, n (%)	13 (12.6)	12 (14.5)	0.115
Missing	*8 (6.7)*	*1 (1.2)*	
Pre-existing hypertension	4 (3.9)	1 (1.2)	0.157
Missing	*0 (0.0)*	*2 (2.4)*	
Pre-existing diabetes mellitus			
Type I	2 (1.9)	0 (0.0)	0.575
Type II	0 (0.0)	0 (0.0)	NA
Pregnancy characteristics			
Mode of conception, n (%)			**<0.001**
Spontaneous	103 (86.6)	0 (0.0)	
IVF	0 (0.0)	33 (39.8)	
ICSI	0 (0.0)	50 (60.2)	
Primigravidity, n (%)	26 (25.2)	43 (51.8)	**<0.001**
Missing	*0 (0.0)*	*1 (1.2)*	
Gestational age at birth in days, median [IQR]	272 [264, 279]	274 [267, 278]	0.277
Neonatal birthweight percentile, median [IQR]	51 [18, 72]	44 [22, 71]	0.924
Pregnancy complication, n (%)			
Preeclampsia	3 (2.9)	3 (3.6)	0.273
with onset <34 weeks	1 (1.0)	2 (2.4)	0.332
Pregnancy induced hypertension	10 (9.7)	4 (4.8)	0.137
Gestational diabetes	14 (13.6)	15 (18.1)	0.189
Missing	*0 (0.0)*	*2 (2.4)*	

REFERENCES

1. Erlebacher A. Immunology of the maternal-fetal interface. Annu Rev Immunol. 2013;31:387-411.
2. Mor G, Aldo P, Alvero AB. The unique immunological and microbial aspects of pregnancy. Nat Rev Immunol. 2017 Aug;17(8):469-82.
3. Steegers-Theunissen RP, Twigt J, Pestinger V, Sinclair KD. The periconceptional period, reproduction and long-term health of offspring: the importance of one-carbon metabolism. Hum Reprod Update. 2013 Nov-Dec;19(6):640-55.
4. Wilcox CR, Holder B, Jones CE. Factors Affecting the FcRn-Mediated Transplacental Transfer of Antibodies and Implications for Vaccination in Pregnancy. Front Immunol. 2017;8:1294.
5. Jauniaux E, Jurkovic D, Gulbis B, Liesnard C, Lees C, Campbell S. Materno-fetal immunoglobulin transfer and passive immunity during the first trimester of human pregnancy. Hum Reprod. 1995 Dec;10(12):3297-300.
6. Woof JM, Russell MW. Structure and function relationships in IgA. Mucosal Immunol. 2011 Nov;4(6):590-7.
7. Seikrit C, Pabst O. The immune landscape of IgA induction in the gut. Semin Immunopathol. 2021 Oct;43(5):627-37.
8. Arnold JN, Wormald MR, Sim RB, Rudd PM, Dwek RA. The impact of glycosylation on the biological function and structure of human immunoglobulins. Annu Rev Immunol. 2007;25:21-50.
9. Zhong J, Li J, Burton GJ, Koistinen H, Cheung KW, Ng EHY, et al. The functional roles of protein glycosylation in human maternal-fetal crosstalk. Hum Reprod Update. 2024 Jan 3;30(1):81-108.
10. Cobb BA. The history of IgG glycosylation and where we are now. Glycobiology. 2020 Mar 20;30(4):202-13.
11. Štambuk T, Klasić M, Zoldoš V, Lauc G. N-glycans as functional effectors of genetic and epigenetic disease risk. Molecular Aspects of Medicine. 2021 2021/6//;79:100891-.
12. Dekkers G, Treffers L, Plomp R, Bentlage AEH, Boer Md, Koeleman CAM, et al. Decoding the human immunoglobulin G-glycan repertoire reveals a spectrum of Fc-receptor- and complement-mediated-effector activities. Frontiers in Immunology. 2017 2017/8//;8(AUG).
13. Kristic J, Lauc G. The importance of IgG glycosylation-What did we learn after analyzing over 100,000 individuals. Immunol Rev. 2024 Nov;328(1):143-70.
14. Memarian E, Heijmans R, Slieker RC, Sierra A, Gornik O, Beulens JWJ, et al. IgG N-glycans are associated with prevalent and incident complications of type 2 diabetes. Diabetes Metab Res Rev. 2023 Oct;39(7):e3685.
15. Gudelj I, Lauc G, Pezer M. Immunoglobulin G glycosylation in aging and diseases. Cell Immunol. 2018 Nov;333:65-79.
16. Selman MH, Derks RJ, Bondt A, Palmblad M, Schoenmaker B, Koeleman CA, et al. Fc specific IgG glycosylation profiling by robust nano-reverse phase HPLC-MS using a sheath-flow ESI sprayer interface. J Proteomics. 2012 Feb 2;75(4):1318-29.
17. Bondt A, Rombouts Y, Selman MH, Hensbergen PJ, Reiding KR, Hazes JM, et al. Immunoglobulin G (IgG) Fab glycosylation analysis using a new mass spectrometric high-throughput profiling method reveals pregnancy-associated changes. Mol Cell Proteomics. 2014 Nov;13(11):3029-39.
18. Bondt A, Nicolardi S, Jansen BC, Stavenhagen K, Blank D, Kammeijer GS, et al. Longitudinal monitoring of immunoglobulin A glycosylation during pregnancy by simultaneous MALDI-FTICR-MS analysis of N- and O-glycopeptides. Sci Rep. 2016 Jun 15;6:27955.
19. Bondt A, Nicolardi S, Jansen BC, Kuijper TM, Hazes JMW, van der Burgt YEM, et al. IgA N- and O-glycosylation profil-

ing reveals no association with the pregnancy-related improvement in rheumatoid arthritis. Arthritis Res Ther. 2017 Jul 5;19(1):160.

20. Ruhaak LR, Uh HW, Deelder AM, Dolhain RE, Wuhrer M. Total plasma N-glycome changes during pregnancy. J Proteome Res. 2014 Mar 7;13(3):1657-68.
21. Redman CW, Sargent IL. Immunology of pre-eclampsia. Am J Reprod Immunol. 2010 Jun;63(6):534-43.
22. Abu-Raya B, Michalski C, Sadarangani M, Lavoie PM. Maternal Immunological Adaptation During Normal Pregnancy. Front Immunol. 2020;11:575197.
23. Rousian M, Schoenmakers S, Eggink AJ, Gootjes DV, Koning AHJ, Koster MPH, et al. Cohort Profile Update: The Rotterdam Periconceptional Cohort and embryonic and fetal measurements using 3D ultrasound and virtual reality techniques. International Journal of Epidemiology. 2021 2021/10//;50(5):1426-7.
24. Steegers-Theunissen RPM, Verheijden-Paulissen JJFM, van Uitert EM, Wildhagen MF, Exalto N, Koning AHJ, et al. Cohort Profile: The Rotterdam Periconceptional Cohort (Predict Study). International journal of epidemiology. 2016 2016/4//;45(2):374-81.
25. Momcilovic A, de Haan N, Hipgrave Ederveen AL, Bondt A, Koeleman CAM, Falck D, et al. Simultaneous Immunoglobulin A and G Glycopeptide Profiling for High-Throughput Applications. Anal Chem. 2020 Mar 17;92(6):4518-26.
26. Falck D, Wuhrer M. GlYcoLISA: antigen-specific and subclass-specific IgG Fc glycosylation analysis based on an immunosorbent assay with an LC-MS readout. Nat Protoc. 2024 Jun;19(6):1887-909.
27. Visconti A, Rossi N, Bondt A, Ederveen AH, Thareja G, Koeleman CAM, et al. The genetics and epidemiology of N- and O-immunoglobulin A glycomics. Genome Med. 2024 Aug 9;16(1):96.
28. Richani K, Soto E, Romero R, Espinoza J, Chaiworapongsa T, Nien JK, et al. Normal pregnancy is characterized by systemic activation of the complement system. J Matern Fetal Neonatal Med. 2005 Apr;17(4):239-45.
29. Baek H, Yang SW, Kim S, Lee Y, Park H, Park M, et al. Development of Anti-Inflammatory Agents Utilizing DC-SIGN Mediated IL-10 Secretion in Autoimmune and Immune-Mediated Disorders: Bridging Veterinary and Human Health. Int J Mol Sci. 2025 Mar 5;26(5).
30. Garcia-Garcia A, Ceballos-Laita L, Serna S, Artschwager R, Reichardt NC, Corzana F, Hurtado-Guerrero R. Structural basis for substrate specificity and catalysis of alpha1,6-fucosyltransferase. Nat Commun. 2020 Feb 20;11(1):973.
31. Pereira NA, Chan KF, Lin PC, Song Z. The "less-is-more" in therapeutic antibodies: Afucosylated anti-cancer antibodies with enhanced antibody-dependent cellular cytotoxicity. MAbs. 2018 Jul;10(5):693-711.
32. Wuhrer M, Selman MH, McDonnell LA, Kumpfel T, Derfuss T, Khademi M, et al. Pro-inflammatory pattern of IgG1 Fc glycosylation in multiple sclerosis cerebrospinal fluid. J Neuroinflammation. 2015 Dec 18;12:235.
33. Einarsdottir HK, Selman MH, Kapur R, Scherjon S, Koeleman CA, Deelder AM, et al. Comparison of the Fc glycosylation of fetal and maternal immunoglobulin G. Glycoconj J. 2013 Feb;30(2):147-57.
34. Gomez-Lopez N, StLouis D, Lehr MA, Sanchez-Rodriguez EN, Arenas-Hernandez M. Immune cells in term and preterm labor. Cell Mol Immunol. 2014 Nov;11(6):571-81.
35. Nikolac Perkovic M, Pucic Bakovic M, Kristic J, Novokmet M, Huffman JE, Vitart V, et al. The association between galactosylation of immunoglobulin G and body mass index. Prog Neuropsychopharmacol Biol Psychiatry. 2014 Jan 3;48:20-5.
36. Farkas A, Suranyi A, Kolcsar B, Gyurkovits Z, Kozinszky Z, Vari SG, Guttman A. Potential Glycobiomarkers in Maternal

Obesity and Gestational Diabetes During Human Pregnancy. J Clin Med. 2025 Feb 27;14(5).

37. Plomp R, Ruhaak LR, Uh HW, Reiding KR, Selman M, Houwing-Duistermaat JJ, et al. Subclass-specific IgG glycosylation is associated with markers of inflammation and metabolic health. Sci Rep. 2017 Sep 26;7(1):12325.
38. Mijakovac A, Juric J, Kohrt WM, Kristic J, Kifer D, Gavin KM, et al. Effects of Estradiol on Immunoglobulin G Glycosylation: Mapping of the Downstream Signaling Mechanism. Front Immunol. 2021;12:680227.
39. Jennewein MF, Goldfarb I, Dolatshahi S, Cosgrove C, Noelette FJ, Krykbaeva M, et al. Fc Glycan-Mediated Regulation of Placental Antibody Transfer. Cell. 2019 Jun 27;178(1):202-15 e14.
40. Kissel T, Toes REM, Huizinga TWJ, Wuhrer M. Glycobiology of rheumatic diseases. Nat Rev Rheumatol. 2023 Jan;19(1):28-43.
41. Birukov A, Plavsa B, Eichelmann F, Kuxhaus O, Hoshi RA, Rudman N, et al. Immunoglobulin G N-Glycosylation Signatures in Incident Type 2 Diabetes and Cardiovascular Disease. Diabetes Care. 2022 Nov 1;45(11):2729-36.
42. Dotz V, Visconti A, Lomax-Browne HJ, Clerc F, Hipgrave Ederveen AL, Medjeral-Thomas NR, et al. O- and N-Glycosylation of Serum Immunoglobulin A is Associated with IgA Nephropathy and Glomerular Function. J Am Soc Nephrol. 2021 Oct;32(10):2455-65.

3

Longitudinal maternal IgG and IgA glycosylation profiles in pregnancy reveal early immune alterations in placenta-related complications: The Rotterdam Periconception Cohort

Lotte W. Voskamp, Melek Rousian, Anna Daniels, Manfred Wuhrer, A.H. Jan Danser, Régine P.M. Steegers-Theunissen, Koen Verdonk

Hypertension. 2026;83:e26531.

ABSTRACT

Background

Placenta-related complications are major contributors to maternal and neonatal morbidity. Immunoglobulin (Ig) glycosylation modulates immune function by altering molecular structure and receptor affinity. During pregnancy, IgG and IgA glycosylation normally shift toward a more anti-inflammatory profile. We hypothesized that this adaptation is attenuated in complicated pregnancies, resulting in altered glycosylation trajectories.

Methods

Women with singleton pregnancies were enrolled in the prospective Rotterdam Periconception Cohort (2017-2018). Serum samples were collected at 9, 11, 13, 22, and 32 weeks gestational age (GA). IgG and IgA glycosylation was measured using LC-MS, and summarized into glycosylation traits (galactosylation, sialylation, fucosylation, bisection). Placenta-related complications (preeclampsia, pregnancy-induced hypertension, preterm birth, or small-for-gestational-age) were identified from medical records. Longitudinal changes and group differences in Ig glycosylation were analyzed using linear mixed-effects models with false discovery rate correction.

Results

Among the included 193 pregnancies, 54 were complicated by at least one placenta-related disorder. In complicated pregnancies, galactosylation and sialylation were consistently lower, whereas fucosylation and bisection were higher. From 9 weeks GA onwards, fucosylation was significantly elevated at IgA Asn327/Asn340 and Asn205 (Z-score difference: 0.45–0.52; q=0.002–0.004 and 0.33–0.37; q=0.038). Bisection increased more steeply at IgG1, IgA Asn144/Asn131 and IgA Asn327/Asn340 (q=0.021–0.027). IgG galactosylation rose less across gestation (q=0.021–0.024), and IgA galactosylation and sialylation declined more sharply at Asn144/Asn131, Asn205, and Asn327/Asn340 (q=0.000–0.026).

Conclusion

Pregnancies with placenta-related complications exhibit more pro-inflammatory glycosylation profiles. These findings suggest a potential mechanistic link between immunoglobulin glycosylation and placental dysfunction, with specific glycopeptides as possible biomarkers.

INTRODUCTION

Placenta-related pregnancy complications remain a major burden in obstetric care. These conditions, including pregnancy-induced hypertension, preeclampsia, preterm birth, and fetal growth restriction, are associated with substantial maternal and neonatal morbidity and mortality [1-3]. Despite advances in prenatal care, effective preventive and therapeutic options remain limited, underscoring the need for a deeper understanding of their pathophysiology.

A hallmark of these disorders is impaired maternal vascular adaptation leading to inadequate placental perfusion. Contributing factors include abnormal spiral artery remodeling, endothelial dysfunction, and increased uterine artery resistance, which is often assessed clinically using uterine artery Doppler velocimetry [1, 4]. However, uterine artery Doppler measurements do not reliably predict all cases of placenta-related complications, suggesting that additional, yet uncharacterized, mechanisms may contribute to the development of placenta-related pregnancy complications.

One such mechanism may involve dysregulation of the maternal immune system, which is increasingly recognized as a central contributor to placental pathology [5]. Immunoglobulins (Ig), key components of the adaptive immune system, modulate immune responses during pregnancy. Their function is regulated by glycosylation, the enzymatic attachment of glycans, which influences their structural conformation, receptor binding affinity, and effector functions (Figure 1) [6]. Specific glycan modifications influence immunoglobulin interactions with Fc gamma receptors (FcγRs) and complement components, shaping downstream immune responses [7]. For example, afucosylation and galactosylation enhance pro-inflammatory pathways such as antibody-dependent cellular cytotoxicity (ADCC) via increased affinity for activating FcγRs (e.g., FcγRIIIa) on natural killer (NK) cells, macrophages and neutrophils [7, 8]. In contrast, sialylation of IgG promotes anti-inflammatory signaling through increasing affinity for receptors such as DC-SIGN and inhibitory FcγRIIb on macrophages [7, 9, 10].

During pregnancy, IgG and IgA glycosylation typically shift toward an anti-inflammatory phenotype, with increased galactosylation and sialylation and reduced bisecting N-acetylglucosamine [11, 12]. These changes occur already within the first trimester and coincide with critical developmental processes such as trophoblast invasion and placentation, which are also highly sensitive to immune regulation [13, 14]. Disruption of this immunological balance may therefore contribute to the pathogenesis of placenta-related complications [5, 15]. Supporting this, our previous analyses showed that maternal characteristics, including elevated body mass index, primigravidity, and conception via assisted reproductive technologies, were associated with distinct immunoglobulin glycosylation patterns across pregnancy [16]. Notably, the same characteristics are known risk factors

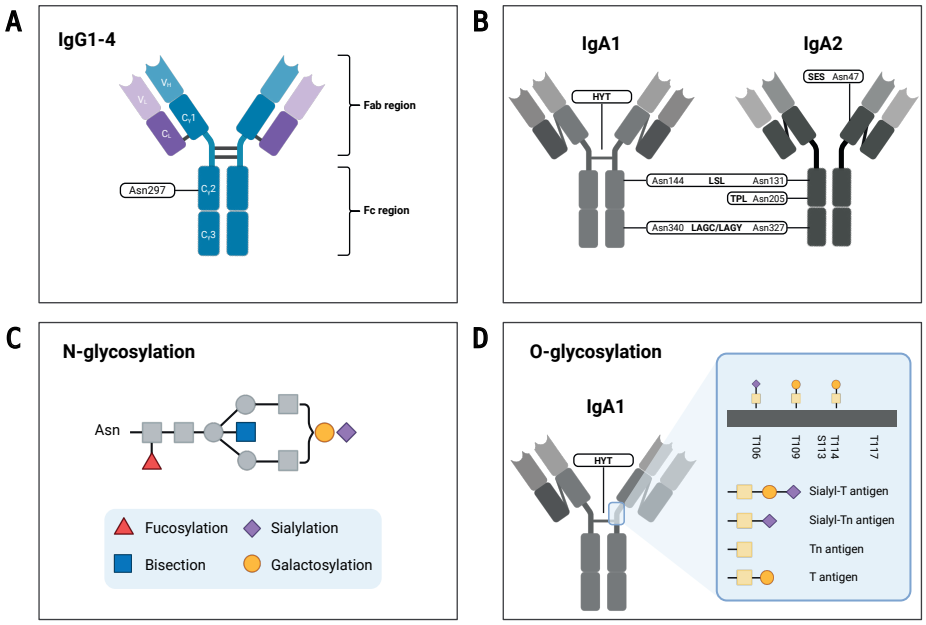


Figure 1. Overview of IgA and IgG glycosylation sites and structures. (A) Schematic IgG molecule showing the N-glycosylation site [6, 19]; (B) Schematic IgA1 and IgA2 molecules showing the N-glycosylation sites and the O-glycosylation site HYT [22]; (C) Example of a complex biantennary N-glycan structure with glycosylation traits (fucosylation, galactosylation, sialylation, bisection); (D) Expanded view of the IgA1 hinge region (HYT peptide) illustrating O-glycan heterogeneity.

for placental disorders [17, 18]. Moreover, altered immunoglobulin glycosylation has been described in autoimmune and cardiovascular diseases, conditions that both share inflammatory and endothelial dysfunction pathways with placental disorders and are associated with increased risk of placental complications [19-21]. These observations indicate that aberrant glycosylation may underlie both immune and vascular maladaptation, representing complementary pathways of interest in the pathogenesis of placental complications.

We hypothesized that pregnancies complicated by placenta-related disorders would exhibit less anti-inflammatory glycosylation profiles, particularly during the first trimester, when placentation occurs. The objective of this study was to characterize longitudinal trajectories of IgG and IgA glycosylation throughout gestation and to determine whether these trajectories differ between pregnancies with and without placenta-related complications. We also examined associations between first-trimester glycosylation and uterine artery pulsatility indices to explore the interplay between vascular and immune adaptation in early pregnancy.

METHODS

Study design

This study was conducted within the Virtual Placenta Study, an ongoing prospective subcohort embedded in the Rotterdam Periconception Cohort (Predict Study), at the Erasmus MC, University Medical Center, a tertiary care hospital in Rotterdam, the Netherlands [22, 23]. Women aged 18 years or older with a singleton pregnancy were included before 10 weeks gestational age (GA) between 2017 and 2018. In case of multiple participation only the first pregnancy per participant was included. Pregnancies ending in miscarriage, with congenital anomalies, or delivered before 24 weeks were excluded. The study was approved by the local ethics committee (MEC-2004-227) and conducted in accordance with the principles of the Declaration of Helsinki. All participants provided written informed consent.

Data collection

Clinical data

At enrolment, participants completed a questionnaire and underwent anthropometric measurements. Pregnancy outcomes were collected from medical records and validated with patient questionnaires. For the primary outcome, pregnancies were classified into two groups: those with placenta-related complications and those without. Placenta complications included pregnancy-induced hypertension, preeclampsia, preterm birth (<37 weeks GA), and small-for-gestational age (SGA). Pregnancy-induced hypertension was defined as new-onset hypertension (systolic ≥140 mmHg and/or diastolic ≥90 mmHg) after 20 weeks GA. Preeclampsia was defined as hypertension with proteinuria, fetal growth restriction and/or maternal organ damage [24]. Small-for-gestational age was defined as birthweight percentile below the 10th percentile compared to a reference curve [25].

Uterine artery Doppler measurements

Ultrasound examinations were performed at 7, 9, 11 and 13 weeks GA using a transvaginal probe and Voluson Expert E8 or E10 systems (GE Healthcare, Zipf, Austria). At each visit, trained sonographers obtained bilateral uterine artery pulsatility index measurements using standardized power Doppler settings [26]. Three measurements per side were recorded, and the mean value per side was used for analysis. GA was determined using last menstrual period for women with regular cycles (21–35 days) or, for conceptions via assisted reproductive technology, by adding 14 days to the date of oocyte retrieval or 19 days to the cryopreserved embryo transfer date [23]. In cases of irregular cycles or difference of >7 days, GA was adjusted based on crown–rump length measurement [22].

Glycosylation measurements

Maternal blood was collected at 9, 11, 13, 22 and 32 weeks GA. Neonatal cord blood was collected immediately after birth. Samples were transported to the analysis laboratory as soon as possible, and always within 48 hours of collection. Samples were allowed to clot for ≥60 minutes at room temperature, centrifuged at 2,000×g for 10 minutes at 4 °C, aliquoted (4 × 0.5 mL) and stored at −80 °C. IgG and IgA glycosylation was profiled at the glycopeptide level using a validated protocol [27]. In short, immunoglobulins were affinity-captured, reduced, alkylated, and digested with trypsin, followed by nanoLC–MS. Raw data were processed and quality-controlled using LaCyTools software and glycosylation traits (e.g., fucosylation, galactosylation, sialylation, bisection) were derived from normalized glycoform abundances (Table S1) and reported per glycosylation site (Figure 1) [28]. Laboratory staff performing LC–MS analyses were blinded to clinical data.

Statistical analysis

Primary outcomes included glycosylation trajectories in pregnancies with and without placenta-related complications. For each Z-standardized glycan trait, we fitted linear mixed-effects models with a natural cubic spline for gestational age (2 degrees of freedom) and its interaction with complication status, including a random intercept for participant. Adjusted models included BMI, primigravidae status, and mode of conception (natural vs. IVF/ICSI) as covariates, based on prior findings [16]. Mode of conception was classified as natural (spontaneous conception, ovulation induction, or intrauterine insemination) or IVF/ICSI (in vitro fertilization with or without intra-cytoplasmatic sperm injection). Estimated marginal means were obtained at 9, 11, 13, 22, and 32 weeks; between-group contrasts (Yes–No) with 95% CIs were summarized at 11, 22, and 32 weeks. False discovery rate (FDR) correction was applied across traits and timepoints, and a q-value <0.05 was considered statistically significant. Sensitivity analyses included regrouping of women with spontaneous preterm birth but without any other placenta-related complications as not having a placenta-related complication. A second analysis compared pregnancies with hypertensive disorders (pregnancy induced hypertension or preeclampsia) to those without complications, excluding isolated SGA or preterm births. Cord blood IgG and IgA glycosylation traits were standardized (Z-scores) and analyzed cross-sectionally. Associations with pregnancy outcomes were assessed using Spearman correlations, with calculation of coefficients (r) and corresponding p-values.

To explore mediation by uterine artery resistance, we first compared mean uterine artery PI between women with and without placental complications using independent-samples t-tests. Subsequently, uterine artery PI trajectories across gestation were analyzed using linear mixed-effects models including gestational age, complication status, and their interaction to assess group differences over time. For mediation analyses, all available UtA-PI measurements (log-transformed) were modelled using a linear mixed-effects model with gestational age (spline), placental complication status, and side (left/right) as

fixed effects, and nested random effects for participants and sides. The predicted mean UtA-PI at 13 weeks was used as mediator in both mediation approaches. First, for each Z-standardized glycan trait, we compared linear mixed-effects models across gestational ages (9–32 weeks) with and without the UtA mediator, evaluating model fit (AIC, BIC, likelihood ratio tests) and explained variance (marginal and conditional R^2). Second, in a forward modelling approach, we assessed whether early changes in glycosylation (9–13 weeks) predicted placental complications, with or without mediation by UtA resistance. Subject-specific 9–13-week slopes were derived from mixed-effects models and used as predictors in logistic regression models for placental complication status. Mediation effects were estimated using the mediate function (R package mediation) with 1,000 bootstraps. FDR correction was applied across traits and q-value below 0.05 was considered significant.

RESULTS

A total of 193 ongoing pregnancies were included. Of these, 54 were complicated by at least one placenta-related disorder: preterm birth occurred in 44% (n=24), preeclampsia in 13% (n=7), gestational hypertension in 28% (n=15), and SGA in 44% (n=24) (Table 1). Co-occurrence of complications is summarized in Table S2. The remaining 139 pregnancies were not affected by any of these complications. Mean maternal age at conception was 32 years and was higher in uncomplicated than in complicated pregnancies (32.6 vs 31.1 years, P=0.033). Baseline characteristics were otherwise comparable, except for a higher uterine artery PI at 13 weeks in the complication group (1.88 vs 1.57, p=0.041; Table 1 and Table S2).

Glycosylation trajectories

In the crude models, when comparing glycosylation trajectories across pregnancy between placenta-complications and uncomplicated pregnancies, several differences were observed (Figure 2). For bisection, a significant interaction term was observed for LAGY, LSL and IgG1 (Figure 2). For LSL and IgG1, bisection is lower in the first and second trimester, and on all three sites, bisection increased more strongly towards the third trimester in pregnancies complicated by placenta-related disorders. For fucosylation, no glycosylation sites showed a significant interaction between gestational age and complication status, indicating that the overall trajectory across pregnancy was similar between women with and without placenta-related complications. However, mean fucosylation levels were consistently higher in the complication group across all measured sites. This difference reached statistical significance at two IgA sites: LAGY (mean Z-score difference across pregnancy: 0.45–0.52; q = 0.002–0.004) and TPL (mean Z-score difference: 0.33–0.37; q = 0.038).

Table 1. Baseline table stratified by placenta-related complications.

	No placenta complication (n=139)	Placenta complication (n=54)
Maternal characteristics		
Maternal age, mean (SD)	32.6 (4.5)	31.1 (4.3)
Maternal BMI, median [IQR]	24.6 [22.1, 28.4]	25.3 [22.6, 29.6]
Geographic origin, n (%)		
Dutch	117 (84.2)	44 (81.5)
Western, not Dutch	5 (3.6)	1 (1.9)
Non-Western	14 (10.1)	6 (11.1)
missing	*3 (2.2)*	*3 (5.6)*
Mode of conception, n (%)		
Natural	78 (56.1)	37 (68.6)
IVF/ICSI	61 (43.9)	17 (31.4)
Primigravida, n (%)	55 (39.6)	22 (40.7)
Nulliparity, n (%)	78 (56.1)	30 (55.6)
Maternal smoking periconceptional, n (%)	16 (11.5)	8 (14.8)
missing	*5 (3.6)*	*4 (7.4)*
Folic acid supplement use, n (%)	132 (95.0)	50 (92.6)
missing	*5 (3.6)*	*4 (7.4)*
Chronic hypertension	3 (2.2)	2 (3.7)
Diabetes type I	1 (0.7)	2 (3.7)
Pregnancy outcomes		
Maternal outcomes		
Uterine artery PI at 13 weeks' GA, mean (SD)	1.57 (0.63)	1.88 (0.95)
Missing, n (%)	*7 (5.0)*	*4 (7.4)*
Gestational diabetes (2 missing NPC)	18 (12.9)	11 (20.4)
Missing, n (%)	*2 (1.4)*	*0*
Birth outcomes		
Gestational age at birth in days, median [IQR]	274 [269, 280]	263 [254, 273]
Neonatal birthweight percentile, median [IQR]	51 [32, 75]	15 [4, 56]
Placenta-related complication, n (%)		
Preterm birth		24 (44.4)
spontaneous		16 (29.6)
Preeclampsia		7 (13.0)
with onset <34 weeks		3 (5.6)
Pregnancy induced hypertension		15 (27.8)
SGA (<10th percentile)		24 (44.4)

BMI = body-mass index; ICSI = intra-cytoplasmatic sperm injection; IQR = interquartile range, IVF = in vitro fertilization; PI = pulsatility index; SD = standard deviation; SGA = small-for-gestational-age.

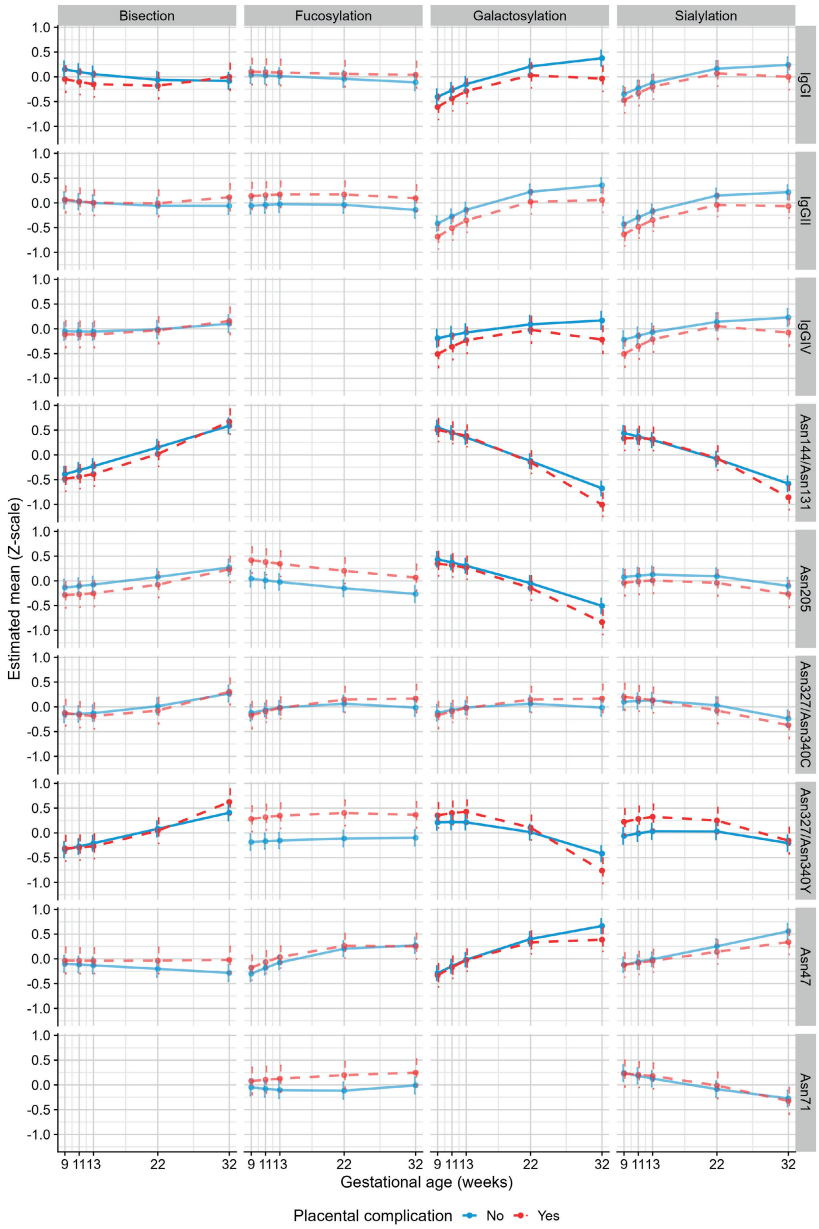


Figure 2. Trajectories of maternal IgG and IgA glycosylation traits across gestation. Lines represent estimated marginal means (Z-standardized values) for pregnancies with placenta-related complications (red, dashed) and without complications (blue, solid), derived from linear mixed-effects models. Each column facet represents a glycosylation trait (fucosylation, bisection, galactosylation, or sialylation), and each row facet represents an individual glycosylation site. Error bars indicate 95% confidence intervals of the estimated marginal means. More intense line colors denote traits with a statistically significant interaction term (placenta complication × gestational age) in the mixed model, while lighter colors indicate non-significant interactions.

A trend towards lower galactosylation throughout pregnancy was observed in women with placenta-related complications across all IgG subtypes, with significant interaction with gestational age (q=0.021-0.025) for IgG1 and IgG4 (Figure 2). At IgA sites LSL, TPL and LAGY, galactosylation decreased more strongly towards the third trimester in the complication group (q = 0.000–0.024). In contrast, galactosylation on LAGC and SES increased over pregnancy in both groups, but significantly less at the SES site for placenta complications (q=0.021) (Figure 2). For sialylation, significant trajectory differences were found at LSL and LAGY (q=0.021-0.026). Women with placenta-related complications showed higher sialylation at LAGY in the first trimester, and a steeper decline on LSL and LAGY after the second trimester (q=0.026). On the other sites, sialylation was generally lower in the complication group, either consistently across pregnancy (IgG I–IV, TPL), with a stronger decline (LAGC), or with a more modest increase (SES) towards the third trimester (Figure 2).

Adjusted models

In the models adjusted for maternal BMI, primigravidae status and mode of conception, trajectories were similar to the unadjusted analyses, with the same interaction terms remaining significant. Local contrasts from the adjusted and unadjusted models were highly consistent in both effect size and direction. The only exception was LSL galactosylation at 32 weeks' GA, which became significant after adjustment (Z-score difference -0.35, q = 0.041).

O-glycosylation (IgA1)

None of the O-glycosylation traits measured at the HYT site (IgA1) showed significantly different trajectories between pregnancies with and without placenta-related complications, either before or after FDR adjustment (Figure 3). Nevertheless, a consistent trend was observed in the complication group, characterized by lower sialylation across pregnancy. This was reflected by reduced disialylated T antigens (mean Z-score difference –0.20;-0.26, q=0.210), lower levels of total sialic acids and sialic acids per galactose (mean difference between -0.20 and -0.29), and reduced sT antigens (-0.20;-0.27). In addition, GalNAc levels were lower (-0.14; -0.28), while T antigens were relatively higher (0.20; 0.30).

Cord blood

Correlation analyses revealed modest but consistent associations between cord blood glycosylation and pregnancy outcomes. Placenta-related complications were negatively correlated with galactosylation and sialylation, and positively with fucosylation and bisection in cord blood (Figure 4). Among IgG traits, IgG2 sialylation showed the strongest positive correlation with birthweight percentile ($r = 0.27$, $p<0.05$). More generally, both sialylation and galactosylation tended to correlate positively with birthweight percentile and negatively with SGA. In contrast, IgG bisection displayed positive correlations with placenta-related complications, particularly pregnancy induced hypertension and pre-

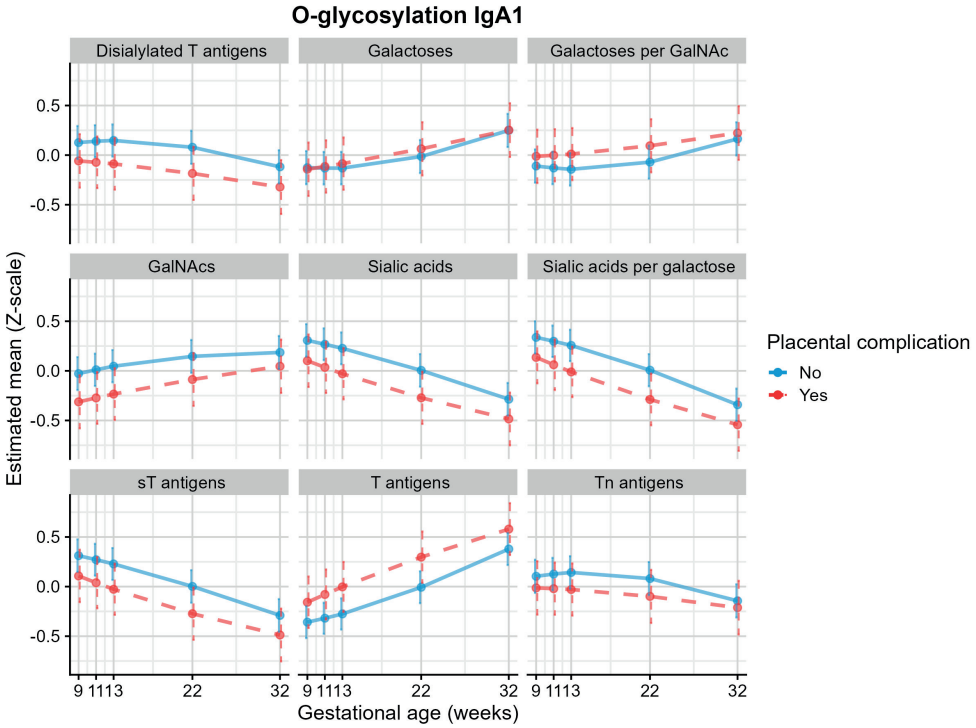


Figure 3. Estimated trajectories of IgA1 O-glycosylation (HYT region) across gestation in pregnancies with and without placenta-related complications. Lines represent estimated marginal means (Z-standardized values) for women with placenta-related complications (red, dashed) and uncomplicated pregnancies (blue, solid), derived from linear mixed-effects models. Each column facet corresponds to an O-glycosylation feature (e.g., sialic acids, GalNAcs, T-antigens, sT-antigens, or disialylated T-antigens). Error bars denote 95% confidence intervals of the estimated marginal means.

eclampsia, with the highest coefficients observed for IgG2. Interestingly, galactosylation showed a mixed pattern: it correlated positively with preeclampsia (r = 0.06–0.17) and preterm birth (r = 0.11–0.21), but negatively with gestational hypertension and SGA (r ≈ –0.05 to –0.20).

For IgA glycosylation, only the SES site (IgA2) could be measured in cord blood. Significant positive correlations with gestational age at birth were observed for more complex SES glycosylation traits (sialylation, galactosylation, and tri-antennary structures; r = 0.26-0.32). In addition, SES galactosylation correlated negatively with overall placenta-related complications (r = –0.29, $p < 0.05$), preterm birth (r = -0.25) and gestational hypertension (r = –0.33, $p<0.01$).

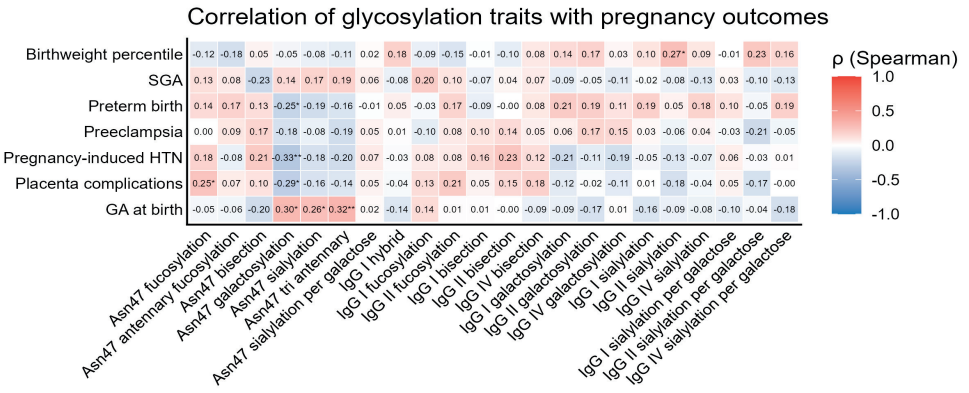


Figure 4. Correlation matrix of glycosylation traits measured in neonatal cord blood (n=75) with clinical pregnancy outcomes. SGA=small-for-gestational-age, HTN=hypertension, GA=gestational age. Significance levels: * p <0.05; ** p < 0.01.

Mediation analysis

Including UtA-PI at 13 weeks in the models did not change the associations between placental complications and any of the 55 IgG glycan trait trajectories. None of the mediation models improved model fit compared with models excluding this variable (all FDR-corrected q-values > 0.1), and the mediator term was not significant in any model. In the forward mediation analyses, no indirect effects through uterine artery resistance were observed between early glycosylation changes and placental complications. Before multiple-testing correction, four glycan traits—IgG1 fucosylation, ENI fucosylation, TPL bisection, and HYT galactoses per GalNAc—showed nominally significant direct effects (ADE p<0.05), corresponding to 13–30% higher odds of placental complications per 1 SD steeper slope between 9 and 13 weeks. However, none of these associations remained significant after FDR correction.

Sensitivity analyses

In a sensitivity analysis reclassifying women with spontaneous preterm birth (n=11) as pregnancies without complications, fewer significant interactions were observed. However, effect directions were comparable to the main analysis (Figure S2). The remaining significant interactions after FDR correction included galactosylation of IgG1, IgG4 and LAGY, IgG1bisection, and SES tri-antennary structures. Glycosylation traits with significant mean differences per trimester after FDR correction were unchanged. When comparing to women with hypertensive complications to those without any placental complications, effect directions were largely consistent with the main analysis, although fewer associations reached statistical significance (Figure S3). Across all IgG subclasses, galactosylation and sialylation remained lower in hypertensive pregnancies, with the pronounced de-

cline in LAGY and TPL galactosylation towards late gestation. In the third trimester, IgG1 galactosylation was significantly reduced in hypertensive pregnancies (–0.58 Z-score, q=0.033), as was TPL galactosylation. LAGY fucosylation was higher throughout pregnancy in the hypertensive group (0.57-0.63 Z-score). Interestingly, number of GalNAcs on the HYT region (O-glycosylation) was significantly lower in hypertensive disorders in all trimesters (-0.64; -0.51 Z-score difference, q=0.011-0.026), Figure S4).

DISCUSSION

Key findings

Specifically, women with complications exhibited higher fucosylation across IgG and IgA sites and lower galactosylation across all IgG subtypes throughout gestation. IgA galactosylation declined more steeply toward the third trimester, while sialylation was consistently lower in the complication group—either across the entire pregnancy (IgG 1–4, TPL) or with a more pronounced decline towards the third trimester (LSL and LAGY). Bisection showed less pronounced differences but tended to be lower for LSL and IgG1 in early pregnancy, increasing more strongly toward the third trimester at several sites in complicated pregnancies. None of the O-glycosylation traits at IgA1 remained significant after FDR correction, although trends toward lower sialylation persisted. The observed associations were not mediated by uterine artery resistance at 13 weeks' gestation. In cord blood, placenta-related complications were similarly associated with lower galactosylation and sialylation, and higher fucosylation and bisection across both IgG and IgA traits, reflecting the observed pro-inflammatory shift at delivery.

Interpretation of findings

Immunological effects

During healthy pregnancy, maternal immunity adapts to promote fetal tolerance while maintaining host defense. This adaptation is reflected in IgG glycosylation, which gradually shifts toward an anti-inflammatory profile with increasing galactosylation and sialylation of the Fc region [12, 16]. In pregnancies complicated by preeclampsia, fetal growth restriction, or preterm birth, this anti-inflammatory shift appears attenuated: Women with placenta-related complications consistently showed lower IgG galactosylation and sialylation, together with higher fucosylation. These glycosylation changes might reflect the underlying immune dysregulation in these disorders, which are characterized by a pro-inflammatory maternal–fetal interface, dominated by M1-like decidual macrophages, increased pro-inflammatory cytokine production (e.g., TNF-α, IL-1β, IL-6), and complement overactivation [15, 29, 30]. Notably, a similar pro-inflammatory IgG glycosylation profile has been described in autoimmune and autoinflammatory conditions such as systemic lupus erythematosus and IgA nephropathy—both of which are associated with increased

risk of placental complications and share clinical and pathophysiological features with preeclampsia [31-33]. Together, these observations suggest that altered glycosylation may play a mechanistic role in placenta complications.

Sialylated IgG generally exerts anti-inflammatory effects by reducing affinity for activating FcγRs and enhancing engagement of inhibitory receptors such as FcγRIIb and Sialic acid-binding immunoglobulin-like lectin (Siglec) 7 [14, 34, 35]. In macrophages, these interactions promote M2-like polarization and suppress inflammatory signaling via DC-SIGN and other lectins [9, 14]. Reduced sialylation may impair these regulatory mechanisms, limiting anti-inflammatory receptor engagement and lowering the threshold for macrophage activation, thereby shifting the decidual immune environment toward inflammation. Increased IgG1 galactosylation enhances C1q binding and facilitates controlled activation of the classical complement pathway, supporting physiological immune and vascular adaptation during pregnancy [35-38]. In addition, galactosylated IgG can inhibit C5a-mediated neutrophil activation through cooperative engagement of FcγRIIB and Dectin-1, suggesting that lower galactosylation may remove an important brake on inflammatory effector functions [36]. We therefore hypothesize that loss of IgG galactosylation contributes to the pathophysiology of placenta-related pregnancy complications through dysregulated complement-mediated vascular injury [37,38]. We also identified higher IgG fucosylation in complicated pregnancies. Lower fucosylation is known to enhance ADCC via FcγRIIIa [8], and elevated fucosylation may attenuate this activating immune response, potentially contributing to immune modulation in these cases. However, the association between fucosylation and inflammatory diseases can differ: a lower fucosylation is observed in women with systemic lupus erythematosus and ulcerative colitis, while in Crohn's disease, IgG fucosylation is higher compared to healthy controls [21, 39]. Moreover, in rheumatoid arthritis, elevated IgG1 fucosylation has been associated with greater disease activity during pregnancy, suggesting a potential link to systemic inflammation [40]. Thus, the potential mechanisms by which increased fucosylation might contribute to placental dysfunction remain to be elucidated.

IgA also showed different glycosylation profiles in pregnancies complicated by preeclampsia, SGA, or PTB, with lower galactosylation and sialylation especially towards the third trimester for LSL, LAGY and TPL (IgA2 only), and increased fucosylation throughout pregnancy for LAGY and TPL. How these changes may contribute to placenta-related complications is less clear, as IgA's role in pro-inflammatory pathways is less well characterized than that of IgG. IgA can engage FcαRI (CD89) on monocytes, macrophages, and neutrophils, triggering inflammatory responses including cytokine release and phagocytosis [41]. Although the influence of IgA glycosylation on FcαRI signaling is not well understood, reduced sialylation may hypothetically lower the threshold for immune activation. Interestingly, in a sensitivity analysis focusing solely on hypertensive disorders, we observed significantly higher GalNAcs on the HYT region of IgA1. Altered GalNAc gly-

cosylation in this region has been implicated in immune complex formation and vascular inflammation, in IgA nephropathy and IgA vasculitis [42]. Patients with IgA nephropathy also have higher risks of pre-eclampsia, suggesting a potential mechanistic link to hypertensive pathology [31]. In systemic lupus erythematosus, a decrease in galactosylation in this region has been observed [41], but this was not seen in our sensitivity analysis. In rheumatoid arthritis pregnancies, IgA glycosylation changes in pregnancy were not associated with disease activity, suggesting a smaller role in modulating systemic inflammation compared to IgG [43]. This may in part reflect IgA's lower abundance in serum (~15% of total immunoglobulin). Although no studies have yet investigated IgA glycosylation in relation to placental dysfunction, its potential role in local immune activation at the maternal–fetal interface warrants further investigation.

Vascular effects

Beyond immune cell modulation, glycosylation may also influence vascular tone. In models of obesity-induced hypertension, hyposialylated IgG induced endothelial dysfunction via FcγRIIB-dependent inhibition of endothelial nitric oxide synthase (eNOS), impairing nitric oxide–mediated vasodilation [44]. As impaired eNOS signaling is implicated in preeclampsia and gestational hypertension, a similar mechanism may be relevant in pregnancy [45]. This is further supported by epidemiologic evidence linking low IgG galactosylation and sialylation to increased cardiovascular risk and endothelial dysfunction in non-pregnant women [19, 46, 47].

In our study, however, the associations between Ig glycosylation and placenta-related complications were not mediated by uterine artery resistance at 13 weeks' gestation. This might suggest more of an immunological rather than vascular contribution of glycosylation to the development of placenta-related complications. However, it is also possible that vascular consequences of altered glycosylation emerge later in pregnancy and are not yet reflected in early Doppler measurements.

Cord blood

Because neonatal IgG is transferred across the placenta, the observed negative correlations between placenta-related disorders and IgG galactosylation and sialylation likely reflect altered maternal glycosylation profiles rather than selective placental transfer, which we could not differentiate due to the absence of maternal samples at delivery. Interestingly, galactosylation showed a mixed pattern: it correlated positively with preeclampsia and preterm birth, but negatively with gestational hypertension and small-for-gestational-age birth. These divergent associations may indicate distinct underlying mechanisms or reflect differences in disease severity. However, as we could not adjust for potential confounding factors, these findings should be interpreted cautiously, and further studies are needed to disentangle the direction and causality of these relation-

ships. SES glycosylation traits in IgA2 also correlated with gestational age, likely reflecting the fetal developmental stage, as IgA is produced endogenously by the fetus.

Strengths and limitations

This study's longitudinal design allowed characterization of glycosylation trajectories from the first trimester onwards and to investigate them in relation to placenta-related complications. The combined assessment of immune and vascular parameters allowed exploration of their potential interplay, and the absence of mediation suggests these processes may act independently, highlighting the value of studying them in parallel. Sensitivity analyses supported the robustness of our findings. Reclassifying spontaneous preterm births did not substantially alter the overall pattern of associations, though significance was attenuated, likely due to reduced statistical power. Notably, 5 of 16 spontaneous preterm births co-occurred with SGA, suggesting shared pathophysiology and supporting their inclusion in the complication group. In hypertensive disorders of pregnancy, consistent differences in IgG galactosylation and sialylation were observed, though limited sample size reduced power for subtler effects. Nonetheless, several limitations should be acknowledged. While we hypothesize that glycosylation changes may precede and contribute to the development of placenta-related complications, the observational nature of this study prevents us from establishing causality, and it remains possible that these changes are a consequence rather than a cause of the complications. Although we adjusted for known confounders such as BMI, mode of conception, and parity, residual confounding from unmeasured factors cannot be excluded inherent to the study design. The sample size, while sufficient for initial exploration, was modest and may have limited statistical power, particularly in subgroup analyses such as those restricted to hypertensive disorders. Furthermore, while false discovery rate correction was applied to account for multiple testing, this conservative approach may have masked biologically relevant associations. To address this, we also examined trends across immunoglobulin subtypes and glycosylation traits, which showed consistency of our findings beyond strict statistical thresholds.

Perspectives

Maternal IgA and IgG glycosylation profiles are altered in placenta-related complications. These changes may not only mirror immune dysregulation toward a proinflammatory state but also contribute functionally to disease pathogenesis through enhanced Fcγ receptor and complement-mediated inflammation. Glycosylation thus represents a promising integrative biomarker linking maternal immune status to placental health. To determine whether these alterations are a cause or consequence of disease, future research should aim to disentangle temporal and mechanistic relationships. Experimental approaches such as glycoengineering and in vitro placental models may help clarify causal pathways. In particular, IgG glycosylation warrants further investigation due to its abundance, established associations with disease activity in pregnancy, and links to

long-term cardiovascular risk [19, 40]. Experimental evidence supports the vascular relevance of IgG sialylation: supplementation with N-acetyl-D-mannosamine (ManNAc) restored IgG sialylation and prevented hypertension in mice, suggesting therapeutic potential through modulation of vascular inflammation and endothelial nitric oxide synthase signaling [44]. These findings warrant further exploration of ManNAc supplementation in human pregnancy, particularly to assess whether it enhances IgG sialylation and how this, in turn, may influence blood pressure. Within IgA, the LAGY and LSL glycopeptides emerged as promising biomarker candidates, showing consistent differences across multiple glycosylation traits and subtypes. The pronounced third-trimester LSL differences may, however, reflect downstream consequences rather than causal involvement. Ultimately, elucidating the mechanistic and temporal dynamics of maternal immunoglobulin glycosylation may enable early risk prediction, personalized prevention, and novel therapeutic approaches to improve maternal and fetal outcomes.

SUPPLEMENTARY MATERIAL

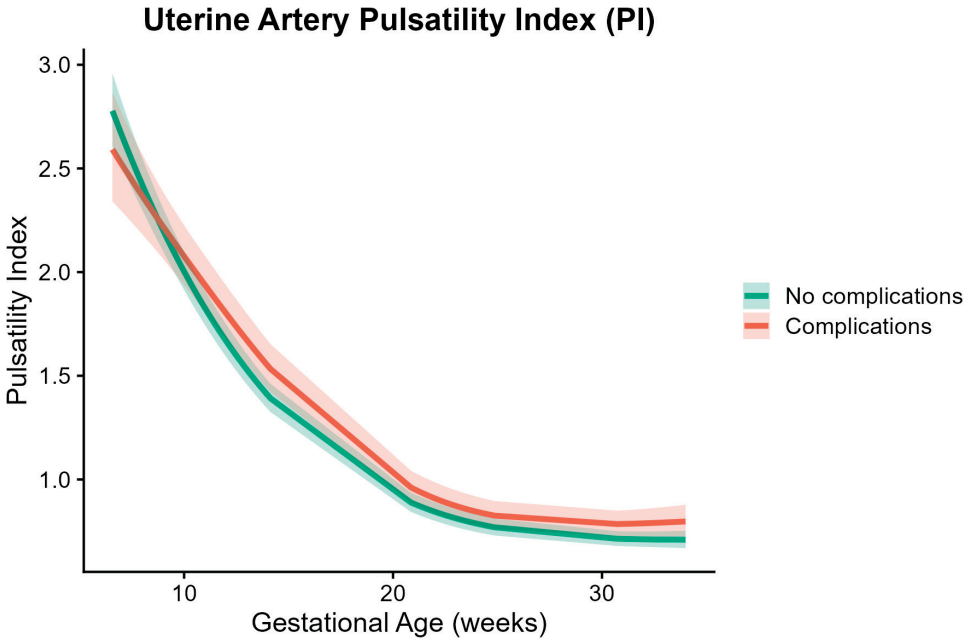


Figure S1. Trajectory of uterine artery pulsatility index in pregnancies with (red) and without (green) placenta-related pregnancy complications. Mixed model analyses of individual trajectories revealed a significant interaction with placenta complications, indicating that uterine artery PI followed a different course in complicated versus uncomplicated pregnancies. The divergence was most pronounced in the first trimester, where PI was initially lower and then decreased less strongly in pregnancies with complications.

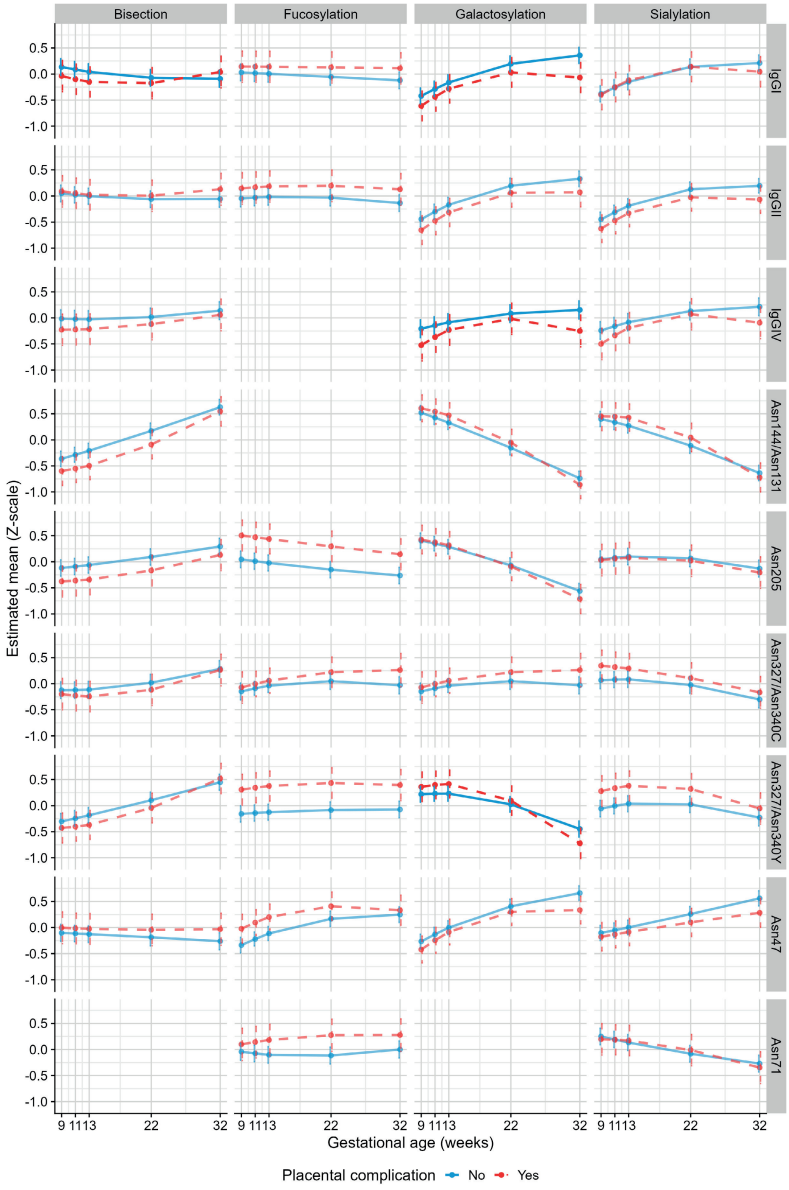


Figure S2: Trajectories of maternal IgG and IgA glycosylation traits across gestation in a sensitivity analysis, in which spontaneous preterm birth without other placenta-related complications was reclassified as not a placenta-related complication. Lines represent estimated marginal means (Z-standardized values) for pregnancies with placenta-related complications (red, dashed) and without complications (blue, solid), derived from linear mixed-effects models. Each column facet represents a glycosylation trait (fucosylation, bisection, galactosylation, or sialylation), and each row facet represents an individual glycosylation site. Error bars indicate 95% confidence intervals of the estimated marginal means. More intense line colors denote traits with a statistically significant interaction term (placenta complication × gestational age) in the mixed model, while lighter colors indicate non-significant interactions.

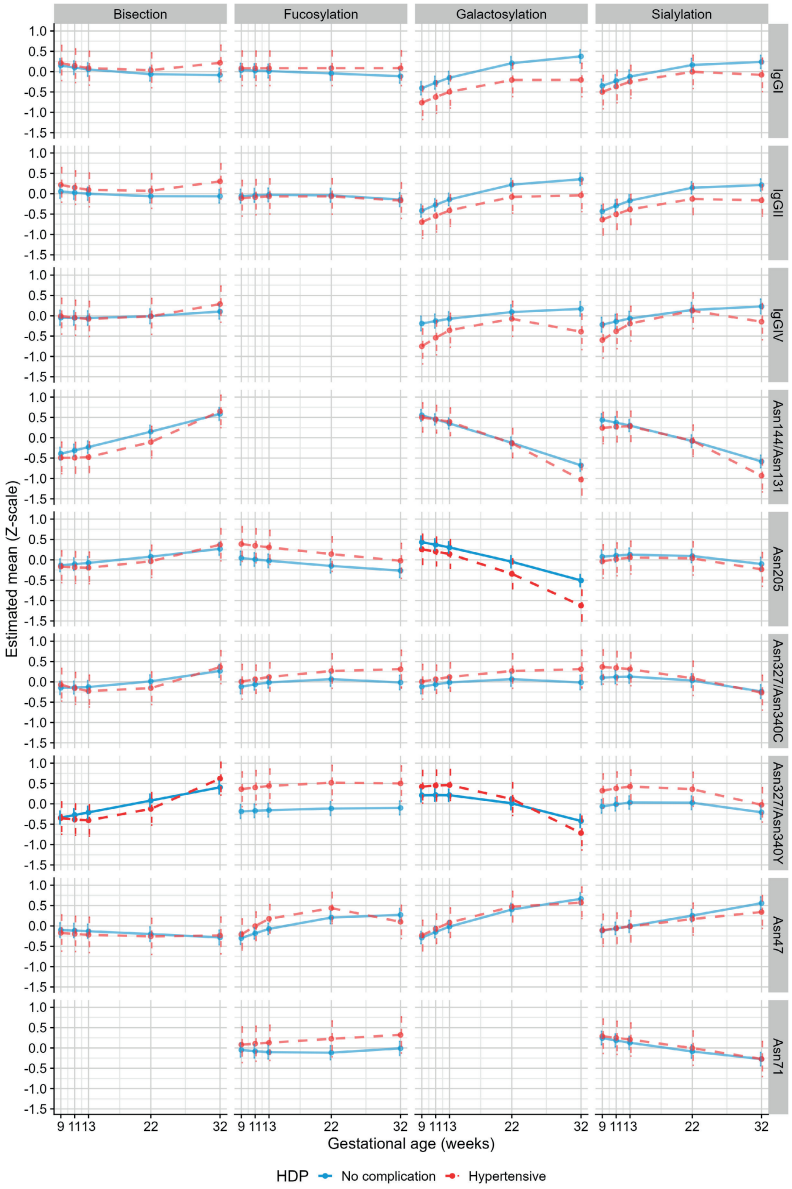


Figure S3: Trajectories of maternal IgG and IgA glycosylation traits across gestation in a sensitivity analysis, comparing hypertensive disorders of pregnancy with uncomplicated pregnancies. Lines represent estimated marginal means (Z-standardized values) for pregnancies with pre-eclampsia or gestational hypertension (red, dashed) and without any placenta-related complication (blue, solid), derived from linear mixed-effects models. Each column facet represents a glycosylation trait (fucosylation, bisection, galactosylation, or sialylation), and each row facet represents an individual glycosylation site. Error bars indicate 95% confidence intervals of the estimated marginal means. More intense line colors denote traits with a statistically significant interaction term (placenta complication × gestational age) in the mixed model, while lighter colors indicate non-significant interactions.

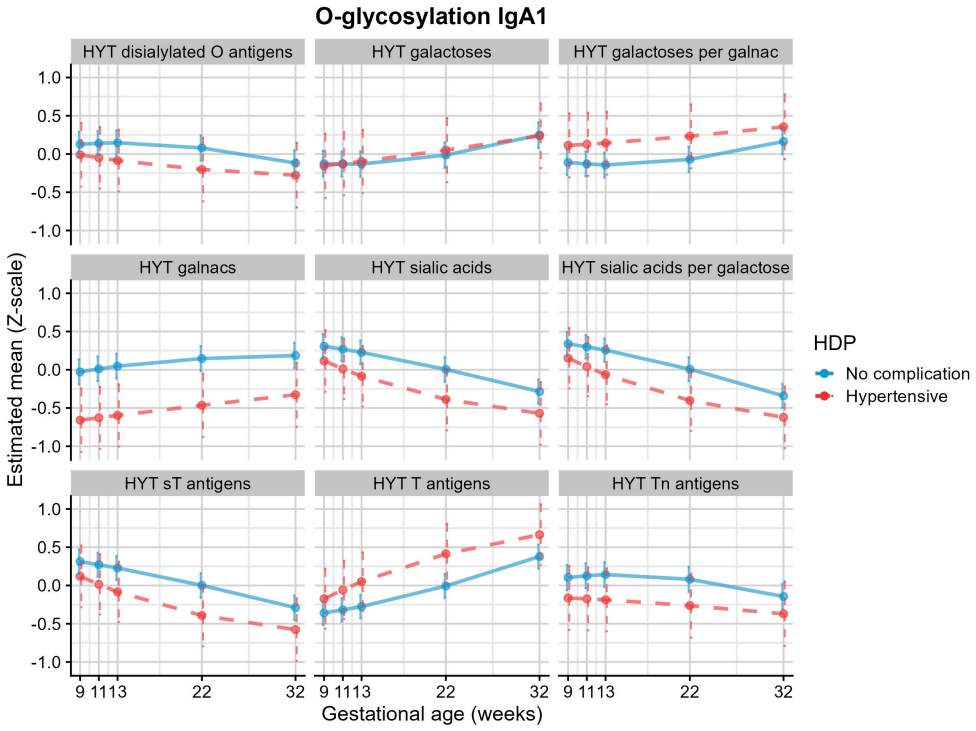


Figure S4. Estimated trajectories of IgA1 O-glycosylation (HYT region) across gestation in pregnancies with hypertensive disorders, compared to those without placenta-related complications. Lines represent estimated marginal means (Z-standardized values) for women with hypertensive disorders (red, dashed) and uncomplicated pregnancies (blue, solid), derived from linear mixed-effects models. Error bars denote 95% confidence intervals of the estimated marginal means.

Table S1: Calculation formulas of composite glycosylation traits.

trait	formula
IgGI_fucosylation	(1 * (IgGI1H3N4F1 + IgGI1H4N4F1 + IgGI1H5N4F1 + IgGI1H4N4F1S1 + IgGI1H5N4F1S1 + IgGI1H5N4F1S2 + IgGI1H3N5F1 + IgGI1H4N5F1 + IgGI1H5N5F1 + IgGI1H4N5F1S1 + IgGI1H5N5F1S1 + IgGI1H4N3F1)) / (IgGI1H3N4F1 + IgGI1H4N4F1 + IgGI1H5N4F1 + IgGI1H4N4F1S1 + IgGI1H5N4F1S1 + IgGI1H5N4F1S2 + IgGI1H3N5F1 + IgGI1H4N5F1 + IgGI1H5N5F1 + IgGI1H4N5F1S1 + IgGI1H5N5F1S1 + IgGI1H3N4 + IgGI1H4N4 + IgGI1H5N4 + IgGI1H5N4S1 + IgGI1H3N5 + IgGI1H4N5 + IgGI1H5N5 + IgGI1H5N5S1 + IgGI1H4N3F1) * 100
IgGI_bisection	(1 * (IgGI1H3N5F1 + IgGI1H4N5F1 + IgGI1H5N5F1 + IgGI1H4N5F1S1 + IgGI1H5N5F1S1 + IgGI1H3N5 + IgGI1H4N5 + IgGI1H5N5 + IgGI1H5N5S1)) / (IgGI1H3N4F1 + IgGI1H4N4F1 + IgGI1H5N4F1 + IgGI1H4N4F1S1 + IgGI1H5N4F1S1 + IgGI1H5N4F1S2 + IgGI1H3N5F1 + IgGI1H4N5F1 + IgGI1H5N5F1 + IgGI1H4N5F1S1 + IgGI1H5N5F1S1 + IgGI1H3N4 + IgGI1H4N4 + IgGI1H5N4 + IgGI1H5N4S1 + IgGI1H3N5 + IgGI1H4N5 + IgGI1H5N5 + IgGI1H5N5S1 + IgGI1H4N3F1) * 100
IgGI_galactosylation	(0.5 * (IgGI1H4N4F1 + IgGI1H4N4F1S1 + IgGI1H4N5F1 + IgGI1H4N5F1S1 + IgGI1H4N4 + IgGI1H4N5) + 1 * (IgGI1H5N4F1 + IgGI1H5N4F1S1 + IgGI1H5N4F1S2 + IgGI1H5N5F1 + IgGI1H5N5F1S1 + IgGI1H5N4 + IgGI1H5N4S1 + IgGI1H5N5 + IgGI1H5N5S1 + IgGI1H4N3F1)) / (IgGI1H3N4F1 + IgGI1H4N4F1 + IgGI1H5N4F1 + IgGI1H4N4F1S1 + IgGI1H5N4F1S1 + IgGI1H5N4F1S2 + IgGI1H3N5F1 + IgGI1H4N5F1 + IgGI1H5N5F1 + IgGI1H4N5F1S1 + IgGI1H5N5F1S1 + IgGI1H3N4 + IgGI1H4N4 + IgGI1H5N4 + IgGI1H5N4S1 + IgGI1H3N5 + IgGI1H4N5 + IgGI1H5N5 + IgGI1H5N5S1 + IgGI1H4N3F1) * 100
IgGI_sialylation	(0.5 * (IgGI1H4N4F1S1 + IgGI1H5N4F1S1 + IgGI1H4N5F1S1 + IgGI1H5N5F1S1 + IgGI1H5N4S1 + IgGI1H5N5S1) + 1 * IgGI1H5N4F1S2) / (IgGI1H3N4F1 + IgGI1H4N4F1 + IgGI1H5N4F1 + IgGI1H4N4F1S1 + IgGI1H5N4F1S1 + IgGI1H5N4F1S2 + IgGI1H3N5F1 + IgGI1H4N5F1 + IgGI1H5N5F1 + IgGI1H4N5F1S1 + IgGI1H5N5F1S1 + IgGI1H3N4 + IgGI1H4N4 + IgGI1H5N4 + IgGI1H5N4S1 + IgGI1H3N5 + IgGI1H4N5 + IgGI1H5N5 + IgGI1H5N5S1 + IgGI1H4N3F1) * 100
IgGI_hybrid	1 * (IgGI1H6N3F1S1 + IgGI1H6N4F1)
IgGII_fucosylation	1 * (IgGII1H3N4F1 + IgGII1H4N4F1 + IgGII1H5N4F1 + IgGII1H4N4F1S1 + IgGII1H5N4F1S1 + IgGII1H3N5F1 + IgGII1H4N5F1 + IgGII1H5N5F1 + IgGII1H5N5F1S1)
IgGII_bisection	1 * (IgGII1H3N5F1 + IgGII1H4N5F1 + IgGII1H5N5F1 + IgGII1H5N5F1S1)
IgGII_galactosylation	0.5 * (IgGII1H4N4F1 + IgGII1H4N4F1S1 + IgGII1H4N5F1 + IgGII1H4N4) + 1 * (IgGII1H5N4F1 + IgGII1H5N4F1S1 + IgGII1H5N5F1 + IgGII1H5N5F1S1)
IgGII_sialylation	0.5 * (IgGII1H4N4F1S1 + IgGII1H5N4F1S1 + IgGII1H5N5F1S1)
IgGIV_bisection	1 * (IgGIV1H3N5F1 + IgGIV1H4N5F1 + IgGIV1H5N5F1)
IgGIV_galactosylation	0.5 * (IgGIV1H4N4F1 + IgGIV1H4N4F1S1 + IgGIV1H4N5F1) + 1 * (IgGIV1H5N4F1 + IgGIV1H5N4F1S1 + IgGIV1H5N5F1)
IgGIV_sialylation	0.5 * (IgGIV1H4N4F1S1 + IgGIV1H5N4F1S1)
IgGI_sialylation_per_galactose	IgGI_sialylation / IgGI_galactosylation * 100
IgGII_sialylation_per_galactose	IgGII_sialylation / IgGII_galactosylation * 100
IgGIV_sialylation_per_galactose	IgGIV_sialylation / IgGIV_galactosylation * 100

Table S1: Calculation formulas of composite glycosylation traits. (continued)

trait	formula
SES_fucosylation	1 * (SES1H4N5F1 + SES1H4N5F1S1 + SES1H4N5F2 + SES1H4N5F2S1 + SES1H5N4F1S2 + SES1H5N5F1S1 + SES1H5N5F1S2 + SES1H5N6F1 + SES1H5N6F2 + SES1H5N6F1S1 + SES1H5N6F1S2 + SES1H5N6F2S2)
SES_antennary_fucosylation	1 * (SES1H4N5F2 + SES1H4N5F2S1 + SES1H5N6F2 + SES1H5N6F2S2)
SES_bisection	1 * (SES1H4N5F1 + SES1H4N5F1S1 + SES1H4N5F2 + SES1H4N5F2S1 + SES1H5N5S1 + SES1H5N5F1S1 + SES1H5N5F1S2 + SES1H5N6F1 + SES1H5N6F2 + SES1H5N6F1S1 + SES1H5N6F1S2 + SES1H5N6F2S2)
SES_galactosylation	0.5 * (SES1H4N5F1 + SES1H4N5F1S1 + SES1H4N5F2 + SES1H4N5F2S1) + 1 * (SES1H5N4F1S2 + SES1H5N5S1 + SES1H5N5F1S1 + SES1H5N5F1S2) + 0.667 * (SES1H5N6F1 + SES1H5N6F2 + SES1H5N6F1S1 + SES1H5N6F1S2 + SES1H5N6F2S2)
SES_sialylation	0.5 * (SES1H4N5F1S1 + SES1H4N5F2S1 + SES1H5N5S1 + SES1H5N5F1S1) + 1 * (SES1H5N4F1S2 + SES1H5N5F1S2) + 0.333 * SES1H5N6F1S1 + 0.667 * (SES1H5N6F1S2 + SES1H5N6F2S2)
SES_tri_antennary	1 * (SES1H5N6F1 + SES1H5N6F2 + SES1H5N6F1S1 + SES1H5N6F1S2 + SES1H5N6F2S2)
SES_sialylation_per_galactose	SES_sialylation / SES_galactosylation * 100
LSL_bisection	1 * (LSL1H3N5 + LSL1H4N5 + LSL1H4N5S1 + LSL1H5N5S1)
LSL_galactosylation	0.5 * (LSL1H4N5 + LSL1H4N5S1) + 1 * (LSL1H5N4S1 + LSL1H5N4S2 + LSL1H5N5S1)
LSL_sialylation	0.5 * (LSL1H4N5S1 + LSL1H5N4S1 + LSL1H5N5S1) + 1 * LSL1H5N4S2
LSL_sialylation_per_galactose	LSL_sialylation / LSL_galactosylation * 100
TPL_fucosylation	1 * (TPL1H4N5F1S1 + TPL1H5N4F1S1 + TPL1H5N4F1S2 + TPL1H5N5F1S1 + TPL1H5N5F1S2 + TPL1H6N5F1S1)
TPL_bisection	1 * (TPL1H4N5F1S1 + TPL1H5N5S1 + TPL1H5N5F1S1 + TPL1H5N5F1S2)
TPL_galactosylation	0.5 * TPL1H4N5F1S1 + 1 * (TPL1H5N4F1S1 + TPL1H5N4F1S2 + TPL1H5N5S1 + TPL1H5N5F1S1 + TPL1H5N5F1S2 + TPL1H6N5F1S1)
TPL_sialylation	0.5 * (TPL1H4N5F1S1 + TPL1H5N4F1S1 + TPL1H5N5S1 + TPL1H5N5F1S1) + 1 * (TPL1H5N4F1S2 + TPL1H5N5F1S2) + 0.333 * TPL1H6N5F1S1
TPL_sialylation_per_galactose	TPL_sialylation / TPL_galactosylation * 100
HYT_sialic_acids	(2 * (HYT1H2N3S2 + HYT1H2N4S2 + HYT1H3N3S2 + HYT1H3N4S2 + HYT1H3N5S2 + HYT1H3N6S2 + HYT1H4N4S2 + HYT1H4N5S2 + HYT1H4N6S2 + HYT1H5N5S2 + HYT1H5N6S2) + 1 * (HYT1H2N5S1 + HYT1H3N3S1 + HYT1H3N4S1 + HYT1H3N5S1 + HYT1H3N6S1 + HYT1H4N4S1 + HYT1H4N5S1 + HYT1H5N5S1) + 3 * (HYT1H3N3S3 + HYT1H3N4S3 + HYT1H3N5S3 + HYT1H3N6S3 + HYT1H4N4S3 + HYT1H4N5S3 + HYT1H4N6S3 + HYT1H5N5S3 + HYT1H5N6S3) + 4 * (HYT1H3N3S4 + HYT1H3N4S4 + HYT1H4N4S4 + HYT1H4N5S4 + HYT1H5N5S4 + HYT1H3N5S4) + 5 * HYT1H4N4S5) / 100

Table S1: Calculation formulas of composite glycosylation traits. (continued)

trait	formula
HYT_galactoses	(2 * (HYT1H2N3S2 + HYT1H2N4S2 + HYT1H2N5S1) + 3 * (HYT1H3N3S1 + HYT1H3N3S2 + HYT1H3N3S3 + HYT1H3N3S4 + HYT1H3N4 + HYT1H3N4S1 + HYT1H3N4S2 + HYT1H3N4S3 + HYT1H3N4S4 + HYT1H3N5 + HYT1H3N5S1 + HYT1H3N5S2 + HYT1H3N5S3 + HYT1H3N6S1 + HYT1H3N6S2 + HYT1H3N6S3 + HYT1H3N5S4) + 4 * (HYT1H4N4 + HYT1H4N4S1 + HYT1H4N4S2 + HYT1H4N4S3 + HYT1H4N4S4 + HYT1H4N4S5 + HYT1H4N5 + HYT1H4N5S1 + HYT1H4N5S2 + HYT1H4N5S3 + HYT1H4N5S4 + HYT1H4N6S2 + HYT1H4N6S3 + HYT1H4N6) + 5 * (HYT1H5N5 + HYT1H5N5S1 + HYT1H5N5S2 + HYT1H5N5S3 + HYT1H5N5S4 + HYT1H5N6S3 + HYT1H5N6S2)) / 100
HYT_galnacs	(3 * (HYT1H2N3S2 + HYT1H3N3S1 + HYT1H3N3S2 + HYT1H3N3S3 + HYT1H3N3S4) + 4 * (HYT1H2N4S2 + HYT1H3N4 + HYT1H3N4S1 + HYT1H3N4S2 + HYT1H3N4S3 + HYT1H3N4S4 + HYT1H4N4 + HYT1H4N4S1 + HYT1H4N4S2 + HYT1H4N4S3 + HYT1H4N4S4 + HYT1H4N4S5) + 5 * (HYT1H2N5S1 + HYT1H3N5 + HYT1H3N5S1 + HYT1H3N5S2 + HYT1H3N5S3 + HYT1H4N5 + HYT1H4N5S1 + HYT1H4N5S2 + HYT1H4N5S3 + HYT1H4N5S4 + HYT1H5N5 + HYT1H5N5S1 + HYT1H5N5S2 + HYT1H5N5S3 + HYT1H5N5S4 + HYT1H3N5S4) + 6 * (HYT1H3N6S1 + HYT1H3N6S2 + HYT1H3N6S3 + HYT1H4N6S2 + HYT1H4N6S3 + HYT1H5N6S3 + HYT1H4N6 + HYT1H5N6S2)) / 100
HYT_Tn_antigens	(1 * (HYT1H2N3S2 + HYT1H3N4 + HYT1H3N4S1 + HYT1H3N4S2 + HYT1H3N4S3 + HYT1H3N4S4 + HYT1H4N5 + HYT1H4N5S1 + HYT1H4N5S2 + HYT1H4N5S3 + HYT1H4N5S4 + HYT1H5N6S3 + HYT1H5N6S2) + 2 * (HYT1H2N4S2 + HYT1H3N5 + HYT1H3N5S1 + HYT1H3N5S2 + HYT1H3N5S3 + HYT1H4N6S2 + HYT1H4N6S3 + HYT1H4N6 + HYT1H3N5S4) + 3 * (HYT1H2N5S1 + HYT1H3N6S1 + HYT1H3N6S2 + HYT1H3N6S3)) / 100
HYT_T_antigens	(1 * (HYT1H2N5S1 + HYT1H3N3S2 + HYT1H3N4S2 + HYT1H3N5S2 + HYT1H3N6S2 + HYT1H4N4S3 + HYT1H4N5S3 + HYT1H4N6S3 + HYT1H5N5S4) + 2 * (HYT1H3N3S1 + HYT1H3N4S1 + HYT1H3N5S1 + HYT1H3N6S1 + HYT1H4N4S2 + HYT1H4N5S2 + HYT1H4N6S2 + HYT1H5N5S3 + HYT1H5N6S3) + 3 * (HYT1H3N4 + HYT1H3N5 + HYT1H4N4S1 + HYT1H4N5S1 + HYT1H5N5S2 + HYT1H5N6S2) + 4 * (HYT1H4N4 + HYT1H4N5 + HYT1H5N5S1 + HYT1H4N6) + 5 * HYT1H5N5) / 100
HYT_sT_antigens	(2 * (HYT1H2N3S2 + HYT1H2N4S2 + HYT1H3N3S2 + HYT1H3N4S2 + HYT1H3N5S2 + HYT1H3N6S2 + HYT1H4N4S2 + HYT1H4N5S2 + HYT1H4N6S2 + HYT1H5N5S2 + HYT1H5N6S2) + 1 * (HYT1H2N5S1 + HYT1H3N3S1 + HYT1H3N4S1 + HYT1H3N5S1 + HYT1H3N6S1 + HYT1H4N4S1 + HYT1H4N5S1 + HYT1H5N5S1) + 3 * (HYT1H3N3S3 + HYT1H3N3S4 + HYT1H3N4S3 + HYT1H3N4S4 + HYT1H3N5S3 + HYT1H3N6S3 + HYT1H4N4S3 + HYT1H4N5S3 + HYT1H4N6S3 + HYT1H5N5S3 + HYT1H5N6S3 + HYT1H3N5S4) + 4 * (HYT1H4N4S4 + HYT1H4N4S5 + HYT1H4N5S4 + HYT1H5N5S4)) / 100
HYT_disialylated_O_antigens	1 * (HYT1H3N3S4 + HYT1H3N4S4 + HYT1H4N4S5 + HYT1H3N5S4)
HYT_sialic_acids_per_galactose	HYT_sialic_acids / HYT_galactoses
HYT_galactoses_per_galnac	HYT_galactoses / HYT_galnacs
ENI_fucosylation	(1 * (ENI1H5N4F1S1 + ENI1H5N4F1S2)) / (ENI1H5N4F1S1 + ENI1H5N4F1S2 + ENI1H5N4S1 + ENI1H5N4S2 + ENI1H5N5S1) * 100

Table S1: Calculation formulas of composite glycosylation traits. (continued)

trait	formula
ENI_sialylation	(0.5 * (ENI1H5N4F1S1 + ENI1H5N4S1 + ENI1H5N5S1) + 1 * (ENI1H5N4F1S2 + ENI1H5N4S2)) / (ENI1H5N4F1S1 + ENI1H5N4F1S2 + ENI1H5N4S1 + ENI-1H5N4S2 + ENI1H5N5S1) * 100
ENI_hybrid	1 * (ENI1H5N3F1S1 + ENI1H5N3S1)
LAGC_fucosylation	1 * (LAGC1H5N4F1S1 + LAGC1H5N4F1S2 + LAGC1H5N5F1S1 + LAGC1H-5N5F1S2 + LAGC1H6N5F1S1 + LAGC1H6N5F1S2 + LAGC1H6N5F1S3 + LAGC1H6N6F1S2 + LAGC1H5N4F1S2Su1 + LAGC1H6N5F1S1Su1)
LAGC_bisection	1 * (LAGC1H5N5F1S1 + LAGC1H5N5F1S2 + LAGC1H5N6S2 + LAGC1H6N6F1S2)
LAGC_galactosylation	1 * (LAGC1H5N4F1S1 + LAGC1H5N4F1S2 + LAGC1H5N5F1S1 + LAGC1H-5N5F1S2 + LAGC1H6N5F1S1 + LAGC1H6N5F1S2 + LAGC1H6N5F1S3 + LAGC1H6N6F1S2 + LAGC1H5N4F1S2Su1 + LAGC1H6N5F1S1Su1) + 0.667 * LAGC1H5N6S2
LAGC_sialylation	0.5 * (LAGC1H5N4F1S1 + LAGC1H5N5F1S1) + 1 * (LAGC1H5N4F1S2 + LAGC1H-5N5F1S2 + LAGC1H6N5F1S3 + LAGC1H5N4F1S2Su1) + 0.333 * (LAGC1H-6N5F1S1 + LAGC1H6N5F1S1Su1) + 0.667 * (LAGC1H6N5F1S2 + LAGC1H5N6S2 + LAGC1H6N6F1S2)
LAGC_tri_antennary	1 * (LAGC1H6N5F1S1 + LAGC1H6N5F1S2 + LAGC1H6N5F1S3 + LAGC1H5N6S2 + LAGC1H6N6F1S2 + LAGC1H6N5F1S1Su1)
LAGY_fucosylation	(1 * (LAGY1H4N5F1 + LAGY1H5N4F1S1 + LAGY1H5N4F1S2 + LAGY1H5N5F1 + LAGY1H5N5F1S1 + LAGY1H5N5F1S2 + LAGY1H6N5F1S1 + LAGY1H6N5F1S2 + LAGY1H5N4F1S2Su1 + LAGY1H5N5F1S2Su1 + LAGY1H6N5F1S2Su1 + LAGY1H6N6F1S2Su1)) / (LAGY1H4N5F1 + LAGY1H5N4S1 + LAGY1H5N4S2 + LAGY1H5N4F1S1 + LAGY1H5N4F1S2 + LAGY1H5N5F1 + LAGY1H5N5F1S1 + LAGY1H5N5F1S2 + LAGY1H6N5F1S1 + LAGY1H6N5F1S2 + LAGY1H5N4F1S-2Su1 + LAGY1H5N5F1S2Su1 + LAGY1H6N5F1S2Su1 + LAGY1H6N6F1S2Su1) * 100
LAGY_bisection	(1 * (LAGY1H4N5F1 + LAGY1H5N5F1 + LAGY1H5N5F1S1 + LAGY1H-5N5F1S2 + LAGY1H5N5F1S2Su1 + LAGY1H6N6F1S2Su1)) / (LAGY1H4N5F1 + LAGY1H5N4S1 + LAGY1H5N4S2 + LAGY1H5N4F1S1 + LAGY1H5N4F1S2 + LAGY1H5N5F1 + LAGY1H5N5F1S1 + LAGY1H5N5F1S2 + LAGY1H6N5F1S1 + LAGY1H6N5F1S2 + LAGY1H5N4F1S2Su1 + LAGY1H5N5F1S2Su1 + LAGY1H-6N5F1S2Su1 + LAGY1H6N6F1S2Su1) * 100
LAGY_galactosylation	(0.5 * LAGY1H4N5F1 + 1 * (LAGY1H5N4S1 + LAGY1H5N4S2 + LAGY1H5N4F1S1 + LAGY1H5N4F1S2 + LAGY1H5N5F1 + LAGY1H5N5F1S1 + LAGY1H5N5F1S2 + LAGY1H6N5F1S1 + LAGY1H6N5F1S2 + LAGY1H5N4F1S2Su1 + LAGY1H-5N5F1S2Su1 + LAGY1H6N5F1S2Su1 + LAGY1H6N6F1S2Su1)) / (LAGY1H4N5F1 + LAGY1H5N4S1 + LAGY1H5N4S2 + LAGY1H5N4F1S1 + LAGY1H5N4F1S2 + LAGY1H5N5F1 + LAGY1H5N5F1S1 + LAGY1H5N5F1S2 + LAGY1H6N5F1S1 + LAGY1H6N5F1S2 + LAGY1H5N4F1S2Su1 + LAGY1H5N5F1S2Su1 + LAGY1H-6N5F1S2Su1 + LAGY1H6N6F1S2Su1) * 100

Table S1: Calculation formulas of composite glycosylation traits. (continued)

trait	formula
LAGY_sialylation	(0.5 * (LAGY1H5N4S1 + LAGY1H5N4F1S1 + LAGY1H5N5F1S1) + 1 * (LAGY1H5N4S2 + LAGY1H5N4F1S2 + LAGY1H5N5F1S2 + LAGY1H5N4F1S2Su1 + LAGY1H5N5F1S2Su1) + 0.333 * LAGY1H6N5F1S1 + 0.667 * (LAGY1H6N5F1S2 + LAGY1H6N5F1S2Su1 + LAGY1H6N6F1S2Su1)) / (LAGY1H4N5F1 + LAGY1H5N4S1 + LAGY1H5N4S2 + LAGY1H5N4F1S1 + LAGY1H5N4F1S2 + LAGY1H5N5F1 + LAGY1H5N5F1S1 + LAGY1H5N5F1S2 + LAGY1H6N5F1S1 + LAGY1H6N5F1S2 + LAGY1H5N4F1S2Su1 + LAGY1H5N5F1S2Su1 + LAGY1H6N5F1S2Su1 + LAGY1H6N6F1S2Su1) * 100
LAGY_tri_antennary	(1 * (LAGY1H6N5F1S1 + LAGY1H6N5F1S2 + LAGY1H6N5F1S2Su1 + LAGY1H6N6F1S2Su1)) / (LAGY1H4N5F1 + LAGY1H5N4S1 + LAGY1H5N4S2 + LAGY1H5N4F1S1 + LAGY1H5N4F1S2 + LAGY1H5N5F1 + LAGY1H5N5F1S1 + LAGY1H5N5F1S2 + LAGY1H6N5F1S1 + LAGY1H6N5F1S2 + LAGY1H5N4F1S2Su1 + LAGY1H5N5F1S2Su1 + LAGY1H6N5F1S2Su1 + LAGY1H6N6F1S2Su1) * 100
LAGC_sialylation_per_galactose	LAGC_sialylation / LAGC_galactosylation * 100
LAGY_sialylation_per_galactose	LAGY_sialylation / LAGY_galactosylation * 100

Table S2. Summary of coexisting placenta-related pregnancy complications.

Complication	N patients
Preeclampsia alone	2
Preeclampsia with preterm birth and SGA	3
Preeclampsia with preterm birth without SGA	2
Pregnancy-induced hypertension alone	12
Pregnancy-induced hypertension with preterm birth without SGA	1
Pregnancy-induced hypertension without preterm birth with SGA	2
Preterm birth alone	12
Preterm birth with SGA	6
SGA alone	14

Supporting information

Uterine artery pulsatility index

At 13 weeks timepoint, there was a significant higher mean uterine artery pulsatility index in women with placenta-related complications (1.88 vs. 1.57, p=0.041, **Table S3**). Mixed model analyses of individual trajectories revealed a significant interaction with placenta complications, indicating that uterine artery PI followed a different course in complicated versus uncomplicated pregnancies. The divergence was most pronounced in the first trimester, where PI was initially lower and then decreased less strongly in pregnancies with complications (**Figure S1**).

Table S3. Uterine artery pulsatility index in pregnancies with and without placenta-related complications. Mean of left and right uterine artery pulsatility index.

	No complication			**Placenta-related complication**			
Weeks GA	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**p-value**
7	82	2,68	0,81	27	2,55	1,14	0,586
9	132	2,17	0,61	49	2,25	1,05	0,605
11	131	1,83	0,86	54	2,01	0,86	0,207
13	132	**1,57**	**0,63**	50	**1,88**	**0,95**	**0,041**
22	132	0,87	0,32	51	0,91	0,30	0,514
32	134	0,72	0,24	45	0,81	0,27	0,079

REFERENCES

1. Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. Lancet. 2010 Aug 21;376(9741):631-44.
2. Flenady V, Koopmans L, Middleton P, Froen JF, Smith GC, Gibbons K, et al. Major risk factors for stillbirth in high-income countries: a systematic review and meta-analysis. Lancet. 2011 Apr 16;377(9774):1331-40.
3. Lawn JE, Kinney M. Preterm birth: now the leading cause of child death worldwide. Sci Transl Med. 2014 Nov 19;6(263):263ed21.
4. Cnossen JS, Morris RK, ter Riet G, Mol BW, van der Post JA, Coomarasamy A, et al. Use of uterine artery Doppler ultrasonography to predict pre-eclampsia and intrauterine growth restriction: a systematic review and bivariable meta-analysis. CMAJ. 2008 Mar 11;178(6):701-11.
5. Jauniaux E, Poston L, Burton GJ. Placental-related diseases of pregnancy: Involvement of oxidative stress and implications in human evolution. Hum Reprod Update. 2006 Nov-Dec;12(6):747-55.
6. Arnold JN, Wormald MR, Sim RB, Rudd PM, Dwek RA. The impact of glycosylation on the biological function and structure of human immunoglobulins. Annu Rev Immunol. 2007;25:21-50.
7. Radovani B, Nimmerjahn F. IgG Glycosylation: Biomarker, Functional Modulator, and Structural Component. J Immunol. 2024 Dec 1;213(11):1573-84.
8. Dekkers G, Treffers L, Plomp R, Bentlage AEH, Boer Md, Koeleman CAM, et al. Decoding the human immunoglobulin G-glycan repertoire reveals a spectrum of Fc-receptor- and complement-mediated-effector activities. Frontiers in Immunology. 2017 2017/8//;8(AUG).
9. Choi H, Yang SW, Joo JS, Park M, Jin Y, Kim JW, et al. Sialylated IVIg binding to DC-SIGN(+) Hofbauer cells induces immune tolerance through the caveolin-1/NF-kB pathway and IL-10 secretion. Clin Immunol. 2023 Jan;246:109215.
10. Pincetic A, Bournazos S, DiLillo DJ, Maamary J, Wang TT, Dahan R, et al. Type I and type II Fc receptors regulate innate and adaptive immunity. Nat Immunol. 2014 Aug;15(8):707-16.
11. Selman MH, Derks RJ, Bondt A, Palmblad M, Schoenmaker B, Koeleman CA, et al. Fc specific IgG glycosylation profiling by robust nano-reverse phase HPLC-MS using a sheath-flow ESI sprayer interface. J Proteomics. 2012 Feb 2;75(4):1318-29.
12. van de Geijn FE, Wuhrer M, Selman MH, Willemsen SP, de Man YA, Deelder AM, et al. Immunoglobulin G galactosylation and sialylation are associated with pregnancy-induced improvement of rheumatoid arthritis and the postpartum flare: results from a large prospective cohort study. Arthritis Res Ther. 2009;11(6):R193.
13. Mor G, Aldo P, Alvero AB. The unique immunological and microbial aspects of pregnancy. Nat Rev Immunol. 2017 Aug;17(8):469-82.
14. Zhong J, Li J, Burton GJ, Koistinen H, Cheung KW, Ng EHY, et al. The functional roles of protein glycosylation in human maternal-fetal crosstalk. Hum Reprod Update. 2024 Jan 3;30(1):81-108.
15. Bezemer RE, Faas MM, van Goor H, Gordijn SJ, Prins JR. Decidual macrophages and Hofbauer cells in fetal growth restriction. Front Immunol. 2024;15:1379537.
16. Voskamp LW, Daniels, A., Rousian, M., van Hoek, M., Wuhrer, M., Steegers-Theunissen, R. P. M., Danser, A. H. J., & Verdonk, K. Longitudinal Profiling of IgG and IgA Glycosylation in Pregnancy Reveals Early Associations with BMI, Primigravidity and Conception Mode: The Rotterdam Periconception Cohort. 2025.
17. Bartsch E, Medcalf KE, Park AL, Ray JG, High Risk of Pre-eclampsia Identification G. Clinical risk factors for pre-eclampsia determined in early pregnancy: system-

atic review and meta-analysis of large cohort studies. BMJ. 2016 Apr 19;353:i1753.
18. Organization WH. Pre-eclamspia. 2025 [updated April 4, 2025; cited 2025 October 6]; Available from: https://www.who.int/news-room/fact-sheets/detail/pre-eclampsia.
19. Štambuk T, Klasić M, Zoldoš V, Lauc G. N-glycans as functional effectors of genetic and epigenetic disease risk. Molecular Aspects of Medicine. 2021 2021/6//;79:100891-.
20. Mayboroda OA, Lageveen-Kammeijer GSM, Wuhrer M, Dolhain R. An Integrated Glycosylation Signature of Rheumatoid Arthritis. Biomolecules. 2023 Jul 12;13(7).
21. Lu X, Wang L, Wang M, Li Y, Zhao Q, Shi Y, et al. Association between immunoglobulin G N-glycosylation and lupus nephritis in female patients with systemic lupus erythematosus: a case-control study. Front Immunol. 2023;14:1257906.
22. Rousian M, Schoenmakers S, Eggink AJ, Gootjes DV, Koning AHJ, Koster MPH, et al. Cohort Profile Update: The Rotterdam Periconceptional Cohort and embryonic and fetal measurements using 3D ultrasound and virtual reality techniques. International Journal of Epidemiology. 2021 2021/10//;50(5):1426-7L.
23. Steegers-Theunissen RPM, Verheijden-Paulissen JJFM, van Uitert EM, Wildhagen MF, Exalto N, Koning AHJ, et al. Cohort Profile: The Rotterdam Periconceptional Cohort (Predict Study). International journal of epidemiology. 2016 2016/4//;45(2):374-81.
24. Magee LA, Brown MA, Hall DR, Gupte S, Hennessy A, Karumanchi SA, et al. The 2021 International Society for the Study of Hypertension in Pregnancy classification, diagnosis & management recommendations for international practice. Pregnancy hypertension. 2022 2022/3//;27:148-69.
25. Hoftiezer L, Hof MHP, Dijs-Elsinga J, Hogeveen M, Hukkelhoven C, van Lingen RA. From population reference to national standard: new and improved birthweight charts. Am J Obstet Gynecol. 2019 Apr;220(4):383 e1- e17.
26. Reijnders IF, Mulders A, Koster MPH, Koning AHJ, Frudiger A, Willemsen SP, et al. New imaging markers for preconceptional and first-trimester uteroplacental vascularization. Placenta. 2018 Jan;61:96-102.
27. Momcilovic A, de Haan N, Hipgrave Ederveen AL, Bondt A, Koeleman CAM, Falck D, et al. Simultaneous Immunoglobulin A and G Glycopeptide Profiling for High-Throughput Applications. Anal Chem. 2020 Mar 17;92(6):4518-26.
28. Falck D, Wuhrer M. GlYcoLISA: antigen-specific and subclass-specific IgG Fc glycosylation analysis based on an immunosorbent assay with an LC-MS readout. Nat Protoc. 2024 Jun;19(6):1887-909.
29. Faas MM, Spaans F, De Vos P. Monocytes and macrophages in pregnancy and preeclampsia. Front Immunol. 2014;5:298.
30. Ravi Prakash G, Vaidhya A, Deepthi V, Rathore LS, Panigrahi M, Madhu CL, et al. Macrophage Switching in Pregnancy: Regulatory Mechanisms Governing Term Labour and Preterm Birth. Am J Reprod Immunol. 2025 Jul;94(1):e70123.
31. Liu Y, Ma X, Zheng J, Liu X, Yan T. A Systematic Review and Meta-Analysis of Kidney and Pregnancy Outcomes in IgA Nephropathy. Am J Nephrol. 2016;44(3):187-93.
32. Smyth A, Oliveira GH, Lahr BD, Bailey KR, Norby SM, Garovic VD. A systematic review and meta-analysis of pregnancy outcomes in patients with systemic lupus erythematosus and lupus nephritis. Clin J Am Soc Nephrol. 2010 Nov;5(11):2060-8.
33. Verdonk K, Visser W, Russcher H, Danser AH, Steegers EA, van den Meiracker AH. Differential diagnosis of preeclampsia: remember the soluble fms-like tyrosine kinase 1/placental growth factor ratio. Hypertension. 2012 Oct;60(4):884-90.

34. Kaneko Y, Nimmerjahn F, Ravetch JV. Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. Science. 2006 Aug 4;313(5787):670-3.
35. Damelang T, Brinkhaus M, van Osch TLJ, Schuurman J, Labrijn AF, Rispens T, Vidarsson G. Impact of structural modifications of IgG antibodies on effector functions. Front Immunol. 2023;14:1304365.
36. Karsten CM, Pandey MK, Figge J, Kilchenstein R, Taylor PR, Rosas M, et al. Anti-inflammatory activity of IgG1 mediated by Fc galactosylation and association of FcgammaRIIB and dectin-1. Nat Med. 2012 Sep;18(9):1401-6.
37. van Osch TLJ, Nouta J, Derksen NIL, van Mierlo G, van der Schoot CE, Wuhrer M, et al. Fc Galactosylation Promotes Hexamerization of Human IgG1, Leading to Enhanced Classical Complement Activation. J Immunol. 2021 Sep 15;207(6):1545-54.
38. Girardi G, Lingo JJ, Fleming SD, Regal JF. Essential Role of Complement in Pregnancy: From Implantation to Parturition and Beyond. Front Immunol. 2020;11:1681.
39. Simurina M, de Haan N, Vuckovic F, Kennedy NA, Stambuk J, Falck D, et al. Glycosylation of Immunoglobulin G Associates With Clinical Features of Inflammatory Bowel Diseases. Gastroenterology. 2018 Apr;154(5):1320-33 e10.
40. Bondt A, Selman MH, Deelder AM, Hazes JM, Willemsen SP, Wuhrer M, Dolhain RJ. Association between galactosylation of immunoglobulin G and improvement of rheumatoid arthritis during pregnancy is independent of sialylation. J Proteome Res. 2013 Oct 4;12(10):4522-31.
41. Ding L, Chen X, Cheng H, Zhang T, Li Z. Advances in IgA glycosylation and its correlation with diseases. Front Chem. 2022;10:974854.
42. Knoppova B, Reily C, Maillard N, Rizk DV, Moldoveanu Z, Mestecky J, et al. The Origin and Activities of IgA1-Containing Immune Complexes in IgA Nephropathy. Front Immunol. 2016;7:117.
43. Bondt A, Nicolardi S, Jansen BC, Kuijper TM, Hazes JMW, van der Burgt YEM, et al. IgA N- and O-glycosylation profiling reveals no association with the pregnancy-related improvement in rheumatoid arthritis. Arthritis Res Ther. 2017 Jul 5;19(1):160.
44. Peng J, Vongpatanasin W, Sacharidou A, Kifer D, Yuhanna IS, Banerjee S, et al. Supplementation With the Sialic Acid Precursor N-Acetyl-D-Mannosamine Breaks the Link Between Obesity and Hypertension. Circulation. 2019 Dec 10;140(24):2005-18.
45. Osol G, Ko NL, Mandala M. Altered Endothelial Nitric Oxide Signaling as a Paradigm for Maternal Vascular Maladaptation in Preeclampsia. Curr Hypertens Rep. 2017 Sep 23;19(10):82.
46. Plomp R, Ruhaak LR, Uh HW, Reiding KR, Selman M, Houwing-Duistermaat JJ, et al. Subclass-specific IgG glycosylation is associated with markers of inflammation and metabolic health. Sci Rep. 2017 Sep 26;7(1):12325.
47. Birukov A, Plavsa B, Eichelmann F, Kuxhaus O, Hoshi RA, Rudman N, et al. Immunoglobulin G N-Glycosylation Signatures in Incident Type 2 Diabetes and Cardiovascular Disease. Diabetes Care. 2022 Nov 1;45(11):2729-36.

4

Corpus luteum number and circulatory adaptation from early pregnancy onwards: The Rotterdam Periconception Cohort

Lotte W. Voskamp, Joni Koerts, Rosalieke E. Wiegel, Koen Verdonk, A.H. Jan Danser, Régine P.M. Steegers-Theunissen, Melek Rousian

Human Reproduction 2025 Nov 1;40(11):2078-2087

ABSTRACT

Study question: Is the number of corpora lutea (CL) associated with maternal circulatory adaptation to pregnancy, as assessed by blood pressure and uterine artery doppler pulsatility and resistance indices?

Summary answer: Pregnancies without a corpus luteum have a higher mean arterial pressure throughout pregnancy and lower uterine artery pulsatility and resistance indices in the first and second trimester, compared to pregnancies where one or more than one corpus luteum is present.

What is known already: Different modes of conception result in varying numbers of corpus luteum in early pregnancy. Previous research has demonstrated significant differences in hypertensive disorders of pregnancy and birthweight in women with 0, 1 and multiple corpora lutea, as well as altered maternal cardiovascular adaptation. Although direct causal evidence is limited, these differences are thought to reflect the presence or absence of corpus luteum-derived hormones, suboptimal decidualization in programmed cycles, or both.

Study design, size, duration: This prospective study used data from the ongoing Rotterdam Periconception Cohort, including women with singleton pregnancies enrolled from 2010 to 2022 at the Erasmus MC, University Medical Center, a tertiary care facility.

Participants/materials, setting, methods: The study population for this research involved pregnancies in 1,986 women: 1,456 with one corpus luteum (1,292 due to natural conception or insemination and 164 due to natural cycle frozen embryo transfer (NC-FET)), 457 with more than one corpus luteum (due to fresh embryo transfer) and 73 with no corpus luteum (due to artificial cycle (AC)-FET). Linear mixed models were adjusted for maternal age, body mass index, nulliparity, smoking, pre-existing hypertension, and uterine artery Doppler outcomes additionally for mean arterial pressure.

Main results and the role of chance: Adjusted mean arterial pressure during pregnancy was significantly higher in women with 0 versus 1 CL (β +2.19 mmHg, 95% CI [0.43 - 3.95], p=0.015) but was not different between those with >1 and 1 CL (β -0.35 mmHg [-1.22–0.53], p=0.438). This was also true for diastolic, but not for systolic blood pressure. Uterine artery doppler indices were available for 624 women. Adjusted uterine artery pulsatility index (PI) and resistance index (RI) were significantly lower in women with 0 CL compared to 1 CL, both at 11 weeks (PI: 1.53, 95% CI [1.38-1.69] vs 1.72 [1.65-1.79], p=0.026; RI: 0.69, [0.66-0.73] vs 0.73 [0.72-0.75], p=0.034) and at 22 weeks gestational age (PI: 0.64 [0.57-0.72] vs 0.81 [0.78-0.85], p<0.001; RI: 0.44 [0.41-0.46] vs 0.51 [0.50-0.53], p<0.001). In pregnancies with >1 CL, uterine artery indices were comparable to the 1 CL group, except

for a slightly higher RI at 22 weeks (0.54 [0.52-0.55], p=0.011). Restricting the analyses to only pregnancies conceived using assisted reproductive technologies did not change the observed directions of the effects.

Limitations, reasons for caution: This study was conducted in a tertiary hospital setting, which may limit generalizability to other populations. Details on luteal support were incomplete, and the corpus luteum number was inferred based on the mode of conception, which could introduce confounding by indication.

Wider implications of the findings*:* These results align with previous literature and provide robust evidence from a large cohort, with adjusting for confounders. Notably, uterine artery models were additionally adjusted for the observed differences in mean arterial pressure. However, despite this adjustment, the differences in uterine artery indices between CL groups persisted, indicating that these cannot be explained by the higher mean arterial pressure and suggesting the involvement of distinct vascular mechanisms. The observed differences in circulatory adaptation to pregnancy between conceptions with corpus luteum numbers may underlie the higher incidence of hypertensive disorders of pregnancy after conception without a corpus luteum. Additionally, these insights further support the preference for certain assisted reproductive treatments, where feasible, to optimize maternal and neonatal outcomes.

Study funding/competing interest(s): This research was funded by the departments of Obstetrics and Gynaecology and Internal Medicine of the Erasmus MC, University Medical Center Rotterdam, the Netherlands. The authors declare no competing interests.

Trial registration number: This study is registered at the Dutch Trial Register (NTR6854).

INTRODUCTION

The use of assisted reproductive technologies (ART) has increased significantly in recent decades, offering many subfertile couples the possibility of achieving pregnancy. However, pregnancies conceived through ART are associated with slightly increased risks for both maternal and neonatal complications compared to spontaneously conceived pregnancies [1, 2]. Several factors inherent to the ART process may contribute to these risks, such as ovarian stimulation protocols, oocyte handling, laboratory techniques, and culture media [3, 4]. Despite the identification of these factors, the pathophysiological mechanisms associating ART protocols with certain pregnancy complications remain poorly understood. In this context, the number of corpora lutea (CL) present during early pregnancy, determined by the specific ART protocol, has emerged as a potential factor influencing differences in pregnancy outcomes [5].

Previous studies have demonstrated significant variation in incidence of hypertensive disorders of pregnancy (HDP) and birthweight outcomes depending on CL number. Specifically, pregnancies without a CL, conceived through programmed-cycle frozen embryo transfer (AC-FET), are associated with nearly double the risk of preeclampsia and all hypertensive disorders of pregnancy, compared to the physiological state of one CL during natural pregnancy [1, 6, 7]. Moreover, evidence suggests that pregnancies after ovarian stimulation treatment and fresh embryo transfer, and thus conceived in the presence of multiple CL, have an increased risk of neonates born small-for-gestational-age (SGA) compared to the physiological state of one CL [1, 8, 9].

To explain the relationship with the number of CL, several mechanisms have been proposed. A key hypothesis centres on the hormonal milieu associated with CL presence or absence. The CL is known to secrete critical hormones such as relaxin, prorenin and progesterone, which are involved in the maternal adaptation to pregnancy [5, 10]. Indeed, our previous work has demonstrated reduced activity of the renin-angiotensin-aldosterone system (RAAS) in pregnancies lacking a CL [11-13]. This altered hormonal environment is suggested to disrupt maternal circulatory adaptation to pregnancy, resulting in abnormal blood pressure regulation and altered uterine artery blood flow.

Two studies have investigated the relationship between CL number and maternal blood pressure across all trimesters of pregnancy, reporting contrasting results [13, 14].Differences in uterine artery Doppler indices, particularly pulsatility index (PI), between ART protocols have been more frequently studied, with lower PI reported in pregnancies with no CL compared to natural conceptions or fresh embryo transfers [13, 15-18]. However, the studies lacked comparisons across all three CL groups (0, 1, and >1 CL) and no study has systematically assessed these differences throughout pregnancy.

By including a larger cohort and adjusting for potential confounders, this study aims to provide improved statistical power and a more accurate understanding of the relationship between CL number and maternal circulatory adaptation, focusing on blood pressure trajectories and uterine artery Doppler indices from early pregnancy onwards. Understanding these associations may provide insights into the pathophysiological mechanisms underlying the increased risk of hypertensive disorders in pregnancies conceived through ART and contribute to optimizing ART protocols and improving maternal and neonatal outcomes.

METHODS

Participant selection

This study utilizes data from the Rotterdam Periconception Cohort, a prospective observational study conducted at the Erasmus MC, University Medical Center, Rotterdam, the Netherlands [19, 20]. Women aged 18 or older with a singleton intrauterine pregnancy and who were proficient in Dutch were eligible for inclusion. Participants were enrolled before 10 weeks of gestation at the outpatient clinic. To ensure data independence, only the first pregnancy was included for women who participated with multiple pregnancies. Exclusion criteria for this study were ovulation induction, structural fetal anomalies, miscarriage, or unknown mode of conception. The study was approved by the Central Committee on Research in The Hague and the Medical Ethical Committee of Erasmus Medical Center (MEC-2004-227), with written informed consent obtained from all participants.

ART treatment protocols

All ART treatments were conducted in a single centre, ensuring uniform procedures. The protocols for ovarian stimulation, oocyte collection, IVF, ICSI and embryo assessment have been outlined previously [21, 22]. Ovarian stimulation involved recombinant follicle-stimulating hormone or human menopausal gonadotropin, paired with either a gonadotropin-releasing hormone agonist or -antagonist. Final oocyte maturation was triggered by a single dose of human chorionic gonadotropin. Fertilization was performed through standard IVF and ICSI procedures [21]. The procedures used for embryo selection and transfer were described previously [7]. Details on luteal support in different ART protocols outlined below.

In the case of fresh embryo transfer, the luteal support protocols have evolved over the years. From the start of the study cohort in 2010, Utrogestan (progesterone) 200 mg was given vaginally for 12 days, starting 3 days after oocyte collection. Starting in June 2018, Rekovelle (recombinant follicle stimulating hormone) was used as stimulating medication. From then on, Utrogestan was given for 15 days, starting the day after oocyte collection. From March 2024 onwards, Utrogestan was given for 7 weeks, starting on the

evening after oocyte collection. In patients with peanut or soy allergies, Duphaston 10 mg 3 times per day or Crinone progesterone gel 1 time per day were used instead of Utrogestan. Patients diagnosed with hypothalamic or functional anovulation received Pregnyl (human chorionic gonadotropin) 1500 units on day 2, 4 and 6 after oocyte collection. In the case of natural cycle frozen embryo transfer, no luteal support was given. In the case of frozen embryo transfer in an artificial cycle, Progynova was given in increasing daily doses, starting at 1 mg until 3 mg on the day of oocyte collection. Utrogestan was given starting 5 days before embryo transfer until the start of the 12th week of gestation. Afterwards, 2 mg Progynova was given 2 times per day.

Data collection

Data on maternal characteristics, fertility treatment, pregnancy outcomes, and blood pressure measurements, taken in clinical settings from the first trimester to the first week postpartum using manual or automated devices, were collected from electronic health records and study databases. At least four study visits were scheduled at specific weeks of gestation, including two in the first trimester (at 7, 9, 11, and/or 13 weeks of gestation) and two in the second and third trimester (at 22–24 weeks and 30–32 weeks of gestation). During the initial visit, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured in mmHg by a nurse or physician using a manual cuff and stethoscope on the left arm, with the patient seated. Mean arterial pressure (MAP) was calculated as: (SBP + 2 × DBP), divided by 3.

For patients included from December 2016 onwards, bilateral measurements of the uterine artery PI and resistance index (RI) were obtained by trained sonographers at 7, 9, 11, 13, 22 and 30 weeks of gestation. Transvaginal ultrasound was performed in the first trimester, and transabdominal ultrasound in the second and third trimesters, using the Voluson Expert E8 or E10 ultrasound system with a 6-12 MHz transvaginal probe or transabdominal 3D probe (GE Healthcare, Austria) using standard power Doppler settings. At each time point, three measurements were taken on both the right and left sides, and the mean values of each side were used for analysis. Uterine artery notching, defined as a brief interruption or drop in velocity in the early diastolic waveform on Doppler ultrasound, was assessed at each study visit. The presence of a notch was recorded for each uterine artery (left and right), and persistent notching was defined as the presence of a notch across multiple visits during pregnancy.

Gestational age (GA) in natural pregnancies was determined from the first day of the last menstrual period (LMP) for women with regular cycles (21–35 days). For pregnancies conceived via in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI), GA was calculated based on the oocyte retrieval day plus 14 days. In pregnancies from frozen embryo transfers, GA was calculated as the embryo transfer date plus 17 or 19 days, depending on the stage of embryo transfer. If there was a discrepancy of more than six

days between the GA based on LMP and the crown-rump length (CRL) measurement, or if cycles were irregular or the LMP was unknown, GA was based on CRL.

Study groups

Consistent with our previous research, pregnancies were categorized by the number of CL present at implantation, determined by the mode of conception [12]. The reference group, defined as 1 CL, included natural conceptions, intrauterine inseminations (IUI), and natural cycle frozen embryo transfers (NC-FET). The 0 CL group included pregnancies conceived through AC-FET, while the >1 CL group involved pregnancies after ovarian stimulation treatment and fresh embryo transfer.

Statistical analysis

Exposure categories were defined based on the number of corpora lutea (0, 1, or >1 CL). When multiple blood pressure measurements were available for a participant during the same visit, the mean of these measurements was calculated. Baseline characteristics, including pregnancy outcomes, were summarized as mean (standard deviation), median (Q1, Q3), or n (%), and compared across groups using t-tests, Mann-Whitney U tests or Chi-square tests as appropriate. Pregnancy outcomes have been studied previously in the same cohort (Koerts et al, 2025). Uterine artery indices were log-transformed to approximate a normal distribution. Linear mixed-effects regression models were used to assess blood pressure trajectories and changes in the uterine artery PI and RI. An interaction term was included in the uterine artery models to account for potential differences in PI and RI trajectories across CL groups. Additionally, a nested hierarchical model was developed, incorporating side (left or right) as a covariate. Associations between CL group and outcomes were first examined with an unadjusted model accounting only for repeated measurements within each patient over gestation. A second, adjusted model controlled for maternal age, nulliparity, body mass index, smoking status and pre-existing hypertension, based on both population characteristics and relevant literature. For uterine artery indices, a third model was further adjusted for the observed differences in mean arterial pressure. Moreover, the presence of uterine artery notches was analysed at each study visit. Sensitivity analyses were performed on ART pregnancies only to address potential confounding due to baseline differences between natural 1 CL pregnancies and NC-FET pregnancies. A separate sensitivity analysis was performed excluding pregnancies involving oocyte donation to account for potential additional confounding factors. After observing the unequal distribution of pregnancies conceived after ICSI across CL groups, a post-hoc analysis was conducted by introducing an interaction term into the model to evaluate potential effect modification. Model comparison was performed using likelihood ratio tests and analysis of variance. All analyses were conducted using R software version 4.3.2.

RESULTS

Study population

During the study period, a total of 3,170 pregnant women met the eligibility criteria for inclusion. The selection process of participants is outlined in the flowchart in Figure 1. Following the exclusion criteria 1,986 patients were included in the final analysis. These could be categorized into the three CL groups: 1,456 women 1 CL, 457 women with >1 CL, and 73 women with 0 CL, including 23 with oocyte donation. Additional information on patient characteristics and mode of conception is shown in Supplementary Table S1.

Baseline characteristics and pregnancy outcomes

The baseline characteristics across the different CL groups are summarized in Table 1. Mean maternal age was significantly higher in the 0 CL and >1 CL groups compared to the 1 CL group (33.5 years and 33.1 years versus 31.7 years, respectively; p<0.001). The

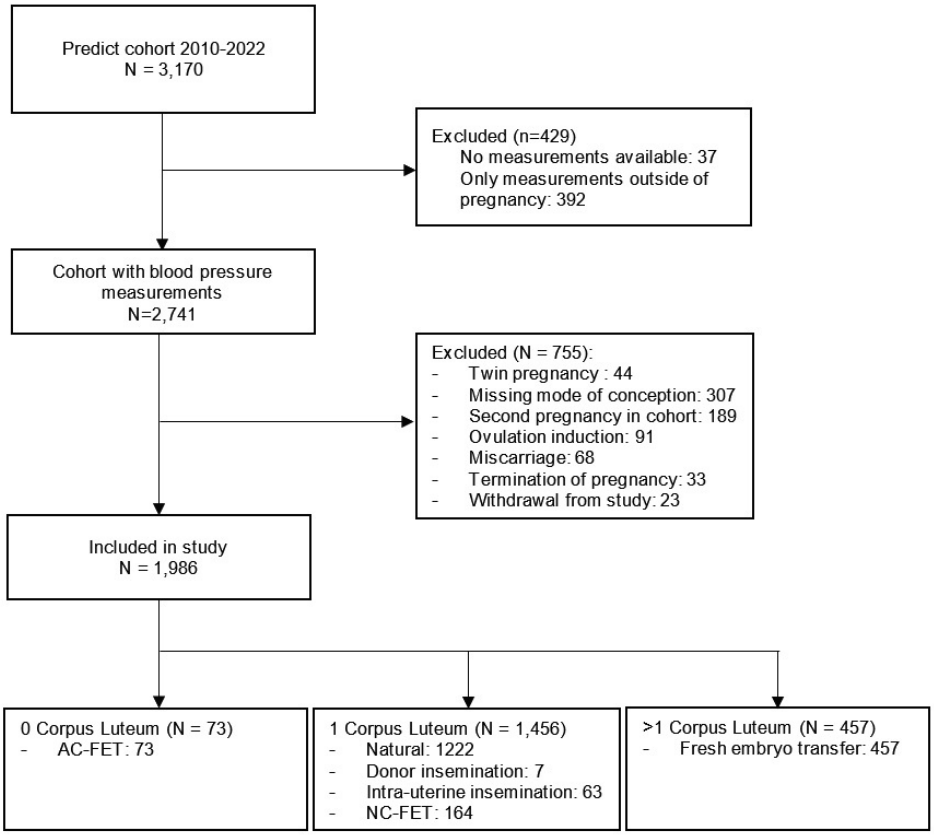


Figure 1. Flowchart of inclusion**.**

Table 1. Baseline and clinical characteristics and pregnancy outcomes of the study population stratified by corpus luteum number.

	0 CL	>1 CL	1 CL	
n	73	457	1,456	p
Maternal age, years (sd)	33.5 (5.86)	33.1 (4.39)	31.7 (4.69)	<0.001
Missing	0 (0.0)	0 (0.0)	0 (0.0)	
Paternal age, years (sd)	35.26 (5.39)	36.38 (6.56)	34.28 (5.84)	<0.001
Missing	4 (5.5)	19 (4.2)	137 (9.4)	
Ethnicity, n (%)				<0.001
Dutch	57 (78.1)	353 (77.2)	872 (59.9)	
Western	3 (4.1)	27 (5.9)	44 (3.0)	
Non-western	7 (9.6)	53 (11.6)	198 (13.6)	
Missing	6 (8.2)	24 (5.3)	342 (23.5)	
Education level, n (%)				<0.001
Low	7 (9.6)	30 (6.6)	100 (6.9)	
Medium	25 (34.2)	159 (34.8)	405 (27.8)	
High	35 (47.9)	243 (53.2)	609 (41.8)	
Missing	6 (8.2)	25 (5.5)	342 (23.5)	
Maternal BMI, kg/m^2 [q1, q3]	23.7 [21.4, 29.3]	24.6 [22.0, 28.0]	25.3 [22.7, 29.4]	<0.001
Missing	0 (0.0)	3 (0.7)	19 (1.3)	
PCOS, n (%)	35 (47.9)	83 (18.2)	154 (10.6)	<0.001
Missing	5 (6.8)	10 (2.2)	1288 (88.5)	
POI, n (%)	13 (17.8)	4 (0.9)	8 (0.5)	<0.001
Missing	2 (2.7)	1 (0.2)	318 (21.8)	
Pre-existing hypertension, n (%)	5 (6.8)	7 (1.5)	69 (4.7)	0.008
Missing	1 (1.4)	19 (4.2)	38 (2.6)	
Pre-existing diabetes, n (%)				
Type 1	0 (0.0)	1 (0.2)	15 (1.0)	<0.001
Type 2	1 (1.4)	0 (0.0)	10 (0.7)	<0.001
Missing	5 (6.8)	64 (14.0)	108 (7.4)	
Nulliparity, n (%)	49 (67.1)	340 (74.4)	637(43.8)	<0.001
Missing	2 (2.7)	24 (5.3)	45 (3.1)	
History of recurrent miscarriages, n (%)	3 (6.7)	12 (3.8)	46 (7.3)	0.114
Missing	28 (38.4)	145 (31.7)	827 (56.8)	
Preeclampsia in a previous pregnancy, n (%)	0 (0.0)	2 (0.4)	71 (6.0)	<0.001
Maternal smoking - preconceptional (%)	2 (2.7)	41 (9.0)	187 (12.8)	<0.001
Missing	6 (8.2)	24 (5.3)	348 (23.9)	
Maternal alcohol use -preconceptional, n (%)	18 (24.7)	74 (16.2)	378 (26.0)	<0.001
Missing	6 (8.2)	25 (5.5)	347 (23.8)	
Folic acid supplement use, n (%)	67 (100.0)	432 (99.8)	1088 (97.7)	<0.001
Missing	6 (8.2)	24 (5.3)	342 (23.5)	

Table 1. Baseline and clinical characteristics and pregnancy outcomes of the study population stratified by corpus luteum number. (continued)

n	0 CL 73	>1 CL 457	1 CL 1,456	p
Mode of conception, n (%)				<0.001
Natural	0 (0.0)	0 (0.0)	1222 (83.9)	
Intra-uterine insemination	0 (0.0)	0 (0.0)	70 (4.8)	
IVF	47 (64.4)	174 (38.1)	69 (4.7)	
IVF + ICSI	23 (31.5)	283 (61.9)	93 (6.4)	
IVF, ICSI unknown	3 (4.1)	0 (0.0)	2 (0.1)	
Hypertensive disorders of pregnancy, n (%)	10 (13.7)	34 (7.4)	165 (11.3)	0.078
Missing	1 (1.4)	19 (4.2)	43 (3.0)	
Preeclampsia, n (%)	7 (9.6)	10 (2.3)	80 (5.6)	**0.007**
	1 (1.4)	18 (3.9)	38 (2.6)	
Early-onset preeclampsia (<34 weeks GA), n (% of preeclampsia)	3 (42.9)	3 (30.0)	25 (31.3)	0.912
Gestational diabetes, n (%)	13 (17.8)	36 (7.9)	127 (8.7)	**0.009**
Missing	1 (1.4)	22 (4.8)	39 (2.7)	
SGA (birthweight <10th percentile), n (%)	6 (8.2)	67 (14.7)	197 (13.5)	0.439
LGA (birthweight >90th percentile), n (%)	6 (8.2)	31 (6.8)	143 (9.8)	0.286
Birthweight missing	4 (5.5)	29 (6.3)	72 (4.9)	

Continuous variables are reported as mean (standard deviation) or median [Q1, Q3]. Categorical variables are reported as n (%), based on the number of non-missing observations. Pregnancy outcomes were partially reported in previously published data (Koerts et al., 2025). Abbreviations: BMI = body mass index, GA = gestational age, PCOS = polycystic ovarian syndrome, POI = premature ovarian insufficiency, IVF = In vitro fertilization, ICSI = Intra-cytoplasmatic sperm injection, SGA = small-for-gestational-age, LGA = large-for-gestational-age.

median maternal body mass index (BMI) was lowest in women with 0 CL compared to those with >1 CL (23.7 kg/m^2 versus 24.6 kg/m^2) and 1 CL (25.3 kg/m^2). The proportion of women who smoked or consumed alcohol within three months prior to conception was significantly higher in the 1 CL group compared to both the 0 CL and >1 CL groups.

The number of pregnancies conceived after ICSI differed across CL groups, with the highest proportion in the >1 CL group (61.9%). Polycystic ovary syndrome (PCOS) was most prevalent in the 0 CL group (47.9%), as was premature ovarian insufficiency (POI) (17.8%), compared to the >1 CL and 1 CL groups, although a high number of missing records in the >1 CL and 1 CL groups may affect direct comparisons. The percentage of nulliparous women was significantly higher in women with >1 CL (74.4%) compared to those with 0 CL and 1 CL (67.1% and 43.8% respectively, p<0.001). In multiparous women, preeclampsia in a previous pregnancy occurred more often in the 1 CL group. Pre-existing hyperten-

sion was observed less frequently in the >1 CL group compared to the 0 CL and 1 CL groups (1.5% versus 6.8% and 4.7%, respectively).

The occurrence of hypertensive disorders of pregnancy varied across the CL groups, with the highest rate observed in the 0 CL group (13.7%, p=0.078) (Table 1). Preeclampsia was significantly more common in the 0 CL group (9.6%) compared to both the 1 CL and >1 CL groups (5.6% and 2.3%, p=0.007), but no difference in the proportion of early- or late-onset preeclampsia cases was observed. Additionally, the 0 CL group had a significantly higher prevalence of gestational diabetes compared to 1 CL and >1 CL (17.8% versus 8.7% and 7.9%, p = 0.009). There were no significant differences for rates of SGA and LGA neonates. Note that some of these findings have been previously published, since the current study was performed within a subgroup of the same cohort (Koerts et al., 2025).

Blood pressure patterns

A total of 8,595 unique measurements were collected, with 2,829 in the first trimester, 2,372 in the second trimester, and 3,394 in the third trimester of pregnancy. These measurements corresponded to 1,777 unique patients in the first trimester, 635 in the second trimester, and 607 in the third trimester.

At 9 weeks, MAP was 82.5 mmHg (95% confidence interval (CI) 82.0; 82.9) in the 1 CL group, compared to 84.8 mmHg (95%CI 83.0; 86.6, p=0.008) in the 0 CL group and 81.4 (95%CI 80.6; 82.1, p=0.020) in the >1 CL group. As there was no significant interaction between gestational age and CL group, the trajectory of MAP over pregnancy was similar across groups, with observed differences reflecting a consistent offset. The unadjusted MAP during pregnancy was significantly higher in women with 0 CL (β 2.54, 95% CI 0.68; 4.41, p=0.008), and lower in women with >1 CL (β -1.06 mmHg, 95%CI -1.93; -0.19,

Table 2. Effect estimates of linear mixed models for difference in blood pressure and for 0 CL and >1 CL.

		0 CL beta (95% CI)	p-value	>1 CL beta (95% CI)	p-value
All trimesters					
Systolic blood pressure	Model 1	1.45 (-1.05 - 3.95)	0.257	-1.05 (-2.22 - 0.12)	0.079
	Model 2	1.08 (-1.33 - 3.48)	0.381	-0.08 (-1.32 - 1.15)	0.894
Diastolic blood pressure	Model 1	2.97 (1.19 - 4.74)	**0.001**	-1.12 (-1.95 - -0.28)	**0.009**
	Model 2	2.70 (1.01 - 4.39)	**0.002**	-0.46 (-1.33 - 0.41)	0.302
Mean arterial pressure	Model 1	2.54 (0.68 - 4.41)	**0.008**	-1.06 (-1.93 - -0.19)	**0.017**
	Model 2	2.19 (0.43 – 3.95)	**0.015**	-0.35 (-1.22 - 0.53)	0.438

1 CL as reference group. Model 1: adjusted for gestational age. Model 2: adjusted for gestational age, maternal age at conception, body-mass index, nulliparity, smoking in periconception period, and pre-existing hypertension. Abbreviations: CI = confidence interval, CL = corpus luteum.

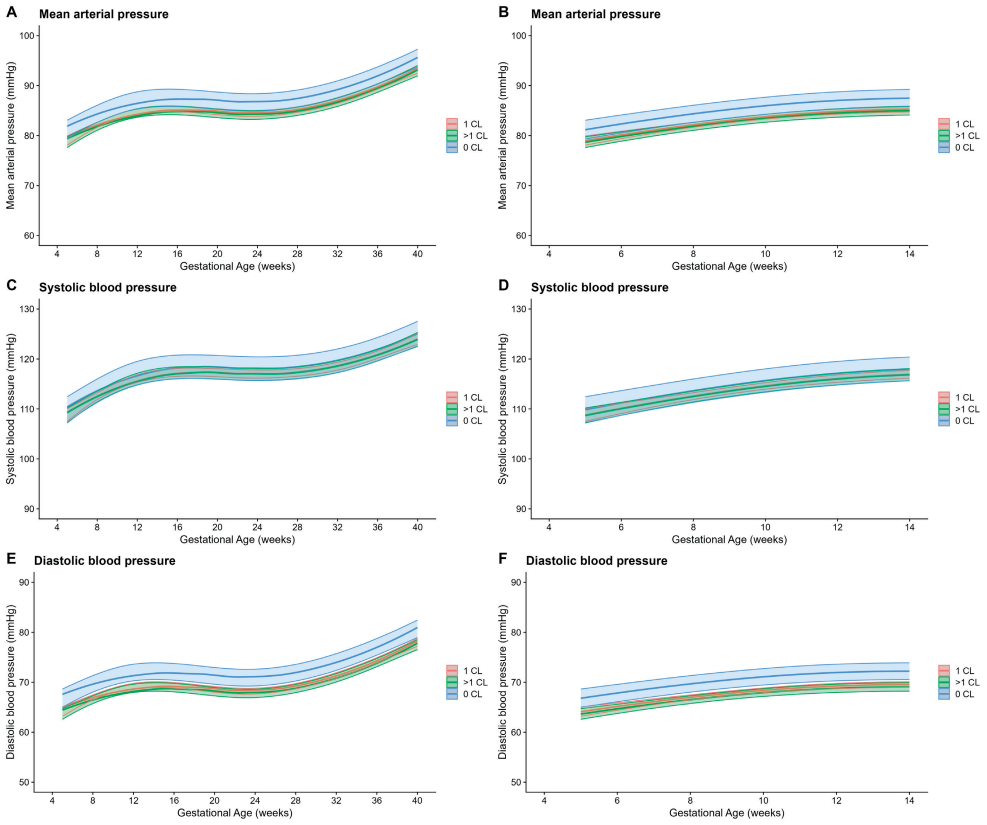


Figure 2. Blood pressure trajectories throughout pregnancy by corpus luteum number. Mean arterial pressure, systolic and diastolic blood pressure throughout pregnancy (A, C and E) and in the first trimester only (B, D and F) in women with 0 CL, 1 CL and >1 CL, after adjusting for gestational age, maternal age at conception, body-mass index, nulliparity

p=0.017), compared to those with 1 CL at any given time during pregnancy (Table 2, Figure 2). After adjusting, MAP remained higher in women with 0 CL, (β 2.19, 95% CI 0.43; 3.95, p=0.015), but was comparable between those with 1 and >1 CL. Similar effects were observed for DBP, but not for SBP.

Uterine artery doppler

Uterine artery measurements were available for a subgroup of 624 patients: n=32 in the 0 CL group, n=398 in the 1 CL group and n=194 in the >1 CL group. A total of 2,473 unique measurements were collected, with 1,418 in the first trimester, specifically 119 in week 7, 580 in week 9, 534 in week 11 and 185 in week 13. In the second and third trimester, respectively 550 and 505 measurements were performed. These measurements corre-

sponded to 608 unique patients in the first trimester, 550 in the second trimester, and 505 in the third trimester. A total of 356 patients completed all four scheduled study visits.

After adjusting for maternal and pregnancy-related characteristics (including maternal age at conception, BMI, nulliparity, smoking and preexisting hypertension), uterine artery PI and RI were lower in pregnancies with 0 CL compared to those with 1 CL in the first and second trimester of pregnancy (Table 3, Figure 3). In pregnancies with >1 CL, uterine artery indices were comparable to the 1 CL group in the first and third trimester, but slightly higher in the second trimester (RI: 0.54 (95% CI 0.52; 0.55) vs 0.51 (95% CI 0.50; 0.53), p-value = 0.009, Table 3, Figure 3).

To evaluate the effect of MAP as investigated in our models (Table 2, Figure 2), we included MAP as a potential confounder in the models for uterine artery PI and RI. This adjustment had minimal impact on most differences observed, as both remained significantly lower in women with 0 CL compared to 1 CL, both at 11 weeks (PI: 1.53, 95% CI [1.38-1.69] vs 1.72 [1.65-1.79], p=0.026; RI: 0.69, 95% CI [0.66-0.73] vs 0.73 [0.72-0.75], p=0.034) and at 22 weeks GA (PI: 0.64 [0.57-0.72] vs 0.81 [0.78-0.85], p<0.001; RI: 0.44 [0.41-0.46] vs 0.51 [0.50-0.53], p<0.001, Table 4). However, the significant difference in uterine artery PI between women with 1 and >1 CL at 22 weeks was no longer present, though it continued to show a trend towards a higher value compared to the 1 CL group. For reference, Tables S2 and S3 present left and averaged uterine artery indices by gestational age and CL group. We compared uterine artery notch presence between groups at all time points and examined

Table 3. **Uterine artery Doppler indices for corpus luteum groups**. Based on adjusted linear mixed models, side = right, no smoking, no pre-existing hypertension, nulliparous, mean BMI and mean age.

		0 CL			>1 CL			1 CL	
		Mean	95% CI	p-value	Mean	95% CI	P-value	Mean	95% CI
7 weeks	UtA PI	2.03	1.74-2.36	**0.010**	2.24	2.08-2.40	**0.026**	2.48	2.34-2.62
	UtA RI	0.79	0.74-0.86	0.209	0.81	0.78-0.84	0.217	0.84	0.81-0.86
9 weeks	UtA PI	1.77	1.59-1.96	**0.005**	1.97	1.88-2.06	0.170	2.06	1.97-2.15
	UtA RI	0.74	0.70-0.78	0.058	0.78	0.76-0.80	0.703	0.78	0.77-0.80
11 weeks	UtA PI	1.52	1.37-1.68	**0.020**	1.73	1.65-1.81	0.871	1.72	1.65-1.79
	UtA RI	0.69	0.65-0.72	**0.025**	0.74	0.72-0.76	0.384	0.73	0.72-0.75
22 weeks	UtA PI	0.63	0.57-0.71	**<0.001**	0.87	0.83-0.91	**0.039**	0.81	0.78-0.85
	UtA RI	0.44	0.41-0.46	**<0.001**	0.54	0.52-0.55	**0.009**	0.51	0.50-0.53
32 weeks	UtA PI	0.68	0.60-0.78	0.781	0.71	0.68-0.75	0.492	0.72	0.69-0.74
	UtA RI	0.46	0.44-0.50	0.812	0.47	0.46-0.49	0.433	0.47	0.46-0.48

1 CL as reference group. Adjusted for maternal age at conception, body-mass index, nulliparity, smoking in periconception period, and pre-existing hypertension. Abbreviations: *CI = confidence interval, CL = corpus luteum, PI = Pulsatility index, RI = Resistance index.*

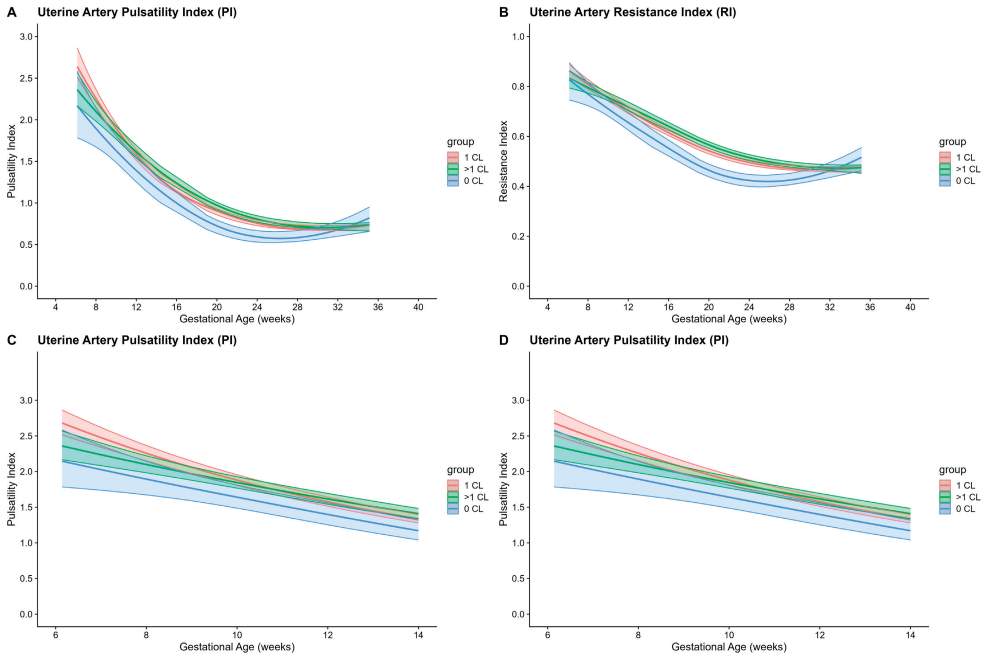


Figure 3. Uterine artery Doppler indices throughout pregnancy by corpus luteum number. Uterine artery pulsatility and resistance indices throughout pregnancy (A and B) and in the first trimester only (C and D) in women with 0 CL, 1 CL and >1 CL, after adjusting for gestational age, maternal age at conception, body-mass index, nulliparity, smoking in the periconception period, and pre-existing hypertension (model 2).

whether first trimester notches persisted into the second and third trimesters. A statistically significant difference in notch presence was observed only at 13 weeks, where the 1 CL group and >1 CL group exhibited higher rates of notching (Supplementary Table S4). At all other gestational stages examined, no significant differences were noted.

Sensitivity analyses

In a sensitivity analysis restricted to ART pregnancies, both blood pressure and uterine artery results were consistent (Supplementary Tables S5 and S6). Excluding pregnancies with oocyte donation did not significantly alter the outcomes (data not shown). Furthermore, post-hoc analysis showed no significant effect modification of the higher proportion of ICSI pregnancies in the >1 CL group (data not shown).

Table 4. Uterine artery Doppler indices for corpus luteum groups, additionally adjusted for mean arterial pressure. Based on adjusted linear mixed models, side = right, no smoking, no pre-existing hypertension, nulliparous, mean BMI and mean age.

		0 CL			>1 CL			1 CL	
		Mean	95% CI	p-value	Mean	95% CI	p-value	Mean	95% CI
7 weeks	UtA PI	2.02	1.73-2.35	**0.012**	2.21	2.05-2.37	**0.025**	2.45	2.32-2.60
	UtA RI	0.79	0.73-0.85	0.237	0.81	0.78-0.84	0.187	0.83	0.81-0.85
9 weeks	UtA PI	1.77	1.59-1.96	**0.007**	1.95	1.86-2.05	0.143	2.05	1.97-2.14
	UtA RI	0.74	0.71-0.78	0.074	0.77	0.76-0.79	0.602	0.78	0.76-0.80
11 weeks	UtA PI	1.53	1.38-1.69	**0.026**	1.72	1.65-1.80	0.954	1.72	1.65-1.79
	UtA RI	0.69	0.66-0.73	**0.034**	0.74	0.72-0.76	0.464	0.73	0.72-0.75
22 weeks	UtA PI	0.64	0.57-0.72	**<0.001**	0.87	0.83-0.91	0.051	0.81	0.78-0.85
	UtA RI	0.44	0.41-0.46	**<0.001**	0.54	0.52-0.55	**0.013**	0.51	0.50-0.53
32 weeks	UtA PI	0.69	0.61-0.79	0.840	0.72	0.68-0.76	0.564	0.70	0.67-0.74
	UtA RI	0.47	0.44-0.51	0.892	0.48	0.46-0.49	0.523	0.47	0.46-0.48

1 CL as reference group. Adjusted for maternal age at conception, body-mass index, nulliparity, smoking in periconception period, and pre-existing hypertension. Abbreviations: *CI = confidence interval, CL = corpus luteum, PI = Pulsatility index, RI = Resistance index.*

DISCUSSION

Principal findings

In this study, we examined the relationship between CL number and maternal circulatory adaptation in pregnancy, as assessed by longitudinal maternal blood pressure and uterine artery doppler indices throughout pregnancy, using adjusted linear mixed model regression. Our analysis showed that MAP and DBP were significantly higher in women with 0 CL compared to those with 1 CL. Pregnancies with 0 CL exhibited significantly lower uterine artery PI and RI during the first and second trimester compared to pregnancies with 1 CL. In contrast, pregnancies with >1 CL showed uterine artery indices that were comparable to the 1 CL group in the first and third trimester but had a slightly higher PI in the second trimester (0.54 vs 0.52, p-value = 0.047). Adjusting the uterine artery models for mean arterial pressure did not substantially alter these findings. Furthermore, there was no observed difference in SBP across CL groups, nor was there a distinct pattern of blood pressure change over the course of pregnancy. These findings suggest that the absence of CL affects both blood pressure and uterine vascular resistance in early to mid-pregnancy.

Comparison with existing literature

Three studies have previously investigated the relationship between CL number and maternal blood pressure. Von Versen-Hoynck et al. (2019) reported a trend toward a higher 3-5 mmHg SBP, DBP and MAP in 0 CL compared to 1 CL pregnancies in the first

and third trimester, based on their supplementary data [23]. Similar to our findings, von Versen-Hoynck (2020) observed no significant differences in blood pressure trajectories between CL groups in their unadjusted mixed model analysis which included preconceptional, first, and third trimester measurements [14]. Lastly, our previous study by Wiegel et al. observed a lower MAP in the third trimester for 0 CL pregnancies, but this was based on unadjusted data from a smaller sample [13]. Taken together, our study extends these findings by providing adjusted, longitudinal blood pressure trajectories by CL number in a larger cohort. Although a mid-pregnancy drop in blood pressure is often described, we observed a slight rise between weeks 6 and 12 followed by stabilization until week 24, which aligns with previous studies reporting more variable or absent mid-gestational declines, particularly in normotensive or low-risk populations [24, 25].

The observed lower uterine artery PI in pregnancies with 0 CL also aligns with several prior studies, even though many did not directly compare based on CL groups [13, 15-18]. However, CL number can often be derived from the study design and the ART treatments that are compared, allowing us to relate their findings to our research focus. For uterine artery Doppler indices, our observation of lower uterine artery PI in 0 CL pregnancies aligns with studies by Inversetti et al. (2018) and Cavoretto et al. (2020), who reported lower PI in donor oocyte pregnancies (typically 0 CL) compared to natural conceptions [16, 17]. Two other studies comparing pregnancies from AC-FET (0 CL) and fresh embryo transfer (>1 CL) further support our findings of a lower PI in pregnancies with 0 CL [15, 18]. However, these studies lacked comparisons with NC-FET and natural pregnancies (1 CL) and did not fully adjust for confounders. One other study investigated PI and RI at the start of progesterone supplementation and 12 weeks of gestation and found a steeper, though non-significant, decline in uterine artery PI and RI in the 0 CL (AC-FET) group compared to 1 CL (NC-FET), consistent with our findings [26]. Our study by Wiegel et al. similarly reported lower uterine artery PI and RI in AC-FET compared to natural and fresh embryo transfer pregnancies [13]. The current study, using a larger, well-adjusted cohort, reinforces these findings across trimesters, providing more robust evidence of lower PI and RI in 0 CL pregnancies.

Uterine artery notching reflects increased resistance to blood flow in the uteroplacental circulation. A persistent notch is associated with impaired vascular remodelling and increased risk of placenta-related pregnancy outcomes [27]. Since CL number is also associated with these outcomes, e.g. SGA pregnancies, notching could be different between CL groups. While one previous study found differences in first trimester notching rates between fresh embryo transfer and natural pregnancies, we observed no significant differences between groups [18]. This suggests that CL number may primarily affect PI and RI rather than notching.

Hypotheses and potential mechanisms

Evidence shows that pregnancies without a corpus luteum exhibit attenuated rises in progesterone, prorenin and renin activity during early pregnancy, as well as absence of circulating relaxin [12, 28]. These hormonal alterations could underlie the different vascular characteristics. In 0 CL pregnancies, reduced levels of relaxin may lead to reduced vascular relaxation and slightly elevated blood pressure [6, 29]. This relative hypertension, combined with low prorenin levels due to the absent CL, appears to suppress the renin-angiotensin system (RAAS). Moreover, lower progesterone or oestrogen could also underlie this reduced RAAS activation [30-32]. The resulting reduced RAAS activation likely contributes to insufficient plasma volume expansion, which is essential for normal pregnancy adaptation [10, 12, 32]. This hypothesis is supported by a prior study showing blunted increases in left atrial size and persistent reduction in E wave velocity in 0 CL pregnancies, suggestive of reduced plasma volume expansion in this group [33]. Another study reported a steeper decline in plasma protein concentrations in the >1 CL group, possibly reflecting greater volume expansion; however, no such difference was observed between 0 and 1 CL groups, and haemoglobin levels declined similarly across all groups [28]. While these observations all support the proposed hypothesis of hormonal alterations underlying circulatory changes, direct evidence that restoring these hormones can reverse these changes remains lacking.

One previous study observed a positive correlation between serum relaxin and uterine artery RI at 10-12 weeks of gestation, which aligns with our finding of higher uterine artery RI in pregnancies with 1 CL or >1 CL compared to 0 CL pregnancies [34]. However, the coexistence of higher MAP and lower uterine artery indices in women with 0 CL, thus in the absence of both relaxin and prorenin, remains complex and unclear. Adjusting the uterine artery models for MAP did not substantially alter the observed effects, suggesting that these measurements may not be directly connected.

The results might suggest the possibility of a compensatory decrease in uterine artery resistance to maintain adequate placental perfusion in 0 CL pregnancies. However, there is currently no evidence to support this hypothesis. One previous study observed that the increase in cardiac output during the first trimester, seen in spontaneous pregnancies (1 CL), is attenuated in 0 CL pregnancies [33]. Future studies could explore cardiac output differences between NC-FET (1 CL) and AC-FET pregnancies to eliminate confounding by natural versus ART conception. In addition, it would be valuable to investigate whether supplementation with CL-derived hormones can restore normal cardiovascular adaptation in 0 CL pregnancies. Additionally, one possible explanation for the observed lower uterine artery indices, as proposed by Conrad et al. (2024), could be greater than normal spiral artery remodelling in 0 CL pregnancies [1]. However, this hypothesis has not yet been investigated.

Strengths and limitations

The large cohort size of this study enhances statistical power, enabling us to adjust for key confounding factors such as maternal age, BMI, pre-existing hypertension, smoking status, and parity. All three CL groups, 0, 1 and >1 CL, were included within one analysis, providing a unique perspective on the role of CL number in pregnancy circulation. Finally, by including only ART-conceived pregnancies in a sensitivity analysis, we minimized variability from natural conception, strengthening our comparisons and reducing potential confounding.

Despite these strengths, there are limitations to consider. The study was conducted within a tertiary hospital setting, which may affect the generalizability of our findings to more diverse, low-risk populations. Additionally, we did not have detailed data on progesterone luteal support. Standard protocols differ across CL groups: fresh embryo transfers typically receive short-term vaginal progesterone supplementation, with additional choriogonadotropin in case of hypothalamic or functional anovulation, while NC-FET cycles do not receive luteal support. In contrast, AC-FET cycles received both progesterone and oestradiol supplementation until 12 weeks of gestation. We should also note here that CL number was based on conception mode rather than ultrasound findings. Another potential limitation is confounding by indication, as fresh embryo transfers are typically attempted first and may selectively represent "successful" pregnancies with favourable outcomes. If fresh transfer is unsuccessful, frozen transfer is often used, and this group may include pregnancies with underlying factors that initially contributed to unsuccessful fresh transfer, potentially impacting pregnancy outcomes. Furthermore, women without a natural cycle, like those with PCOS, are more likely to be in the 0 CL group, and studies have shown a higher risk of preeclampsia and elevated blood pressure in women with PCOS [35, 36]. Lastly, data on antihypertensive medication use during pregnancy or preconceptional blood pressure measurements were not available in this study. Therefore, we cannot rule out the possibility that the observed higher blood pressure in the 0 CL group may partly reflect pre-existing differences, as reported in prior studies [23, 33].

Conclusion

In this study, we examined maternal blood pressure across pregnancies with 0, 1 and >1 corpus luteum (CL) throughout gestation. We found that women with 0 CL had significantly higher MAP and DBP compared to those with 1 CL, while no differences were observed between the 1 and >1 CL groups. Additionally, uterine artery Doppler indices showed that 0 CL pregnancies had lower PI and RI in the first and second trimesters compared to 1 CL pregnancies, even after adjusting for the higher mean arterial pressure. These findings suggest distinct vascular adaptations in 0 CL pregnancies, potentially influenced by embryo transfer protocols and absence of the CL.

SUPPLEMENTARY MATERIAL

Supplementary Table S1. Baseline and clinical characteristics and pregnancy outcomes of the study population stratified by mode of conception.

	0 CL		>1 CL	1 CL		
	AC-FET without OD	AC-FET with OD	Fresh ET	Spontaneous	IUI	NC-FET
n	50	23	457	1224	70	162
Maternal age, years (SD)	31.6 (4.18)	37.7 (6.86)	33.1 (4.39)	31.4 (4.75)	33.9 (3.24)	33.1 (4.25)
Missing	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Paternal age, years (SD)	33.7 (4.40)	38.6 (5.89)	36.4 (6.56)	33.9 (5.74)	37.2 (6.60)	35.9 (5.79)
Missing	3 (6.0)	1 (4.3)	19 (4.2)	108 (8.8)	17 (24.3)	12 (7.4)
Ethnicity, n (%)						
Dutch	38 (76.0)	19 (82.6)	353 (77.2)	710 (58.0)	51 (72.9)	111 (68.5)
Western	1 (2.0)	2 (8.7)	27 (5.9)	34 (2.8)	5 (7.1)	5 (3.1)
Non-Western	6 (12.0)	1 (4.3)	53 (11.6)	150 (12.3)	11 (15.7)	37 (22.8)
Missing	5 (10.0)	1 (4.3)	24 (5.3)	330 (27.0)	3 (4.3)	9 (5.6)
Education level, n (%)						
Low	4 (8.0)	3 (13.0)	30 (6.6)	85 (6.9)	3 (4.3)	12 (7.4)
Medium	20 (40.0)	5 (21.7)	159 (34.8)	329 (26.9)	22 (31.4)	54 (33.3)
High	21 (42.0)	14 (60.9)	243 (53.2)	480 (39.2)	42 (60.0)	87 (53.7)
Missing	5 (10.0)	1 (4.3)	25 (5.5)	330 (27.0)	3 (4.3)	9 (5.6)
Maternal BMI, kg/m^2 [q1, q3]	24.7 [21.5, 29.4]	22.3 [21.0, 25.1]	24.6 [22.0, 28.0]	25.3 [22.7, 29.6]	25.8 [23.0, 29.6]	25.1 [22.5, 28.7]
Missing	0 (0.0)	0 (0.0)	3 (0.7)	15 (1.2)	3 (4.3)	1 (0.6)
PCOS, N (%)	32 (64.0)	1 (4.3)	83 (18.2)	3 (0.2)	0 (0.0)	11 (6.8)
Missing	1 (2.0)	4 (17.4)	10 (2.2)	1216 (99.3)	70 (100.0)	2 (1.2)
POI, N (%)	0 (0.0)	13 (56.5)	4 (0.9)	7 (0.6)	0 (0.0)	1 (0.6)
Missing	1 (2.0)	1 (4.3)	1 (0.2)	315 (25.7)	3 (4.3)	0 (0.0)
Pre-existing hypertension, n (%)	3 (6.0)	2 (8.7)	7 (1.5)	59 (4.8)	5 (7.1)	5 (3.1)
Missing	1 (2.0)	0 (0.0)	19 (4.2)	26 (2.1)	4 (5.7)	8 (4.9)
Pre-existing diabetes, n (%)						
Type 1	0 (0.0)	0 (0.0)	1 (0.2)	14 (1.1)	0 (0.0)	1 (0.6)
Type 2	0 (0.0)	1 (4.3)	0 (0.0)	10 (0.8)	0 (0.0)	0 (0.0)
Missing	5 (10.0)	0 (0.0)	64 (14.0)	76 (6.2)	12 (17.1)	20 (12.3)
Nulliparity, n (%)	29 (58.0)	13 (56.5)	174 (38.1)	889 (72.6)	30 (42.9)	89 (54.9)
Missing	0 (0.0)	0 (0.0)	7 (1.5)	22 (1.8)	1 (1.4)	4 (2.5)
History of recurrent miscarriages, n (%)	12 (3.8)	2 (20.0)	12 (3.8)	38 (8.3)	1 (2.1)	7 (5.6)
Missing	145 (31.7)	13 (56.6)	145 (31.7)	765 (62.6)	23 (32.9)	37 (22.8)
Preeclampsia in a previous pregnancy, n (%)	0 (0.0)	0 (0.0)	2 (0.4)	66 (5.4)	1 (1.4)	4 (2.5)

Supplementary Table S1. Baseline and clinical characteristics and pregnancy outcomes of the study population stratified by mode of conception. (continued)

	0 CL		>1 CL	1 CL		
	AC-FET without OD	AC-FET with OD	Fresh ET	Spontaneous	IUI	NC-FET
n	50	23	457	1224	70	162
Missing	1 (2.0)	2 (8.7)	29 (6.3)	253 (20.7)	6 (8.6)	17 (10.5)
Maternal smoking - preconceptional (%)	2 (4.0)	0 (0.0)	41 (9.0)	163 (13.3)	3 (4.3)	21 (13.0)
Missing	5 (10.0)	1 (4.3)	24 (5.3)	335 (27.4)	4 (5.7)	9 (5.6)
Maternal alcohol use - preconceptional, n (%)	14 (28.0)	4 (17.4)	74 (16.2)	316 (25.8)	24 (34.3)	38 (23.5)
Missing	5 (10.0)	1 (4.3)	25 (5.5)	334 (27.3)	4 (5.7)	9 (5.6)
Folic acid supplement use, n (%)	45 (90.0)	22 (95.7)	432 (94.5)	869 (71.0)	66 (94.3)	153 (94.4)
Missing	5 (10.0)	1 (4.3)	24 (5.3)	329 (26.9)	4 (5.7)	9 (5.6)
Mode of conception, n (%)						
Natural	0 (0.0)	0 (0.0)	0 (0.0)	1224 (100.0)	0 (0.0)	0 (0.0)
IUI	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	70 (100.0)	0 (0.0)
IVF	31 (62.0)	16 (69.6)	174 (38.1)	0 (0.0)	0 (0.0)	69 (42.6)
IVF+ ICSI	16 (32.0)	7 (30.4)	283 (61.9)	0 (0.0)	0 (0.0)	93 (57.4)
IVF, with or without ICSI unknown	3 (6.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Hypertensive disorders of pregnancy, n (%)	6 (12.0)	4 (17.4)	34 (7.4)	142 (11.6)	7 (10.0)	16 (9.9)
Missing	1 (2.0)	0 (0.0)	19 (4.2)	30 (2.5)	4 (5.7)	9 (5.6)
Preeclampsia, n (%)	4 (8.0)	3 (13.0)	10 (2.2)	65 (5.3)	6 (8.6)	9 (5.6)
Missing	1 (2.0)	0 (0.0)	18 (3.9)	26 (2.1)	4 (5.7)	8 (4.9)
Early-onset preeclampsia (<34 weeks GA), n (% of preeclampsia)	2 (50.0)	1 (33.3)	3 (30.0)	21 (32.3)	2 (33.3)	2 (22.2)
Gestational diabetes, n (%)	10 (20.0)	3 (13.0)	36 (7.9)	102 (8.3)	8 (11.4)	17 (10.5)
Missing	1 (2.0)	0 (0.0)	22 (4.8)	27 (2.2)	4 (5.7)	8 (4.9)
SGA (Birthweight <10th Percentile), n (%)	1 (2.0)	5 (21.7)	67 (14.7)	173 (14.1)	10 (14.3)	14 (8.6)
LGA (Birthweight >90th Percentile), n (%)	4 (8.0)	2 (8.7)	31 (6.8)	119 (9.7)	6 (8.6)	18 (11.1)
Birthweight missing	4 (8.0)	0 (0.0)	29 (6.3)	53 (4.3)	5 (7.1)	14 (8.6)

Continuous variables are reported as mean (standard deviation) or median [Q1, Q3]. Pregnancy outcomes were partially reported in previously published data. Abbreviations: AC-FET = artificial cycle frozen embryo transfer, OD = oocyte donation, Fresh ET = Fresh embryo transfer, IUI = intra-uterine insemination, NC-FET = natural cycle frozen embryo transfer, BMI = body mass index, GA = gestational age, PCOS = polycystic ovarian syndrome, POI = premature ovarian insufficiency, IVF = In vitro fertilization, ICSI = Intracytoplasmatic sperm injection, SGA = small-for-gestational-age, LGA = large-for-gestational age.

Supplementary Table S2. Effect estimates of linear mixed models for difference in blood pressure and for 0 CL and >1 CL, for ART pregnancies only.

		0 CL		>1 CL	
All trimesters		beta (95% CI)	p-value	beta (95% CI)	p-value
Systolic blood pressure	Model 1	1.53 (-1.29 – 4.35)	0.286	-0.73 (-2.61 – 1.14)	0.445
	Model 2	1.56 (-1.14 – 4.26)	0.256	0.21 (-1.64 – 2.07)	0.821
Diastolic blood pressure	Model 1	3.50 (1.52 – 5.47)	**0.001**	-0.63 (-1.95 – 0.68)	0.345
	Model 2	3.20 (1.30 – 5.11)	**0.001**	-0.18 (-1.49 – 1.13)	0.784
Mean arterial pressure	Model 1	2.93 (0.85 - 5.00)	**0.006**	-0.64 (-2.01 - 0.74)	0.363
	Model 2	2.66 (0.70 – 4.63)	**0.008**	-0.06 (-1.41 – 1.29)	0.930

1 CL as reference group. Model 1: adjusted for gestational age. Model 2: adjusted for gestational age, maternal age at conception, body-mass index, nulliparity, smoking in periconception period, and pre-existing hypertension. Abbreviations: CI = confidence interval, CL = corpus luteum.

Supplementary Table S3. Mean predicted uterine artery pulsatility index and resistance index for corpus luteum groups when restricted to ART pregnancies only. Based on adjusted linear mixed models, side = right, no smoking, no pre-existing hypertension, mean BMI and mean age.

		0 CL			>1 CL			1 CL	
		Mean	95% CI	p-value	Mean	95% CI	p-value	Mean	95% CI
7 weeks	UtA PI	2.01	1.73-2.34	**0.011**	2.25	2.09-2.41	0.067	2.57	2.28-2.89
	UtA RI	0.79	0.74-0.86	0.116	0.82	0.79-0.85	0.182	0.86	0.81-0.91
9 weeks	UtA PI	1.76	1.59-1.94	**0.005**	1.98	1.89-2.08	0.228	2.09	1.94-2.26
	UtA RI	0.74	0.71-0.78	**0.031**	0.78	0.76-0.80	0.450	0.79	0.77-0.83
11 weeks	UtA PI	1.51	1.37-1.67	**0.033**	1.74	1.66-1.82	0.786	1.72	1.61-1.84
	UtA RI	0.69	0.66-0.72	**0.025**	0.74	0.73-0.76	0.633	0.74	0.71-0.76
22 weeks	UtA PI	0.63	0.56-0.71	**<0.001**	0.88	0.84-0.92	0.045	0.80	0.74-0.86
	UtA RI	0.44	0.41-0.46	**<0.001**	0.54	0.53-0.55	**0.008**	0.51	0.49-0.53
32 weeks	UtA PI	0.68	0.60-0.77	0.917	0.72	0.68-0.76	0.340	0.69	0.64-0.74
	UtA RI	0.46	0.44-0.50	0.951	0.48	0.46-0.49	0.235	0.46	0.45-0.48

1 CL as reference group. Adjusted for maternal age at conception, body-mass index, nulliparity, smoking in periconception period, and pre-existing hypertension. Abbreviations: CI = confidence interval, CL = corpus luteum, PI = Pulsatility index, RI = Resistance index.

Supplementary Table S4. Presence of notching in uterine artery.

		0 CL	>1 CL	1 CL	p-value
		n=32	n=194	n=398	
7 weeks	No	0 (0.0%)	1 (0.5%)	2 (0.5%)	0.318
	Yes	10 (31.2%)	38 (19.6%)	65 (16.6%)	
	Missing	22 (68.8%)	155 (79.9%)	350 (82.9%)	
9 weeks	No	5 (15.6%)	29 (14.9%)	42 (10.6%)	0.416
	Yes	23 (71.9%)	125 (64.4%)	270 (67.8%)	
	Missing	4 (12.5%)	40 (20.6%)	86 (21.6%)	
11 weeks	No	12 (37.5%)	45 (23.2%)	79 (19.8%)	0.214
	Yes	15 (46.9%)	116 (59.8%)	248 (62.3%)	
	Missing	5 (15.6%)	33 (17.0%)	71 (17.8%)	
13 weeks	No	8 (25.0%)	17 (8.8%)	26 (6.3%)	**<0.001**
	Yes	2 (6.2%)	31 (16.0%)	102 (25.6%)	
	Missing	22 (68.8%)	146 (75.3%)	287 (68.1%)	
22 weeks	No	28 (87.5%)	143 (73.7%)	287 (72.1%)	0.266
	Yes	0 (0.0%)	23 (11.9%)	51 (12.8%)	
	Missing	4 (12.5%)	28 (14.4%)	60 (15.1%)	
30 weeks	No	22 (68.8%)	144 (74.2%)	267 (67.1%)	0.123
	Yes	0 (0.0%)	13 (6.7%)	38 (9.5%)	
	Missing	10 (31.2%)	37 (19.1%)	93 (23.4%)	
Persisting notch	No	21 (65.6%)	117 (60.3%)	227 (56.9%)	0.561
	Yes	0 (0.0%)	6 (3.1%)	19 (4.8%)	
	Missing	11 (34.4%)	71 (36.6%)	152 (38.2%)	

Abbreviations: CL = corpus luteum.

Supplementary Table S5. Uterine artery Doppler indices for corpus luteum groups
Based on adjusted linear mixed models, side = left, no smoking, no pre-existing hypertension, nulliparous, mean BMI and mean age.

		0 CL			**>1 CL**			**1 CL**	
		Mean	95% CI	p-value	Mean	95% CI	p-value	Mean	95% CI
7 weeks	UtA PI	2.08	1.79-2.43	**0.010**	2.30	2.14-2.47	**0.026**	2.55	2.41-2.69
	UtA RI	0.81	0.75-0.87	0.209	0.83	0.80-0.85	0.217	0.85	0.83-0.87
9 weeks	UtA PI	1.82	1.63-2.02	**0.005**	2.02	1.93-2.12	0.170	2.12	2.03-2.21
	UtA RI	0.75	0.72-0.79	0.058	0.79	0.77-0.81	0.703	0.79	0.80-0.81
11 weeks	UtA PI	1.56	1.41-1.73	**0.020**	1.78	1.70-1.86	0.871	1.77	1.70-1.84
	UtA RI	0.70	0.66-0.73	**0.025**	0.75	0.74-0.77	0.385	0.74	0.73-0.76
22 weeks	UtA PI	0.65	0.58-0.73	**<0.001**	0.90	0.85-0.94	**0.039**	0.84	0.80-0.87
	UtA RI	0.44	0.42-0.47	**<0.001**	0.55	0.53-0.56	**0.009**	0.52	0.51-0.53
32 weeks	UtA PI	0.70	0.62-0.80	0.781	0.74	0.70-0.77	0.492	0.72	0.69-0.75
	UtA RI	0.47	0.44-0.50	0.816	0.48	0.47-0.49	0.433	0.48	0.47-0.49

1 CL as reference group. Adjusted for maternal age at conception, body-mass index, nulliparity, smoking in periconception period, and pre-existing hypertension. Abbreviations: CI = confidence interval, CL = corpus luteum, PI = Pulsatility index, RI = Resistance index.

Supplementary Materials and methods

Luteal support in different artificial reproductive technology protocols

In the case of fresh embryo transfer, the luteal support protocols have evolved over the years. From the start of the study cohort in 2010, Utrogestan (progesterone) 200 mg was given vaginally for 12 days, starting 3 days after oocyte collection. Starting in June 2018, Rekovelle (recombinant follicle stimulating hormone) was used as stimulating medication. From then on, Utrogestan was given for 15 days, starting the day after oocyte collection. Starting in March 2024, Utrogestan is now given for 7 weeks, starting on the evening after oocyte collection. In patients with peanut or soy allergies Duphaston 10 mg 3 times per day or Crinone progesterone gel 1 time per day were used instead of Utrogestan. Patients diagnosed with WHO type 1 anovulation received Pregnyl (human chorionic gonadotropin) 1500 units on day 2, 4 and 6 after oocyte collection. In the case of natural cycle frozen embryo transfer, no luteal support was given. In the case of frozen embryo transfer in an artificial cycle, Progynova was given in increasing daily doses start at 1 mg until 3 mg on the day of oocyte collection. Utrogestan was given starting 5 days before embryo transfer until the start of the 12th week of gestation. Afterwards, 2 mg Progynova was given 2 times per day.

Supplementary Table S6. **Left, right, and averaged uterine artery Doppler indices by gestational age and CL group**. Means from raw measurements.

		0 CL			>1 CL			1 CL		
		Right	Left	Average	Right	Left	Average	Right	Left	Average
7 weeks	UtA PI	2.47	1.94	2.23	2.33	2.41	2.36	2.73	2.74	2.76
	UtA RI	0.83	0.79	0.81	0.83	0.83	0.83	0.87	0.86	0.87
9 weeks	UtA PI	2.03	1.98	2.03	2.01	2.11	2.04	2.15	2.20	2.17
	UtA RI	0.78	0.76	0.77	0.78	0.79	0.79	0.79	0.80	0.79
11 weeks	UtA PI	1.61	1.60	1.61	1.85	1.79	1.83	1.86	1.84	1.86
	UtA RI	0.69	0.68	0.68	0.75	0.74	0.75	0.74	0.74	0.74
22 weeks	UtA PI	0.68	0.65	0.66	0.91	0.93	0.92	0.87	0.92	0.90
	UtA RI	0.46	0.44	0.45	0.54	0.55	0.55	0.53	0.54	0.54
32 weeks	UtA PI	0.73	0.65	0.68	0.77	0.80	0.78	0.76	0.80	0.78
	UtA RI	0.48	0.45	0.46	0.49	0.50	0.50	0.49	0.50	0.49

Abbreviations: CL = corpus luteum, PI = Pulsatility index, RI = Resistance index.

REFERENCES

1. Conrad KP, von Versen-Hoynck F, Baker VL. Pathologic maternal and neonatal outcomes associated with programmed embryo transfer. J Assist Reprod Genet. 2024 Apr;41(4):821-42.
2. Qin J, Liu X, Sheng X, Wang H, Gao S. Assisted reproductive technology and the risk of pregnancy-related complications and adverse pregnancy outcomes in singleton pregnancies: a meta-analysis of cohort studies. Fertil Steril. 2016 Jan;105(1):73-85 e1-6.
3. Santos MA, Kuijk EW, Macklon NS. The impact of ovarian stimulation for IVF on the developing embryo. Reproduction. 2010 Jan;139(1):23-34.
4. Youssef MM, Mantikou E, van Wely M, Van der Veen F, Al-Inany HG, Repping S, Mastenbroek S. Culture media for human pre-implantation embryos in assisted reproductive technology cycles. Cochrane Database Syst Rev. 2015 Nov 20;2015(11):CD007876.
5. Conrad KP, Baker VL. Corpus luteal contribution to maternal pregnancy physiology and outcomes in assisted reproductive technologies. American journal of physiology Regulatory, integrative and comparative physiology. 2013 2013/1//;304(2):R69-72.
6. Conrad KP, von Versen-Hoynck F, Baker VL. Potential role of the corpus luteum in maternal cardiovascular adaptation to pregnancy and preeclampsia risk. Am J Obstet Gynecol. 2022 May;226(5):683-99.
7. Koerts JJ, Voskamp LW, Rousian M, Steegers-Theunissen RPM, Wiegel RE. Impact of corpus luteum number on maternal pregnancy and birth outcomes: the Rotterdam Periconception Cohort. Fertil Steril. 2025 Jun;123(6):1039-50.
8. Elias FTS, Weber-Adrian D, Pudwell J, Carter J, Walker M, Gaudet L, et al. Neonatal outcomes in singleton pregnancies conceived by fresh or frozen embryo transfer compared to spontaneous conceptions: a systematic review and meta-analysis. Arch Gynecol Obstet. 2020 Jul;302(1):31-45.
9. Landsverk E, Westvik-Johari K, Romundstad LB, Opdahl S. Birth size after embryo cryopreservation: larger by all measures? Hum Reprod. 2023 Jul 5;38(7):1379-89.
10. Chapman AB, Abraham WT, Zamudio S, Coffin C, Merouani A, Young D, et al. Temporal relationships between hormonal and hemodynamic changes in early human pregnancy. Kidney Int. 1998 Dec;54(6):2056-63.
11. Derkx FH, Alberda AT, Zeilmaker GH, Schalekamp MA. High concentrations of immunoreactive renin, prorenin and enzymatically-active renin in human ovarian follicular fluid. Br J Obstet Gynaecol. 1987 Jan;94(1):4-9.
12. Wiegel RE, Jan Danser AH, Steegers-Theunissen RPM, Laven JSE, Willemsen SP, Baker VL, et al. Determinants of Maternal Renin-Angiotensin-Aldosterone-System Activation in Early Pregnancy: Insights From 2 Cohorts. J Clin Endocrinol Metab. 2020 Nov 1;105(11):3505-17.
13. Wiegel RE, Karsten MJH, Reijnders IF, van Rossem L, Willemsen SP, Mulders AGMGJ, et al. Corpus luteum number and the maternal renin-angiotensin-aldosterone system as determinants of utero-placental (vascular) development: the Rotterdam Periconceptional Cohort. Reprod Biol Endocrinol. 2021 Nov 4;19(1):164.
14. von Versen-Hoynck F, Hackl S, Selamet Tierney ES, Conrad KP, Baker VL, Winn VD. Maternal Vascular Health in Pregnancy and Postpartum After Assisted Reproduction. Hypertension. 2020 Feb;75(2):549-60.
15. Cavoretto PI, Farina A, Gaeta G, Sigismondi C, Spinillo S, Casiero D, et al. Uterine artery Doppler in singleton pregnancies conceived after in-vitro fertilization or intracytoplasmic sperm

injection with fresh vs frozen blastocyst transfer: longitudinal cohort study. Ultrasound in Obstetrics and Gynecology. 2020 2020/10//;56(4):603-10.
16. Cavoretto PI, Farina A, Miglio R, Zamagni G, Girardelli S, Vanni VS, et al. Prospective longitudinal cohort study of uterine arteries Doppler in singleton pregnancies obtained by IVF/ICSI with oocyte donation or natural conception. Human Reproduction. 2020 2020/11//;35(11):2428-38.
17. Inversetti A, Mandia L, Candiani M, Cetin I, Larcher A, Savasi V, et al. Uterine artery Doppler pulsatility index at 11-38 weeks in ICSI pregnancies with egg donation. Journal of Perinatal Medicine. 2018 2018/1//;46(1):21-7.
18. Choux C, Ginod P, Barberet J, Rousseau T, Bruno C, Sagot P, et al. Placental volume and other first-trimester outcomes: are there differences between fresh embryo transfer, frozen-thawed embryo transfer and natural conception? Reproductive BioMedicine Online. 2019 2019/4//;38(4):538-48.
19. Rousian M, Schoenmakers S, Eggink AJ, Gootjes DV, Koning AHJ, Koster MPH, et al. Cohort Profile Update: the Rotterdam Periconceptional Cohort and embryonic and fetal measurements using 3D ultrasound and virtual reality techniques. Int J Epidemiol. 2021 Nov 10;50(5):1426-7l.
20. Steegers-Theunissen RP, Verheijden-Paulissen JJ, van Uitert EM, Wildhagen MF, Exalto N, Koning AH, et al. Cohort Profile: The Rotterdam Periconceptional Cohort (Predict Study). Int J Epidemiol. 2016 Apr;45(2):374-81.
21. Heijnen EM, Eijkemans MJ, De Klerk C, Polinder S, Beckers NG, Klinkert ER, et al. A mild treatment strategy for in-vitro fertilisation: a randomised non-inferiority trial. Lancet. 2007 Mar 3;369(9563):743-9.
22. Hohmann FP, Macklon NS, Fauser BC. A randomized comparison of two ovarian stimulation protocols with gonadotropin-releasing hormone (GnRH) antagonist cotreatment for in vitro fertilization commencing recombinant follicle-stimulating hormone on cycle day 2 or 5 with the standard long GnRH agonist protocol. J Clin Endocrinol Metab. 2003 Jan;88(1):166-73.
23. von Versen-Hoynck F, Narasimhan P, Selamet Tierney ES, Martinez N, Conrad KP, Baker VL, Winn VD. Absent or Excessive Corpus Luteum Number Is Associated With Altered Maternal Vascular Health in Early Pregnancy. Hypertension. 2019 Mar;73(3):680-90.
24. Nama V, Antonios TF, Onwude J, Manyonda IT. Mid-trimester blood pressure drop in normal pregnancy: myth or reality? J Hypertens. 2011 Apr;29(4):763-8.
25. Shen M, Tan H, Zhou S, Smith GN, Walker MC, Wen SW. Trajectory of blood pressure change during pregnancy and the role of pre-gravid blood pressure: a functional data analysis approach. Sci Rep. 2017 Jul 24;7(1):6227.
26. Lawrenz B, Markova D, Melado L, Vitorino RL, Digma S, Samir S, Fatemi HM. Prospective observational comparison of arteria uterina blood flow between two frozen embryo transfer cycle regimens: natural cycle versus hormonal replacement cycle. Arch Gynecol Obstet. 2022 Dec;306(6):2177-85.
27. Cnossen JS, Morris RK, ter Riet G, Mol BW, van der Post JA, Coomarasamy A, et al. Use of uterine artery Doppler ultrasonography to predict pre-eclampsia and intrauterine growth restriction: a systematic review and bivariable meta-analysis. CMAJ. 2008 Mar 11;178(6):701-11.
28. Conrad KP, Graham GM, Chi YY, Zhai X, Li M, Williams RS, et al. Potential influence of the corpus luteum on circulating reproductive and volume regulatory hormones, angiogenic and immunoregulatory factors in pregnant women. Am J Physiol Endocrinol Metab. 2019 Oct 1;317(4):E677-E85.
29. Conrad KP. Emerging role of relaxin in the maternal adaptations to normal preg-

nancy: implications for preeclampsia. Semin Nephrol. 2011 Jan;31(1):15-32.
30. Komukai K, Mochizuki S, Yoshimura M. Gender and the renin-angiotensin-aldosterone system. Fundam Clin Pharmacol. 2010 Dec;24(6):687-98.
31. O'Donnell E, Floras JS, Harvey PJ. Estrogen status and the renin angiotensin aldosterone system. Am J Physiol Regul Integr Comp Physiol. 2014 Sep 1;307(5):R498-500.
32. Wiegel RE, von Versen-Hoynck F, Steegers-Theunissen RPM, Steegers EAP, Danser AHJ. Prorenin periconceptionally and in pregnancy: Does it have a physiological role? Mol Cell Endocrinol. 2021 Feb 15;522:111118.
33. Conrad KP, Petersen JW, Chi YY, Zhai X, Li M, Chiu KH, et al. Maternal Cardiovascular Dysregulation During Early Pregnancy After In Vitro Fertilization Cycles in the Absence of a Corpus Luteum. Hypertension. 2019 Sep;74(3):705-15.
34. Anumba DO, El Gelany S, Elliott SL, Li TC. Serum relaxin levels are reduced in pregnant women with a history of recurrent miscarriage, and correlate with maternal uterine artery Doppler indices in first trimester. Eur J Obstet Gynecol Reprod Biol. 2009 Nov;147(1):41-5.
35. Qin JZ, Pang LH, Li MJ, Fan XJ, Huang RD, Chen HY. Obstetric complications in women with polycystic ovary syndrome: a systematic review and meta-analysis. Reprod Biol Endocrinol. 2013 Jun 26;11:56.
36. Valdimarsdottir R, Vanky E, Elenis E, Lindstrom L, Junus K, Jonsson M, et al. Polycystic ovary syndrome and risk of pre-eclampsia: A national register-based cohort study. BJOG. 2024 Jun;131(7):985-95.

5

Impact of corpus luteum number on maternal pregnancy and birth outcomes: The Rotterdam Periconception Cohort

Joni J. Koerts, Lotte W. Voskamp, Melek Rousian,
Régine P.M. Steegers-Theunissen, Rosalieke E. Wiegel

Fertility and Sterility.2025 Jun;123(6):1039-1050.

ABSTRACT

Objective: To investigate associations between assisted reproductive technology (ART) -induced alterations in corpus luteum (CL) number during implantation, and maternal pregnancy and birth outcomes. Pregnancies conceived through ART are associated with increased risks of adverse obstetric and perinatal outcomes, with discrepancies in outcomes between different ART treatment protocols. We hypothesize this is due to the periconceptional hormonal environment regulated by the CL. ART protocols affect CL quantity at conception resulting in CL absence or a supraphysiological number of CL.

Design: This study is embedded in the Rotterdam Periconceptional Cohort, an ongoing tertiary center prospective cohort at the Erasmus University Medical Center in Rotterdam, the Netherlands.

Subjects: Women with a singleton pregnancy with data on CL.

Exposure: The number of CL, based on mode of conception: 0 CL (artificial cycle frozen embryo transfer (AC-FET), n=72); >1 CL (ovarian stimulated fresh embryo transfer, n=462) and 1 CL (natural cycle frozen embryo transfer (NC-FET) and natural conceptions, n=1327).

Main outcome measures: Hypertensive disorders of pregnancy, gestational diabetes, gestational age at birth and birthweight, derived from medical records.

Results: We included 1,861 pregnancies. The results were adjusted for maternal age, maternal body mass index, nulliparity and obstetric history. In comparison with natural conceptions (1 CL), a pregnancy with CL absence (0 CL) was associated with a higher risk of gestational diabetes (aOR 2.59 [95% CI 1.31;5.15]), and a higher risk of preeclampsia, albeit non-significantly (aOR 2.02 [95% CI 0.91;4.51]). In comparison with pregnancies with >1 CL the risk of preeclampsia was significantly lower (aOR 0.36 [95% CI 0.18;0.72]). Post hoc analyses revealed that in male neonates, >1 CL was associated with a lower birthweight percentile (aβ -6.18 [95% CI -11.16;-1.20]). In contrast, female neonates showed no association with >1 CL, whereas CL absence was associated with a higher birthweight percentile (aβ 12.93 [95% CI 2.52;23.34]).

Conclusion: Risks of hypertensive disorders of pregnancy, gestational diabetes and relative birthweight differ between CL groups. These findings support the hypothesis that an aberrant number of CL impacts maternal pregnancy and or birth outcomes. Additional studies need to be conducted to investigate causes and underlying pathophysiology.

INTRODUCTION

Pregnancies conceived through assisted reproductive technology (ART) techniques have been linked to higher risks of adverse maternal pregnancy and birth outcomes [1]. As the complication risk is not identical across ART protocols, it is important to investigate which patients are at risk. ART consists of conception through in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI). The ART protocols can be divided into fresh embryo transfer and frozen embryo transfer. Fresh embryo transfer has been associated with an increased risk of preterm birth, small for gestational age (SGA) and lower birthweight, whereas frozen embryo transfer has been associated with an increased risk of hypertensive disorders of pregnancy and neonates born large for gestational age (LGA) [2-6]. In frozen embryo transfer, artificial cycle frozen embryo transfer (AC-FET) has been associated with a higher risk of preeclampsia compared to natural cycle frozen embryo transfer (NC-FET) [7-9]. These differences might be caused by a variety of factors, such as laboratory techniques, cryopreservation methods or maternal characteristics. However, direct causes and underlying pathways remain elusive.

It is known that the number of corpora lutea (CL) during the periconceptional period is dependent on the ART protocol [10]. In pregnancies conceived in a natural cycle, either spontaneous conception or NC-FET, one CL is present. In AC-FET the CL is absent, due to repression of ovarian function by the administration of exogenous hormones. Contrastingly, fresh embryo transfer involves a large number of CL due to controlled ovarian hyperstimulation used for oocyte retrieval. We hypothesize that the differences in CL number contribute to complication risks in ART protocols, due to derangements in involved hormonal pathways. The CL is the primary source of reproductive hormone production during the first trimester, including estrogen, progesterone, relaxin, vasopressin and prorenin [11-13]. These hormones are vital for placentation and maternal cardiovascular and renal adaptation [14]. Relaxin induces renal and systemic vasodilation and is particularly associated with vascular adaptation in the first trimester [15]. Disruption of this adaptation has been linked to hypertensive disorders of pregnancy, fetal growth restriction and preterm birth [16-20].

Previous research has shown that in CL absence a lack of vasoactive products was found, including relaxin and prorenin [13, 21]. In contrast, a large number of CL revealed high hormone concentrations, including prorenin and relaxin [13, 22]. The associations between these hormonal differences and maternal pregnancy and birth outcomes are understudied. Understanding the association between CL number and these outcomes is crucial for optimizing ART protocols. Our aim is to further understand the associations between the number of CL and adverse maternal pregnancy and birth outcomes.

METHODS

Study participants

The data used in this study were collected from the Rotterdam Periconceptional Cohort (Predict Study, November 2010 to December 2022), an ongoing prospective observational study embedded in tertiary care at the Erasmus MC, University Medical Center, in Rotterdam, the Netherlands [23, 24]. Recruitment of patients took place during their initial preconception or pregnancy consultation before 10^{+0} weeks of pregnancy at the outpatient clinic. Eligible participants included women aged 18 or older with an intrauterine singleton pregnancy and Dutch language proficiency. Ethical approval for the study was obtained from the Central Committee on Research in The Hague and the Medical Ethical Committee of the Erasmus Medical Center (MEC-2004-227). Informed consent was obtained from all participants. Excluded cases involved ovulation induction or mild ovarian hyperstimulation with or without IUI or donor insemination, structural anomalies, or missing exposure or outcome data, i.e., missing mode of conception, miscarriages, termination of pregnancy and loss to follow-up. Women treated with ovulation induction or mild ovarian hyperstimulation were excluded due to the potential maturation of multiple oocytes, leading to an uncertain number of CL.

Data collection

During the study intake visit, anthropometric measurements were performed by trained researchers. Maternal body mass index (BMI) was calculated using periconceptional weight and length measurements. Detailed questionnaires gathered information on general maternal and paternal characteristics, lifestyle, and obstetrical history. Pregnancy complications were identified through questionnaires and verified in medical records. Details regarding mode of conception, subfertility diagnosis, fertility treatment and birth outcomes were retrieved from medical records.

Study groups

The study groups were assessed by the number of CL present during the implantation stage, determined through mode of conception. These groups comprised AC-FET conceptions (0 CL) with suppressed ovarian function resulting in CL absence and ovarian stimulated fresh embryo transfer (>1 CL) resulting in multiple CL. Within this group, the number of CL was defined as the number of follicles punctured during ovum retrieval or, if missing, the number of retrieved oocytes. The reference group of a natural conception (1 CL) included conception without interventions, intrauterine insemination (IUI) or donor insemination (DI) in a natural cycle, and NC-FET. Thus, pregnancies were categorized as either 0, >1 or 1 CL.

ART treatment groups

The procedures used for ovarian stimulation, oocyte retrieval, IVF, ICSI and the assessment of embryo morphology were described previously [25, 26]. Ovarian stimulation was performed using recombinant follicle stimulating hormone (rFSH) or human menopausal gonadotropin (hMG) with either gonadotropin-releasing hormone (GnRH) -agonist or -antagonist co-treatment. Final oocyte maturation was induced using a single dose of human chorionic gonadotropin (HCG). Oocytes were inseminated following routine IVF, ICSI with ejaculated sperm or TESE-ICSI procedures [26]. Embryos were cultured in Vitrolife G5 series (Vitrolife, Goteborg, Sweden) until November 2014, thereafter in SAGE 1-StepTM (CooperSurgical, Trumbull, CT, USA). Until April 1, 2019, fresh embryo transfers were performed on day 3 and thereafter on day 5 after fertilization. Remaining embryos were selected for cryopreservation on day 4 or 5 based on developmental stage and morphology. In NC-FET, frozen embryo transfer was performed 4 days after detecting a spontaneous ovulatory surge by urinary luteinizing hormone (LH) testing. In AC-FET, the endometrium was prepared using estrogens, followed by increasing doses of intravaginal progesterone and daily GnRH agonist injections. Embryo transfer occurred when the endometrial lining measured at least 4 mm on ultrasound or after 19 days of endometrial preparation.

Maternal pregnancy and birth outcomes

All primary outcomes were predefined. As maternal complications, hypertensive disorders of pregnancy and gestational diabetes were investigated. Hypertensive disorders of pregnancy encompassed pregnancy-induced hypertension, preeclampsia, and hemolysis, elevated liver enzymes, and low platelets syndrome (HELLP syndrome). Pregnancy-induced hypertension was defined as new-onset hypertension (systolic ≥140 mmHg or diastolic ≥90 mmHg), after 20 weeks of gestation [27]. Preeclampsia was initially defined as gestational hypertension after 20 weeks combined with urine protein/creatinine ratio of ≥30 mg/mmol [27]. In 2018, the clinical definition of preeclampsia was expanded to include gestational hypertension with fetal growth restriction or other maternal organ dysfunction [28]. Early-onset preeclampsia was defined as occurring before 34 weeks. Preeclampsia superimposed on chronic hypertension was defined as preeclampsia in patients with hypertension diagnosed before pregnancy or before 20 weeks of gestation [28]. HELLP syndrome was defined as a platelet count below 100x109/L, serum aspartate aminotransferase and serum alanine aminotransferase concentrations >70 IU/L and lactic dehydrogenase >600 IU/L. Gestational diabetes was defined as fasting plasma glucose ≥7.0 mmol/L or 2-hour plasma glucose ≥7.8 mmol/L after a 75g oral glucose tolerance test, excluding pre-existing diabetes mellitus.

Birth outcomes comprised gestational age at birth and birthweight. In spontaneous pregnancies, gestational age was based on the last menstrual period in women with a known regular cycle, or on the crown-rump length from first-trimester ultrasounds otherwise.

In pregnancies conceived through fresh embryo transfer, frozen embryo transfer or IUI, gestational age was based on the date of oocyte retrieval plus 14 days, transfer date plus 19 days, or the insemination date, respectively. Missing data were supplemented with the gestational age from the delivery report. Preterm birth was defined as birth before 37^{+0} weeks. Birthweight was computed to relative birthweight in Hoftiezer percentiles using Dutch reference growth curves with adjustments for neonatal sex and gestational age at birth, with $<10^{th}$ percentile as SGA and $>90^{th}$ percentile as LGA [29]. Subcategories based on absolute birthweight included high birthweight (>4000g) and low birthweight (<2500g).

Statistical analyses

Baseline characteristics and birth outcomes, stratified by CL number, were presented as mean with standard deviation (SD) or median with interquartile range (IQR) for continuous variables, and frequency and proportion for binary variables. Statistical differences in numerical data were assessed using appropriate tests: Kruskal-Wallis for three groups and Mann-Whitney test for two groups. Categorical data comparisons used the Chi-squared test, or the Fisher-exact test in case of at least one count below 5. Multivariable linear regression models were used for continuous outcomes and binary logistic regression models for binary outcomes, using the 1 CL group as reference category. Results were reported as crude and adjusted effect sizes or crude and adjusted odds ratios (OR and aOR), respectively. Confounders in the adjusted models were selected using impactful variables known in literature, baseline population differences and the purposeful selection of variables method [30]. In all outcomes, adjustments were made for maternal age, maternal BMI and nulliparity. Depending on the outcome, additional adjustments were performed for obstetric history of preeclampsia, preterm birth, low birthweight, or macrosomia; preconceptional smoking; and the origin of subfertility.

A subgroup analysis was conducted for pregnancies conceived through ART, with NC-FET (1 CL) as reference category. This approach aimed to address potential confounding from ART treatment and differences in characteristics and underlying disease between ART and non-ART conceptions. Additionally, this approach allows for separate interpretation of the results within the ART subgroup. Post hoc sensitivity analyses were conducted to validate the findings. The male-female difference was not predefined and was explored in these analyses. A dose-response analysis using a linear regression model examined the relationship between >1 CL and male birthweight, with CL number as a continuous exposure. This approach was not feasible in other outcomes due to sample size limitations.

Nonlinear associations and clinically plausible interactions were assessed using partial likelihood ratio tests. Analyses were performed in R version 4.2.1 for Windows, with significance set at $p<0.05$.

RESULTS

Participants

From the 3,098 pregnancies of the Predict study, 1,935 were included for this study (Figure 1). After excluding 74 cases with missing outcome data, 1,861 pregnancies were analyzed, including 72 in the 0 CL group after AC-FET, 462 in the >1 CL group after fresh embryo transfer and 1327 in the 1 CL group (with 1141 after natural conception and 186 after NC-FET). The >1 CL group had a median of 9 CL [IQR 6-12].

The baseline characteristics of the study population are depicted in Table 1. Women in the 1 CL group were on average younger, more often multiparous, had higher BMI and were more frequently diagnosed with chronic hypertension or previous pregnancy complications. They were also more likely to smoke or consume alcohol preconceptionally. Between the ART groups fewer significant differences were observed.

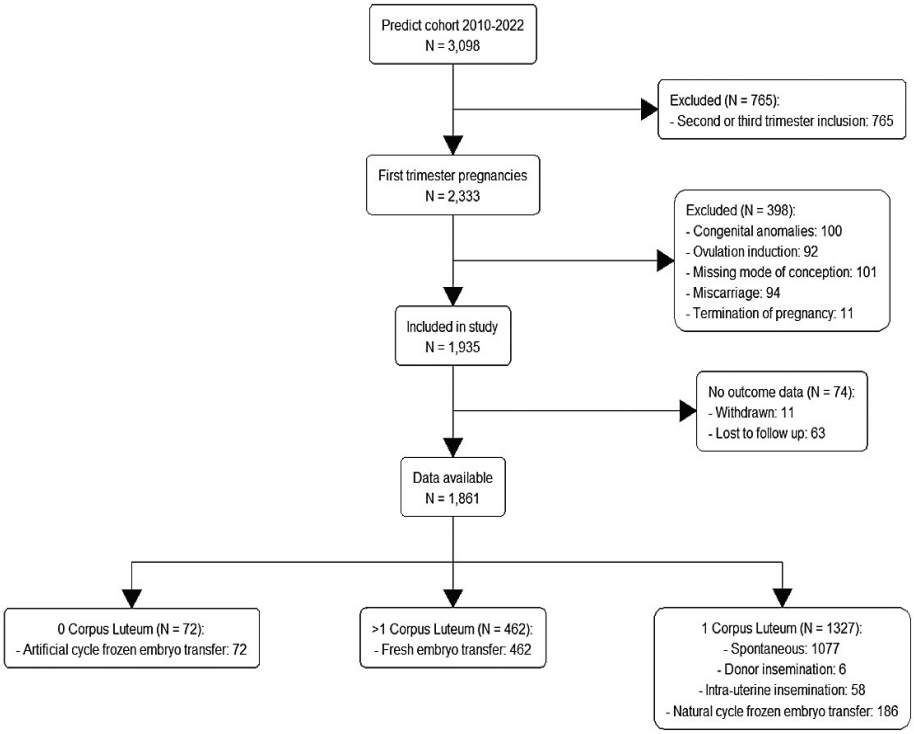


Figure 1. Flow chart of study participants.

Table 1. Baseline, medical and reproductive characteristics of the total study population.

	0 CL AC-FET	**>1 CL** Fresh ET	**1 CL** NAT/ NC-FET		**1 CL** NC-FET	
	N = 72	N = 462	N = 1327	P-value	N = 186	P-value
Maternal characteristics						
Age at conception (years), mean ± SD	32.7 ± 5.0	33.3 ± 4.4	31.9 ± 4.6	**<0.001**	33.5 ± 4.2	0.414
Nulliparity (n, %)	50 (70.4)	332 (72.3)	533 (40.9)	**<0.001**	101 (55.8)	**<0.001**
Ethnicity (n, %)				**0.015**		**0.002**
Dutch	61 (87.1)	362 (81.3)	935 (79.2)		131 (74.0)	
Western	3 (4.3)	29 (6.5)	46 (3.9)		5 (2.8)	
Non-western	6 (8.6)	54 (12.1)	199 (16.9)		41 (23.2)	
Education level (n, %)				0.598		0.501
Low	8 (11.4)	28 (6.3)	94 (8.0)		13 (7.3)	
Medium	24 (34.3)	164 (36.9)	421 (35.7)		58 (32.6)	
High	38 (54.3)	252 (56.8)	664 (56.3)		107 (60.1)	
BMI at study entry (kg/m^2), median [IQR]	23.5 [21.6-27.9]	24.7 [22.0-28.0]	25.2 [22.5-29.5]	**<0.001**	25.2 [22.5-28.7]	0.117
MAP (mmHg), mean ± SD	83.5 ± 8.9	79.7 ± 7.5	81.0 ± 8.3	**<0.001**	79.8 ± 8.0	**0.001**
Pre-existing hypertension (n, %)	4 (5.6)	6 (1.3)	68 (5.1)	**0.002**	5 (2.7)	0.051
Polycystic Ovary Syndrome (n, %)	35 (50.0)	89 (19.6)	41 (19.5)	**<0.001**	14 (7.7)	**<0.001**
Primary Ovarian Insufficiency (n, %)	14 (19.4)	4 (0.9)	12 (1.0)	**<0.001**	1 (0.5)	**<0.001**
Obstetric outcomes in prior pregnancy (n, %)						
Preeclampsia	0 (0.0)	2 (0.4)	78 (6.0)	**<0.001**	4 (2.3)	0.056
Pregnancy-induced hypertension	0 (0.0)	1 (0.2)	18 (1.4)	0.076	0 (0.0)	0.761
Preterm birth	0 (0.0)	6 (1.3)	95 (7.3)	**<0.001**	3 (1.7)	0.548
Low birthweight	0 (0.0)	5 (1.1)	51 (3.9)	**0.004**	2 (1.2)	0.664
Macrosomia	0 (0.0)	2 (0.4)	6 (0.5)	0.847	0 (0.0)	0.579
Lifestyle factors (n, %)						
Smoking preconceptionally	2 (2.9)	43 (9.6)	187 (16.0)	**<0.001**	20 (11.2)	0.118
Smoking first trimester	0 (0.0)	5 (3.1)	40 (6.6)	0.102	2 (3.2)	0.635
Alcohol use preconceptionally	19 (27.1)	76 (17.1)	390 (33.2)	**<0.001**	43 (24.2)	**0.038**
Alcohol use first trimester	0 (0.0)	1 (0.8)	9 (2.6)	0.443	1 (1.8)	0.769
Folic acid use initiated preconceptionally	70 (100.0)	444 (99.8)	1149 (97.5)	**0.004**	178 (100.0)	0.756
Paternal characteristics						
Age at conception (years), mean ± SD	35.04 ± 5.5	36.5 ± 6.6	34.6 ± 5.8	**<0.001**	36.2 ± 5.7	0.123
BMI at study entry (kg/m^2), median [IQR]	25.8 [23.2-28.3]	26.4 [23.9-28.7]	26.0 [23.7-28.3]	0.304	26.8 [24.2-29.1]	0.266
MAP (mmHg), mean ± SD	91.2 ± 8.1	90.4 ± 9.2	90.4 ± 9.0	0.613	89.6 ± 8.1	0.456

Table 1. Baseline, medical and reproductive characteristics of the total study population. (continued)

	0 CL AC-FET	**>1 CL** Fresh ET	**1 CL** NAT/ NC-FET		**1 CL** NC-FET	
	N = 72	N = 462	N = 1327	P-value	N = 186	P-value
Subfertility treatment						
Origin of subfertility (n, %)			NA	NA		**<0.001**
Female factor	45 (64.3)	111 (24.3)			48 (26.2)	
Male factor	4 (5.7)	199 (43.6)			88 (48.1)	
Combined	19 (27.1)	78 (17.1)			21 (11.5)	
Unexplained	2 (2.9)	68 (14.9)			26 (14.2)	
Origin semen (n, %)			NA	NA		**0.005**
Ejaculate	17 (81.0)	325 (76.1)			17 (48.6)	
MESA/PESA	1 (4.8)	50 (11.7)			7 (20.0)	
TESE	3 (14.3)	52 (12.2)			11 (31.4)	
Technique (n, %)			NA	NA		**<0.001**
IVF	50 (69.4)	176 (38.1)			77 (41.6)	
ICSI	22 (30.6)	286 (61.9)			108 (58.4)	
Embryo stage at transfer (n, %)			NA	NA		0.270
Cleavage stage (day 3)	52 (74.3)	305 (66.2)			115 (63.5)	
Blastocyst stage (day 5)	18 (25.7)	156 (33.8)			66 (36.5)	

Data are presented as n (%), mean ± SD or median [IQR]. Participants with missing data were excluded from the total sum of participants during analyses by variable. ART: Artificial Reproductive Technology. CL: Corpus Luteum. AC-FET: Artificial-Cycle Frozen Embryo Transfer. ET: Embryo Transfer. NAT: Natural conception. NC-FET: Natural-Cycle Frozen Embryo Transfer. BMI: Body Mass Index. MAP: Mean arterial pressure. MESA: Microepididymal Sperm Aspiration. PESA: Percutaneous Epididymal Sperm Aspiration. TESE: Testicular Sperm Extraction. IVF: In Vitro Fertilization. ICSI: Intracytoplasmic Sperm Injection. NA: Not available.

Maternal pregnancy outcomes

The incidence of hypertensive disorders of pregnancy and preeclampsia was significantly different across the groups. Pregnancies with 0 CL showed an increased risk of preeclampsia compared to 1 CL (11.1% vs 5.4%), albeit non-significant after adjustment (aOR 2.02 [95% CI 0.91; 4.51]). Conversely, >1 CL was associated with significantly reduced risk of hypertensive disorders of pregnancy compared to 1 CL (7.6% versus 11.7%, aOR 0.59 [95% CI 0.39; 0.90], Table 2 and 3). These results remained consistent after additional adjustment for smoking (data not shown). Concerning preeclampsia specifically, >1 CL was associated with an even stronger protective effect (2.2% vs 5.4%, aOR 0.36 [95% CI 0.18; 0.72]). Comparable results were observed in analyses restricted to ART patients, and after excluding patients with preeclampsia superimposed on chronic hypertension (Table 2).

Table 2. Maternal pregnancy and birth outcomes stratified by the number of corpora lutea. N=1861.

	0 CL AC-FET	**>1 CL Fresh ET**	**1 CL NAT NC-FET**	P-value 0 vs 1 CL	P-value 1 vs >1 CL	**1 CL NC-FET**	P-value 0 vs 1 CL	P-value 1 vs >1 CL
	N = 72	N = 462	N = 1327			N = 186		
Maternal outcomes								
Hypertensive disorders of pregnancy (n, %)	12/72 (16.7)	35/460 (7.6)	155/1322 (11.7)	0.209	**0.014**	17/183 (9.3)	0.095	0.481
Pregnancy-induced hypertension (n, %)	4/64 (6.2)	25/449 (5.6)	84/1247 (6.7)	1.000	0.387	7/172 (4.1)	0.495	0.450
Preeclampsia (n, %)	8/72 (11.1)	10/460 (2.2)	71/1322 (5.4)	**0.040**	**0.005**	10/183 (5.5)	0.113	**0.030**
Early-onset preeclampsia (n, %)	3/7 (42.9)	3/7 (42.9)	17/58 (29.3)	0.667	0.667	2/6 (33.3)	1.000	1.000
Without chronic hypertension (n, %)	6/68 (8.8)	8/453 (1.8)	50/1253 (4.0)	0.054	**0.025**	7/177 (4.0)	0.128	0.105
Gestational age preeclampsia (days), median [IQR]	256 [226, 262]	257 [219, 269]	252 [235, 260]	0.975	0.775	251 [239, 253]	0.836	0.731
HELLP syndrome (n, %)	1/72 (1.4)	2/460 (0.4)	9/1322 (0.7)	0.413	0.739	0/183 (0.0)	0.282	1.000
Gestational diabetes (n, %)	12/72 (16.7)	42/456 (9.2)	123/1322 (9.3)	**0.040**	0.953	21/183 (11.5)	0.266	0.385

Table 2. Maternal pregnancy and birth outcomes stratified by the number of corpora lutea. N=1861. (continued)

	0 CL AC-FET	**>1 CL Fresh ET**	**1 CL NAT NC-FET**	P-value 0 vs 1 CL	P-value 1 vs >1 CL	**1 CL NC-FET**	P-value 0 vs 1 CL	P-value 1 vs >1 CL
	N = 72	N = 462	N = 1327			N = 186		
Birth outcomes								
Gestational age at birth (days), median [IQR])	275 [270, 283]	275 [268, 282]	273 [265, 280]	**0.042**	**<0.001**	275 [267, 281]	0.384	0.233
Preterm birth (n, %)	9/71 (12.7)	35/453 (7.7)	138/1292 (10.7)	0.598	0.070	17/178 (9.6)	0.467	0.453
Non-preeclampsia cases (n, %)	5/63 (7.9)	32/441 (7.3)	108/1220 (8.9)	0.803	0.301	13/167 (7.8)	0.970	0.824
Birthweight (kg), median [IQR]	3.5 [3.2, 3.8]	3.3 [3.0, 3.7]	3.3 [3.0, 3.7]	0.076	0.938	3.4 [3.1, 3.8]	0.739	**0.045**
High birthweight (>4000g), (n, %)	4/71 (5.6)	40/454 (8.8)	115/1293 (8.9)	0.514	0.957	23/180 (12.8)	0.117	0.132
Low birthweight (<2500g), (n, %)	7/71 (9.9)	35/454 (7.7)	104/1293 (8.0)	0.586	0.821	15/180 (8.3)	0.700	0.793
Relative birthweight (p), median [IQR]	52.3 [26.0, 77.0]	41.1 [19.5, 71.3]	49.4 [23.9, 76.9]	0.525	**0.006**	54.2 [26.4, 79.7]	0.974	**0.008**
LGA (>90th), (n, %)	5/70 (7.1)	36/449 (8.0)	143/1275 (11.2)	0.289	0.056	21/174 (12.1)	0.259	0.116
SGA (<10th), (n, %)	7/70 (10.0)	62/449 (13.8)	162/1275 (12.7)	0.506	0.550	16/174 (9.2)	0.846	0.119
Non-preeclampsia/PTB cases (n,%)	1/57 (1.8)	51/408 (12.5)	121/1103 (11.0)	**0.024**	0.406	11/152 (7.2)	0.187	
Neonatal sex (male), (n, %)	38/71 (53.5)	225/456 (49.3)	637/1299 (49.0)	0.462	0.911	78/180 (43.3)	0.145	
Birthweight, male (kg), median [IQR]	3.4 [3.1, 3.7]	3.4 [3.0, 3.7]	3.4 [3.0, 3.8]	0.795	0.431	3.6 [3.3, 3.8]	**0.049**	**0.003**
Relative birthweight, male (p), median [IQR]	45.9 [20.6, 67.9]	38.7 [17.3, 72.5]	52.6 [24.5, 78.4]	0.259	**0.003**	61.9 [33.5, 85.8]	**0.028**	**<0.001**
Birthweight, female (kg), median [IQR]	3.6 [3.3, 3.8]	3.3 [3.0, 3.6]	3.3 [2.9, 3.6]	**0.005**	0.328	3.3 [3.0, 3.6]	**0.023**	0.888
Relative birthweight, female (p), median [IQR]	70.1 [44.8, 82.8]	43.7 [20.7, 69.6]	46.2 [22.9, 75.0]	**0.030**	0.351	43.5 [22.1, 75.2]	**0.035**	0.796

Data are presented as n (%) or median [IQR]. Relative birthweight is noted as percentiles*100. ART: Artificial Reproductive Technology. CL: Corpus Luteum. AC-FET: Artificial-Cycle Frozen Embryo Transfer. NAT: Natural conception. NC-FET: Natural-Cycle Frozen Embryo Transfer. ET: Embryo Transfer. SGA: Small for gestational age. HELLP: Hemolysis, Elevated Liver Enzymes and Low Platelets syndrome. PCOS: Polycystic ovary syndrome. LGA: Large for Gestational Age. PTB: Preterm birth. P: Percentiles*100.

Gestational diabetes was more prevalent in the 0 CL group (16.7% vs 9.3%, aOR 2.59 [95% CI 1.31; 5.15]). The presence of >1 CL was not associated with gestational diabetes risk.

Birth outcomes

The >1 CL group had a higher average gestational age than the 1 CL group (275 vs 273 days, aβ 2.08 days [95% CI 0.37; 3.79]). Concordantly, the risk of preterm birth was lower in the >1 CL group, albeit not significant after adjustments (7.7% vs 10.7%, aOR 0.73 [95% CI 0.47; 1.13]).

Absolute birthweight was comparable across groups (Table 2). Relative birthweight was lower in the pregnancies conceived with >1 CL, although non-significant (41.1 vs 49.9 percentiles, aβ -3.06 [95% CI -6.52; 0.40], Tables 2 and 3). Post hoc stratification by neonatal sex revealed diversity in the associations. Female neonates with CL absence had higher birthweight percentiles (aβ 12.93 [95% CI 2.52; 23.34]), while male neonates showed no significant associations between the 0 and 1 CL groups. However, in males >1 CL was associated with significantly lower birthweight percentiles (aβ -6.18 [95% CI -11.16; -1.20]), while no association was found in females. ART analysis confirmed these findings with even stronger effect sizes (Table 2 and Supplementary Table 1). A graphical representation of the birthweight differences among study groups is shown in Figure S1.

Sensitivity analyses

All results remained consistent after excluding patients with polycystic ovary syndrome (PCOS) and premature ovarian insufficiency (POI). Additionally, the results remained consistent after excluding those who underwent IUI, after including only the first pregnancy for each woman, and after stratification for the timing of embryo transfer, respectively (Supplementary Table 2). The associations with hypertensive disorders were not affected by excluding patients with chronic hypertension or gestational diabetes mellitus, respectively. No significantly nonlinear associations or interactions were found. Analyzing CL numbers as a continuous variable in a dose-response manner in 462 patients showed the same direction of the association as previously demonstrated: higher CL number was associated with a lower relative male birthweight (crude β -0.24 [95% CI -1.19; 0.70], aβ -0.44 [95% CI -1.43; 0.55]).

To assess heterogeneity in the 1 CL group, pregnancies conceived through NC-FET were compared to naturally conceived pregnancies. No significant differences between these groups were found across all outcomes, except for birthweight (3.3 kg vs 3.4 kg respectively, P=0.014).

Table 3. Regression analysis of maternal pregnancy and birth outcomes after pregnancies conceived after exposure to different CL numbers. N=1861.

		Crude model OR (95% CI)	**P-value**	**Adjusted model** OR (95% CI)	**P-value**
Maternal outcomes	*1 CL*	*Reference*		*Reference*	
Hypertensive disorders of pregnancy[1]	0 CL	1.51 (0.79; 2.86)	0.212	1.59 (0.82; 3.09)	0.174
	>1 CL	0.62 (0.42; 0.91)	**0.015**	0.59 (0.39; 0.90)	**0.014**
Pregnancy-induced hypertension[1]	0 CL	0.92 (0.33; 2.60)	0.880	1.08 (0.37; 3.11)	0.889
	>1 CL	0.82 (0.52; 1.29)	0.387	0.83 (0.49; 1.41)	0.496
Preeclampsia[1]	0 CL	2.20 (1.02; 4.77)	**0.045**	2.02 (0.91; 4.51)	0.086
	>1 CL	0.39 (0.20; 0.77)	**0.006**	0.36 (0.18; 0.72)	**0.004**
Gestational diabetes[2]	0 CL	1.95 (1.02; 3.72)	**0.043**	2.59 (1.31; 5.15)	**0.006**
	>1 CL	0.99 (0.68; 1.43)	0.953	1.23 (0.82; 1.86)	0.320
Birth outcomes					
Gestational age at birth (days)[3]	0 CL	1.97 (-1.57; 5.51)	0.275	0.56 (-2.98; 4.09)	0.757
	>1 CL	3.02 (1.44; 4.61)	**<0.001**	2.08 (0.37; 3.79)	**0.017**
Birthweight (kg)[4]	0 CL	0.07 (-0.08; 0.21)	0.350	0.03 (-0.07; 0.14)	0.549
	>1 CL	0.01 (-0.05, 0.07)	0.761	-0.04 (-0.09; 0.01)	0.110
Relative birthweight (p)[2]	0 CL	2.48 (-4.75; 9.71)	0.501	3.35 (-3.90; 10.59)	0.365
	>1 CL	-4.59 (-7.82; -1.36)	**0.005**	-3.06 (-6.52; 0.40)	0.083
Birthweight, male (kg)[4]	0 CL	-0.04 (-0.25; 0.16)	0.668	-0.07 (-0.23; 0.08)	0.338
	>1 CL	-0.02 (-0.11; 0.08)	0.739	-0.09 (-0.16; -0.01)	**0.024**
Relative birthweight male (p)[2]	0 CL	-5.75 (-15.71; 4.22)	0.258	-5.89 (-16.01; 4.23)	0.254
	>1 CL	-7.08 (-11.75; -2.42)	**0.003**	-6.18 (-11.16; -1.20)	**0.015**
Birthweight, female (kg)[4]	0 CL	0.19 (-0.02; 0.39)	0.078	0.13 (-0.02; 0.28)	0.080
	>1 CL	0.04 (-0.05; 0.12)	0.433	0.00 (-0.07; 0.07)	0.999
Relative birthweight female (p)[2]	0 CL	11.90 (1.40; 22.41)	**0.026**	12.93 (2.52; 23.34)	**0.015**
	>1 CL	-2.17 (-6.64; 2.30)	0.340	-0.10 (-4.92; 4.71)	0.967
Preterm birth[3]	0 CL	1.21 (0.59; 2.50)	0.598	1.44 (0.69; 3.03)	0.332
	>1 CL	0.70 (0.48; 1.03)	0.071	0.73 (0.47; 1.13)	0.162
LGA (>90th)[5]	0 CL	0.61 (0.24; 1.54)	0.294	0.60 (0.21; 1.70)	0.338
	>1 CL	0.69 (0.47; 1.01)	0.057	0.85 (0.56; 1.29)	0.442
SGA (<10th)[6]	0 CL	0.76 (0.34; 1.70)	0.507	0.80 (0.36; 1.82)	0.601
	>1 CL	1.10 (0.80; 1.51)	0.550	1.04 (0.73; 1.49)	0.816

Data are presented as Odds Ratios (95% CI) for binary variables and Effect estimates of the linear mixed model (Beta, 95% CI) for continuous variables. Relative birthweight is noted as percentiles*100. CL: Corpus Luteum. SGA: Small for Gestational Age. LGA: Large for Gestational Age. ART: Artificial Reproductive Technology. Data are adjusted for maternal age, BMI, and nulliparity. Also adjusted for: 1. Preeclampsia in prior pregnancy. 2. Smoking. 3. Smoking and preterm birth in prior pregnancy. 4. Smoking and gestational age at birth. 5. Smoking and LGA in prior pregnancy. 6. Smoking and SGA in prior pregnancy.

DISCUSSION

In this prospective observational cohort, we demonstrated associations between CL number and adverse maternal pregnancy and neonatal outcomes. In pregnancies with 0 CL, we found a nonsignificant 1.6-fold increase of hypertensive disorders of pregnancy and a significant 2.6-fold increase of gestational diabetes. Conversely, >1 CL pregnancies showed a significant 0.6-fold decreased risk of hypertensive disorders of pregnancy and lower birthweights, with post hoc analyses revealing that the lower birthweights were particularly pronounced in male neonates. Overall, we demonstrate that the periconceptional number of CL is associated with maternal pregnancy and neonatal outcomes.

Maternal pregnancy outcomes

Consistent with existing literature, we observed a higher incidence of hypertensive disorders of pregnancy in CL absence, with an OR of 1.59 (p=0.174) [8, 9, 31-36]. Interestingly, we also discovered a lower incidence of hypertensive disorders of pregnancy in the >1 CL group, with an odds ratio of 0.59. While some studies support this finding, comparison studies between different IVF protocols have not specifically focused on the >1 CL group in relation to preeclampsia [5-7, 33]. These associations cannot be explained by PCOS or POI, which was demonstrated by exclusion of these patients. Similarly, the effects persisted in the subgroup analysis restricted to ART patients. This population is more homogeneous than the full cohort, as all patients visited the tertiary care center with a subfertility diagnosis. This minimizes the effects of confounding by indication, thus indicating a true effect of the CL on the development of hypertensive disorders of pregnancy.

In relation to gestational diabetes, our study identified a significantly higher incidence in the cohort lacking a CL, with an odds ratio of 2.59. Existing literature shows mixed findings, reporting positive, negative, and neutral associations [5, 9, 34-37]. However, most studies align with our observation of higher incidence of gestational diabetes in absence of a CL. The findings were not explained by high-risk patients with PCOS, as the effect persisted after exclusion of these pregnancies.

A possible explanation for our findings is the influence of luteal hormones on maternal cardiovascular adaptation and placentation, which have been associated with both hypertensive disorders of pregnancy and gestational diabetes [17, 38, 39]. The CL plays a significant role in cardiovascular adaptation, as it is decreased in CL absence [40, 41]. This association could be exerted through relaxin, which is produced primarily by the CL and has an impact on vasodilation decidualization and reduced vascular inflammation [42-44]. Elimination of relaxin in gravid rats resulted in a total absence of cardiovascular adaptations, possibly explaining the increase in maternal complications found in the 0 CL group in this cohort [40]. Besides relaxin, vascular endothelial growth factor (VEGF) production by the CL affects early pregnancy, mainly by improving placentation [21]. Moreover, pregnancies conceived

in CL absence have lower hormone concentrations of the renin-angiotensin-aldosterone system (RAAS). Decreased RAAS activity is known to be associated with higher risks of hypertensive disorders of pregnancy [45]. Similarly, the increased concentrations of relaxin and RAAS components in pregnancies with >1 CL could be protective against hypertensive disorders of pregnancy. Differences in the etiology of early-onset and late-onset preeclampsia have been proposed, but it remains unclear whether CL number influences one or both types [46]. Although analyzing these groups separately could offer insights into underlying pathways, our cohort lacked sufficient PE cases for such an analysis.

Birth outcomes

Consistent with previous publications, we found no association between 0 CL and gestational age at birth [8, 31, 37]. We did observe a positive association between >1 CL and gestational age at birth, though the clinical implications are limited due to the small effect size. Our findings show a trend towards less preterm birth in pregnancies conceived in the presence of >1 CL, which is supported by literature [34-37]. Higher powered studies are needed to confirm this effect.

Previous research consistently describes a lower birthweight in pregnancies with multiple CL, but the effects of CL absence are debated [5, 31, 33, 35, 47-52]. Our findings from the post hoc analysis highlight neonatal sex diversity as a possible explanation for the discrepancies in literature. We observed that female neonates with CL absence had significantly higher birthweights, estimated at 12.9 percentiles. However, this association was not established in male neonates; in fact, their average birthweight was lower in CL absence. Interestingly, the presence of multiple CL solely affected males, resulting in a reduced average birthweight. These findings align with a previous study by Waschkies et al., but the literature on effect modification by neonatal sex is sparse [31]. We hypothesize that differences between male and female neonates are due to differences in response to placental conditions, as sex-specific placental function and adaptation have been proposed previously [53]. Females may prioritize placenta development, while males invest more in growth [54]. This could explain sex-specific effects, with the female response potentially mitigating the effects of disrupted hormone levels. RAAS signaling might be part of the underlying pathway. CL absence is associated with reduced RAAS activity, whereas multiple CL increase its activity [13, 22]. Increased angiotensin II concentrations in pregnancies with females suggest they could be more affected by low RAAS activity in CL absence [55]. Prorenin concentrations negatively correlate with birthweight, fitting with higher birthweight in CL absence in susceptible females and lower birthweight in the case of multiple CL in susceptible males [49].

An alternative hypothesis concerns placental growth factor (PlGF) and soluble fms-like tyrosine kinase-1 (sFlt-1) signaling. In CL absence, increased sFlt-1/PlGF ratios and decreased VEGF concentrations are found, which are associated with increased incidence of

preeclampsia and fetal growth restriction [22, 56-58]. However, the sex-dependent nature of the sFlt-1/PlGF ratio remains debated, with conflicting results across studies [59-65].

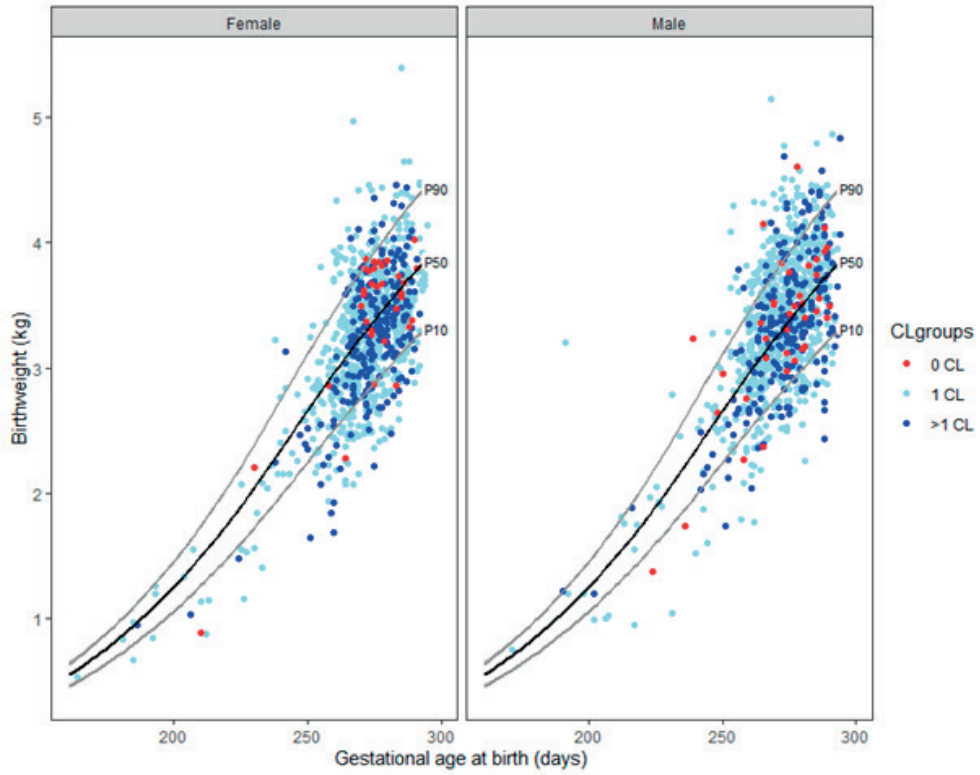
Strengths and limitations

Our study addresses a gap in literature by examining the impact of both CL absence and the presence of multiple CL. Unlike existing literature, which often compares embryo transfer protocols without distinguishing CL numbers, our stratified approach allowed for individual interpretation of these effects. We minimized selection bias and recall bias through a prospective observational cohort design, and all clinical outcomes were verified using medical records. This study was conducted within a single institution, ensuring high internal generalizability, and the use of consistent ART and study recruitment protocols. Several limitations should be acknowledged. The context of a tertiary care hospital may slightly weaken external generalizability. Women could participate in the cohort with subsequent pregnancies, but we corrected for parity and prior pregnancy outcomes to limit confounding. A sensitivity analysis excluding subsequent pregnancies showed no significant impact. Small sample sizes, particularly in the 0 CL group, limited the statistical power of our analysis. This likely contributed to the observed attenuation of the association between 0 CL and preeclampsia risk in the adjusted model, where the statistical significance was no longer observed. Additionally, due to limited available data, we were unable to conduct a dose-response analysis for our primary binary outcomes. While confounding by underlying conditions is possible, results remained consistent after adjusting for known confounders and in subgroup and sensitivity analyses. While the effects of the CL and mode of conception and its indications could not be disentangled completely, significant confounding by indication is unlikely. In addition, residual confounding inherent to the observational study design cannot be excluded. Future high-powered studies are needed to support these findings and to measure first-trimester CL hormones, vasoactive products, and outcomes related to placental function and cardiovascular adaptation to clarify underlying pathways.

Conclusions

This study supports our hypothesis that CL number is associated with maternal pregnancy and birth outcomes, with modification by neonatal sex. CL absence was associated with elevated risks of hypertensive disorders of pregnancy, gestational diabetes, and higher birthweight in females. An increased number of CL was associated with a lower risk of hypertensive disorders of pregnancy and lower birthweight in males. While further evidence is needed to confirm the underlying pathways, it appears that enhancing our understanding of the early, demanding maternal-embryo-placenta interaction, along with optimizing the ART protocol to enhance maternal physiological conditions, contributes to improved pregnancy outcomes.

SUPPLEMENTARY MATERIAL



Supplementary Figure 1. Birthweight percentiles by gestational age, stratified by corpus luteum category and neonatal sex. P: Hoftiezer percentile. CL: Corpus luteum.

Supplementary Table 1. Regression analysis of birth outcomes after pregnancies conceived after exposure to different CL numbers, focusing only on ART patients (n=720).

		Crude model	P-value	Adjusted model	P-value
		Beta (95% CI)		Beta (95% CI)	
	1 CL	*Reference*		*Reference*	
Gestational age at birth (days)[1]	0 CL	0.36 (-3.40; 4.11)	0.851	-0.01 (-3.96; 3.95)	0.997
	>1 CL	1.41 (-0.96; 3.78)	0.242	1.25 (-1.19; 3.69)	0.316
Birthweight (kg)[2]	0 CL	-0.02 (-0.18; 0.14)	0.827	-0.01 (-0.14; 0.12)	0.871
	>1 CL	-0.08 (-0.18; 0.02)	0.138	-0.09 (-0.17; -0.01)	**0.022**
Relative birthweight (p)[3]	0 CL	-0.09 (-8.33; 8.15)	0.982	0.47 (-8.36; 9.30)	0.917
	>1 CL	-7.16 (-12.36; -1.96)	**0.007**	-6.59 (-11.99; -1.20)	**0.017**
Birthweight, male (kg)[2]	0 CL	-0.23 (-0.47; 0.00)	0.050	-0.18 (-0.37; 0.01)	0.057
	>1 CL	-0.21 (-0.36; -0.05)	**0.010**	-0.21 (-0.33; -0.09)	**0.001**
Relative birthweight male (p)[3]	0 CL	-13.43 (-25.11; -1.74)	**0.024**	-12.74 (-25.41; -0.07)	**0.049**
	>1 CL	-14.76 (-22.60; -6.93)	**<0.001**	-14.36 (-22.34; -6.38)	**<0.001**
Birthweight, female (kg)[2]	0 CL	0.16 (-0.06; 0.39)	0.154	0.13 (-0.04; 0.31)	0.135
	>1 CL	0.01 (-0.12; 0.15)	0.858	0.00 (-0.10; 0.11)	0.927
Relative birthweight female (p)[3]	0 CL	12.89 (1.25; 24.54)	**0.030**	12.92 (0.46; 25.38)	**0.042**
	>1 CL	-1.18 (-8.08; 5.72)	0.736	0.20 (-7.16; 7.55)	0.958

Data are presented as effect estimates of the linear mixed model (Beta, 95% confidence interval) Relative birthweight is noted as percentiles*100. ART: Artificial Reproductive Technology. CL: Corpus Luteum. All data are adjusted for maternal age, maternal BMI, nulliparity, smoking, origin of subfertility. 1: Additionally adjusted for preterm birth in previous pregnancy. 2: Additionally adjusted for gestational age at birth. 3: Not additionally adjusted.

Supplementary Table 2. Birth outcomes after pregnancies conceived after exposure to different CL numbers, stratified by embryo transfer stage.

	0 CL AC-FET	>1 CL Fresh ET	1 CL NC-FET	P-value 0 vs. 1 CL	P-value 1 vs. >1 CL
All ART-conceived pregnancies	N = 72	N = 462	N = 186		
Day-3	N = 52	N = 305	N = 115		
Day-5	N = 18	N = 156	N = 66		
Birth outcomes					
Gestational age at birth (days), median [IQR])	275 [270, 283]	275 [268, 282]	275 [267, 281]	0.384	0.233
Day-3	275 [270, 285]	275 [269, 282]	274 [266, 281]	0.181	0.102
Day-5	274 [270, 280]	276 [268, 282]	275 [267, 283]	0.455	0.788
Preterm birth (n, %)	9/71 (12.7)	35/453 (7.7)	17/178 (9.6)	0.467	0.453
Day-3	6/51 (11.8)	19/299 (6.4)	13/107 (12.1)	0.945	0.056
Day-5	3/18 (16.7)	15/153 (9.8)	3/66 (4.5)	0.109	0.284
Birthweight (kg), median [IQR]	3.5 [3.2, 3.8]	3.3 [3.0, 3.7]	3.4 [3.1, 3.8]	0.739	0.045
Day-3	3.5 [3.1, 3.8]	3.3 [3.0, 3.6]	3.4 [3.1, 3.7]	0.530	0.382
Day-5	3.6 [3.3, 3.8]	3.4 [2.9, 3.7]	3.6 [3.2, 3.9]	0.938	0.023
High birthweight (>4000g), (n, %)	4/71 (5.6)	40/454 (8.8)	23/180 (12.8)	0.117	0.132
Day-3	1/51 (2.0)	25/299 (8.4)	11/110 (10.0)	0.105	0.604
Day-5	3/18 (16.7)	15/154 (9.7)	12/65 (18.5)	1.000	0.073
Low birthweight (<2500g), (n, %)	7/71 (9.9)	35/454 (7.7)	15/180 (8.3)	0.700	0.793
Day-3	7/51 (13.7)	22/299 (7.4)	13/110 (11.8)	0.733	0.153
Day-5	0/18 (0.0)	12/154 (7.8)	2/65 (3.1)	1.000	0.240
Relative birthweight (p), median [IQR]	52.3 [26.0, 77.0]	41.1 [19.5, 71.3]	54.2 [26.4, 79.7]	0.974	0.008
Day-3	49.8 [22.7, 75.9]	40.1 [19.8, 67.4]	43.5 [24.0, 74.9]	0.919	0.190
Day-5	70.9 [48.4, 83.7]	44.3 [19.5, 76.6]	63.9 [33.7, 82.4]	0.351	0.010
LGA (>90th), (n, %)	5/70 (7.1)	36/449 (8.0)	21/174 (12.1)	0.259	0.116
Day-3	1/51 (2.0)	17/296 (5.7)	9/105 (8.6)	0.168	0.312
Day-5	4/17 (23.5)	19/152 (12.5)	11/64 (17.2)	0.506	0.073
SGA (<10th), (n, %)	7/70 (10.0)	62/449 (13.8)	16/174 (9.2)	0.846	0.119
Day-3	7/51 (13.7)	42/296 (14.2)	11/105 (10.5)	0.551	0.335
Day-5	0/17 (0.0)	19/152 (12.5)	3/64 (4.7)	1.000	0.091
Neonatal sex (male), (n, %)	38/71 (53.5)	225/456 (49.3)	78/180 (43.3)	0.145	0.172
Day-3	26/51 (51.0)	151/301 (50.2)	52/109 (47.7)	0.699	0.660
Day-5	11/18 (61.1)	74/154 (48.1)	24/66 (36.4)	0.059	0.110
Birthweight, male (kg), median [IQR]	3.4 [3.1, 3.7]	3.4 [3.0, 3.7]	3.6 [3.3, 3.8]	0.049	0.003

Supplementary Table 2. Birth outcomes after pregnancies conceived after exposure to different CL numbers, stratified by embryo transfer stage. (continued)

	0 CL AC-FET	>1 CL Fresh ET	1 CL NC-FET	P-value 0 vs. 1 CL	P-value 1 vs. >1 CL
Day-3	3.4 [3.1, 3.6]	3.4 [3.1, 3.7]	3.5 [3.2, 3.8]	0.152	0.213
Day-5	3.5 [3.3, 4.0]	3.4 [2.9, 3.7]	3.8 [3.6, 4.1]	0.211	<0.001
Relative birthweight, male (p), median [IQR]	45.9 [20.6, 67.9]	38.7 [17.3, 72.5]	61.9 [33.5, 85.8]	0.028	<0.001
Day-3	35.9 [18.2, 63.5]	39.0 [20.0, 72.7]	55.3 [32.1, 80.9]	0.031	0.040
Day-5	64.6 [44.8, 87.9]	38.4 [15.8, 69.2]	75.8 [55.3, 91.7]	0.772	<0.001
Birthweight, female (kg), median [IQR]	3.6 [3.3, 3.8]	3.3 [3.0, 3.6]	3.3 [3.0, 3.6]	0.023	0.888
Day-3	3.6 [3.3, 3.8]	3.3 [2.9, 3.5]	3.3 [2.9, 3.5]	0.017	0.973
Day-5	3.6 [3.3, 3.7]	3.4 [3.1, 3.7]	3.4 [3.1, 3.8]	0.627	0.951
Relative birthweight, female (p), median [IQR]	70.1 [44.8, 82.8]	43.7 [20.7, 69.6]	43.5 [22.1, 75.2]	0.035	0.796
Day-3	61.0 [27.9, 81.1]	41.1 [19.5, 61.8]	37.5 [16.6, 67.6]	0.052	0.844
Day-5	82.0 [57.6, 83.5]	47.7 [28.3, 81.7]	55.5 [30.3, 82.3]	0.170	0.799

Data are presented as n (%) or median [IQR]. Relative birthweight is noted as percentiles*100. ART: Artificial Reproductive Technology. CL: Corpus Luteum. AC-FET: Artificial-Cycle Frozen Embryo Transfer. NC-FET: Natural-Cycle Frozen Embryo Transfer. ET: Embryo Transfer. SGA: Small for gestational age. LGA: Large for Gestational Age. P: Percentiles*100.

REFERENCES

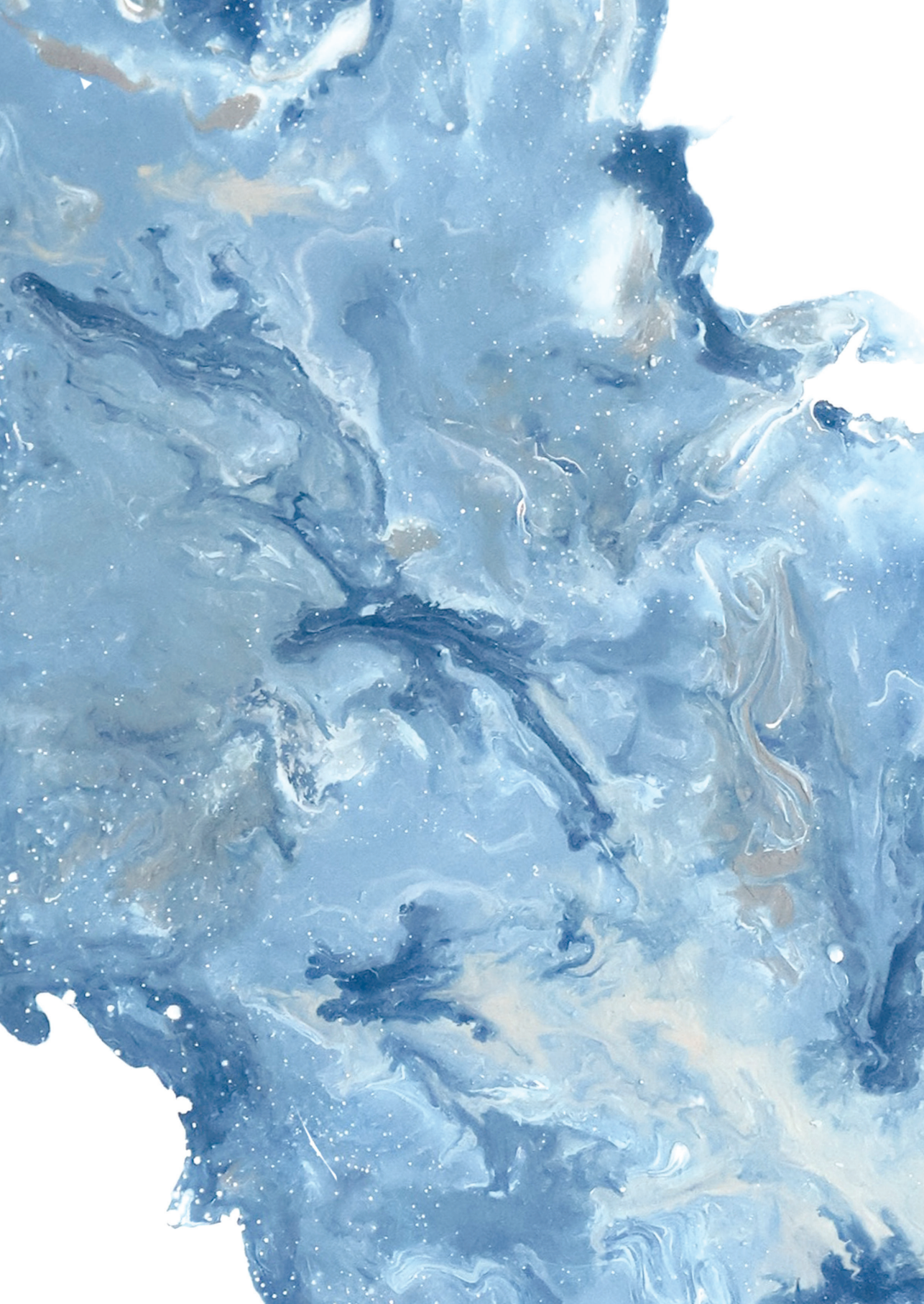
1. Jackson RA, Gibson KA, Wu YW, Croughan MS. Perinatal outcomes in singletons following in vitro fertilization: a meta-analysis. Obstet Gynecol. 2004 Mar;103(3):551-63.
2. Maheshwari A, Pandey S, Raja EA, Shetty A, Hamilton M, Bhattacharya S. Is frozen embryo transfer better for mothers and babies? Can cumulative meta-analysis provide a definitive answer? Human Reproduction Update. 2018;24(1):35-58.
3. Ishihara O, Araki R, Kuwahara A, Itakura A, Saito H, Adamson GD. Impact of frozen-thawed single-blastocyst transfer on maternal and neonatal outcome: an analysis of 277,042 single-embryo transfer cycles from 2008 to 2010 in Japan. Fertil Steril. 2014 Jan;101(1):128-33.
4. Opdahl S, Henningsen AA, Tiitinen A, Bergh C, Pinborg A, Romundstad PR, et al. Risk of hypertensive disorders in pregnancies following assisted reproductive technology: a cohort study from the CoNARTaS group. Hum Reprod. 2015 Jul;30(7):1724-31.
5. Sha T, Yin X, Cheng W, Massey IY. Pregnancy-related complications and perinatal outcomes resulting from transfer of cryopreserved versus fresh embryos in vitro fertilization: a meta-analysis. Fertility and Sterility. 2018 2018/2//;109(2):330-42.e9.
6. Sites CK, Wilson D, Barsky M, Bernson D, Bernstein IM, Boulet S, Zhang Y. Embryo cryopreservation and preeclampsia risk. Fertility and Sterility. 2017 2017/11//;108(5):784-90.
7. von Versen-Hoynck F, Schaub AM, Chi YY, Chiu KH, Liu J, Lingis M, et al. Increased Preeclampsia Risk and Reduced Aortic Compliance With In Vitro Fertilization Cycles in the Absence of a Corpus Luteum. Hypertension. 2019 Mar;73(3):640-9.
8. Zaat TR, Brink AJ, de Bruin JP, Goddijn M, Broekmans FJM, Cohlen BJ, et al. Increased obstetric and neonatal risks in artificial cycles for frozen embryo transfers? Reproductive BioMedicine Online. 2021 2021/5//;42(5):919-29.
9. Saito K, Kuwahara A, Ishikawa T, Morisaki N, Miyado M, Miyado K, et al. Endometrial preparation methods for frozen-thawed embryo transfer are associated with altered risks of hypertensive disorders of pregnancy, placenta accreta, and gestational diabetes mellitus. Hum Reprod. 2019 Aug 1;34(8):1567-75.
10. Conrad KP. Evidence for Corpus Luteal and Endometrial Origins of Adverse Pregnancy Outcomes in Women Conceiving with or Without Assisted Reproduction. Obstetrics and Gynecology Clinics of North America: W.B. Saunders; 2020. p. 163-81.
11. Conrad KP, Baker VL. Corpus luteal contribution to maternal pregnancy physiology and outcomes in assisted reproductive technologies. Am J Physiol Regul Integr Comp Physiol. 2013 Jan 15;304(2):R69-72.
12. Schams D. Luteal peptides and intercellular communication. J Reprod Fertil Suppl. 1987;34:87-99.
13. Wiegel RE, Jan Danser AH, Steegers-Theunissen RPM, Laven JSE, Willemsen SP, Baker VL, et al. Determinants of Maternal Renin-Angiotensin-Aldosterone-System Activation in Early Pregnancy: Insights From 2 Cohorts. J Clin Endocrinol Metab. 2020 Nov 1;105(11):3505-17.
14. Singh B, Reschke L, Segars J, Baker VL. Frozen-thawed embryo transfer: the potential importance of the corpus luteum in preventing obstetrical complications. Fertility and Sterility: Elsevier Inc.; 2020. p. 252-7.
15. Conrad KP. Maternal vasodilation in pregnancy: The emerging role of relaxin. American Journal of Physiology - Regulatory Integrative and Comparative Physiology. 2011 2011/8//;301(2).
16. Bosio PM, McKenna PJ, Conroy R, O'Herlihy C. Maternal central hemo-

dynamics in hypertensive disorders of pregnancy. Obstet Gynecol. 1999 Dec;94(6):978-84.
17. Khalil A, Garcia-Mandujano R, Maiz N, Elkhouli M, Nicolaides KH. Longitudinal changes in maternal hemodynamics in a population at risk for pre-eclampsia. Ultrasound in Obstetrics and Gynecology. 2014;44(2):197-204.
18. Khaw A, Kametas NA, Turan OM, Bamfo JE, Nicolaides KH. Maternal cardiac function and uterine artery Doppler at 11-14 weeks in the prediction of pre-eclampsia in nulliparous women. BJOG. 2008 Feb;115(3):369-76.
19. Vasapollo B, Valensise H, Novelli GP, Altomare F, Galante A, Arduini D. Abnormal maternal cardiac function precedes the clinical manifestation of fetal growth restriction. Ultrasound Obstet Gynecol. 2004 Jul;24(1):23-9.
20. Valensise H, Farsetti D, Lo Presti D, Pisani I, Tiralongo GM, Gagliardi G, et al. Preterm delivery and elevated maternal total vascular resistance: signs of suboptimal cardiovascular adaptation to pregnancy? Ultrasound Obstet Gynecol. 2016 Oct;48(4):491-5.
21. von Versen-Höynck F, Strauch NK, Liu J, Chi YY, Keller-Woods M, Conrad KP, Baker VL. Effect of Mode of Conception on Maternal Serum Relaxin, Creatinine, and Sodium Concentrations in an Infertile Population. Reproductive Sciences. 2019 2019/3//;26(3):412-9.
22. Conrad KP, Graham GM, Chi YY, Zhai X, Li M, Williams RS, et al. Potential influence of the corpus luteum on circulating reproductive and volume regulatory hormones, angiogenic and immunoregulatory factors in pregnant women. Am J Physiol Endocrinol Metab. 2019 Oct 1;317(4):E677-E85.
23. Steegers-Theunissen RP, Verheijden-Paulissen JJ, van Uitert EM, Wildhagen MF, Exalto N, Koning AH, et al. Cohort Profile: The Rotterdam Periconceptional Cohort (Predict Study). Int J Epidemiol. 2016 Apr;45(2):374-81.
24. Rousian M, Schoenmakers S, Eggink AJ, Gootjes DV, Koning AHJ, Koster MPH, et al. Cohort Profile Update: the Rotterdam Periconceptional Cohort and embryonic and fetal measurements using 3D ultrasound and virtual reality techniques. Int J Epidemiol. 2021 Nov 10;50(5):1426-7l.
25. Hohmann FP, Macklon NS, Fauser BC. A randomized comparison of two ovarian stimulation protocols with gonadotropin-releasing hormone (GnRH) antagonist cotreatment for in vitro fertilization commencing recombinant follicle-stimulating hormone on cycle day 2 or 5 with the standard long GnRH agonist protocol. J Clin Endocrinol Metab. 2003 Jan;88(1):166-73.
26. Heijnen EM, Eijkemans MJ, De Klerk C, Polinder S, Beckers NG, Klinkert ER, et al. A mild treatment strategy for in-vitro fertilisation: a randomised non-inferiority trial. Lancet. 2007 Mar 3;369(9563):743-9.
27. Brown MA, Lindheimer MD, de Swiet M, Van Assche A, Moutquin JM. The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). Hypertens Pregnancy. 2001;20(1):IX-XIV.
28. Brown MA, Magee LA, Kenny LC, Karumanchi SA, McCarthy FP, Saito S, et al. Hypertensive Disorders of Pregnancy: ISSHP Classification, Diagnosis, and Management Recommendations for International Practice. Hypertension. 2018 Jul;72(1):24-43.
29. Hoftiezer L, Hukkelhoven CW, Hogeveen M, Straatman HM, van Lingen RA. Defining small-for-gestational-age: prescriptive versus descriptive birthweight standards. Eur J Pediatr. 2016 Aug;175(8):1047-57.
30. Bursac Z, Gauss CH, Williams DK, Hosmer DW. Purposeful selection of variables in logistic regression. Source Code Biol Med. 2008 Dec 16;3:17.

31. Waschkies F, Kroning L, Schill T, Chandra A, Schippert C, Töpfer D, et al. Pregnancy Outcomes After Frozen-Thawed Embryo Transfer in the Absence of a Corpus Luteum. Frontiers in Medicine. 2021 2021/9//;8.
32. Asserhoj LL, Spangmose AL, Aaris Henningsen AK, Clausen TD, Ziebe S, Jensen RB, Pinborg A. Adverse obstetric and perinatal outcomes in 1,136 singleton pregnancies conceived after programmed frozen embryo transfer (FET) compared with natural cycle FET. Fertil Steril. 2021 Apr;115(4):947-56.
33. Ginstrom Ernstad E, Wennerholm UB, Khatibi A, Petzold M, Bergh C. Neonatal and maternal outcome after frozen embryo transfer: Increased risks in programmed cycles. Am J Obstet Gynecol. 2019 Aug;221(2):126 e1- e18.
34. Makhijani R, Bartels C, Godiwala P, Bartolucci A, Nulsen J, Grow D, et al. Maternal and perinatal outcomes in programmed versus natural vitrified–warmed blastocyst transfer cycles. Reproductive BioMedicine Online. 2020 2020/8//;41(2):300-8.
35. Hu KL, Zhang D, Li R. Endometrium preparation and perinatal outcomes in women undergoing single-blastocyst transfer in frozen cycles. Fertil Steril. 2021 Jun;115(6):1487-94.
36. Zong L, Liu P, Zhou L, Wei D, Ding L, Qin Y. Increased risk of maternal and neonatal complications in hormone replacement therapy cycles in frozen embryo transfer. Reproductive Biology and Endocrinology. 2020 2020/5//;18(1).
37. Wang Z, Liu H, Song H, Li X, Jiang J, Sheng Y, Shi Y. Increased Risk of Pre-eclampsia After Frozen-Thawed Embryo Transfer in Programming Cycles. Frontiers in Medicine. 2020 2020/4//;7.
38. Weissgerber TL, Milic NM, Milin-Lazovic JS, Garovic VD. Impaired Flow-Mediated Dilation Before, During, and After Preeclampsia: A Systematic Review and Meta-Analysis. Hypertension. 2016 Feb;67(2):415-23.
39. Wiegel RE, von Versen-Hoynck F, Steegers-Theunissen RPM, Steegers EAP, Danser AHJ. Prorenin periconceptionally and in pregnancy: Does it have a physiological role? Mol Cell Endocrinol. 2021 Feb 15;522:111118.
40. Conrad KP. Unveiling the vasodilatory actions and mechanisms of relaxin. Hypertension2010. p. 2-9.
41. Conrad KP, Petersen JW, Chi YY, Zhai X, Li M, Chiu KH, et al. Maternal cardiovascular dysregulation during early pregnancy after in vitro fertilization cycles in the absence of a corpus luteum. Hypertension. 2019 2019/9//;74(3):705-15.
42. Smith MC, Danielson LA, Conrad KP, Davison JM. Influence of recombinant human relaxin on renal hemodynamics in healthy volunteers. J Am Soc Nephrol. 2006 Nov;17(11):3192-7.
43. Conrad KP, Davison JM. The renal circulation in normal pregnancy and preeclampsia: Is there a place for relaxin? American Journal of Physiology - Renal Physiology: American Physiological Society; 2014.
44. Du XJ, Bathgate RA, Samuel CS, Dart AM, Summers RJ. Cardiovascular effects of relaxin: from basic science to clinical therapy. Nat Rev Cardiol. 2010 Jan;7(1):48-58.
45. Verdonk K, Visser W, Van Den Meiracker AH, Danser AH. The renin-angiotensin-aldosterone system in pre-eclampsia: the delicate balance between good and bad. Clin Sci (Lond). 2014 Apr;126(8):537-44.
46. Conrad KP, von Versen-Hoynck F, Baker VL. Pathologic maternal and neonatal outcomes associated with programmed embryo transfer. J Assist Reprod Genet. 2024 Apr;41(4):821-42.
47. Vidal M, Vellvé K, González-Comadran M, Robles A, Prat M, Torné M, et al. Perinatal outcomes in children born after fresh or frozen embryo transfer: a Catalan cohort

study based on 14,262 newborns. Fertility and Sterility. 2017 2017/4//;107(4):940-7.
48. Baker VL, Brown MB, Luke B, Conrad KP, editors. Association of number of retrieved oocytes with live birth rate and birth weight: An analysis of 231,815 cycles of in vitro fertilization. Fertility and Sterility; 2015: Elsevier Inc.
49. Wiegel RE, Aoulad Fares D, Willemsen SP, Steegers EAP, Jan Danser A, Steegers-Theunissen RPM. First-trimester maternal renin–angiotensin–aldosterone system activation and fetal growth and birthweight: the Rotterdam Periconceptional Cohort. 2022.
50. van Duijn L, Hoek J, Rousian M, Baart EB, Willemsen SP, Laven JSE, et al. Prenatal growth trajectories and birth outcomes after frozen-thawed extended culture embryo transfer and fresh embryo transfer: the Rotterdam Periconception Cohort. Reprod Biomed Online. 2021 Aug;43(2):279-87.
51. Weiss G, Goldsmith LT, Sachdev R, Von Hagen S, Lederer K. Elevated first-trimester serum relaxin concentrations in pregnant women following ovarian stimulation predict prematurity risk and preterm delivery. Obstet Gynecol. 1993 Nov;82(5):821-8.
52. Nakashima A, Araki R, Tani H, Ishihara O, Kuwahara A, Irahara M, et al. Implications of assisted reproductive technologies on term singleton birth weight: an analysis of 25,777 children in the national assisted reproduction registry of Japan. Fertil Steril. 2013 Feb;99(2):450-5.
53. Clifton VL. Review: Sex and the human placenta: mediating differential strategies of fetal growth and survival. Placenta. 2010 Mar;31 Suppl:S33-9.
54. Buckberry S, Bianco-Miotto T, Bent SJ, Dekker GA, Roberts CT. Integrative transcriptome meta-analysis reveals widespread sex-biased gene expression at the human fetal-maternal interface. Mol Hum Reprod. 2014 Aug;20(8):810-9.
55. Sykes SD, Pringle KG, Zhou A, Dekker GA, Roberts CT, Lumbers ER, Consortium S. The balance between human maternal plasma angiotensin II and angiotensin 1-7 levels in early gestation pregnancy is influenced by fetal sex. J Renin Angiotensin Aldosterone Syst. 2014 Dec;15(4):523-31.
56. Lee MS, Cantonwine D, Little SE, McElrath TF, Parry SI, Lim KH, Wilkins-Haug LE. Angiogenic markers in pregnancies conceived through in vitro fertilization. Am J Obstet Gynecol. 2015 Aug;213(2):212 e1-8.
57. Verlohren S, Brennecke SP, Galindo A, Karumanchi SA, Mirkovic LB, Schlembach D, et al. Clinical interpretation and implementation of the sFlt-1/PlGF ratio in the prediction, diagnosis and management of preeclampsia. Pregnancy Hypertens. 2022 Mar;27:42-50.
58. Pei J, Li Y, Min Z, Dong Q, Ruan J, Wu J, Hua X. MiR-590-3p and its targets VEGF, PlGF, and MMP9 in early, middle, and late pregnancy: their longitudinal changes and correlations with risk of fetal growth restriction. Ir J Med Sci. 2022 Jun;191(3):1251-7.
59. Andersen LB, Jorgensen JS, Herse F, Andersen MS, Christesen HT, Dechend R. The association between angiogenic markers and fetal sex: Implications for preeclampsia research. J Reprod Immunol. 2016 Sep;117:24-9.
60. Brown ZA, Schalekamp-Timmermans S, Tiemeier HW, Hofman A, Jaddoe VW, Steegers EA. Fetal sex specific differences in human placentation: a prospective cohort study. Placenta. 2014 Jun;35(6):359-64.
61. Taylor BD, Haggerty CL, Ness RB, Hougaard DM, Skogstrand K, Roberts JM, Olsen J. Fetal sexual dimorphism in systemic soluble fms-like tyrosine kinase 1 among normotensive and preeclamptic women. Am J Reprod Immunol. 2018 Nov;80(5):e13034.

62. Enninga EA, Nevala WK, Creedon DJ, Markovic SN, Holtan SG. Fetal sex-based differences in maternal hormones, angiogenic factors, and immune mediators during pregnancy and the postpartum period. Am J Reprod Immunol. 2015 Mar;73(3):251-62.
63. Arenas GA, Docheva N, Lopes Perdigao J, Mueller A, Dada T, Rana S. Association of fetal sex with angiogenic factors in normotensive and hypertensive pregnancy states. Pregnancy Hypertens. 2022 Aug;29:108-15.
64. Myers JE, Thomas G, Tuytten R, Van Herrewege Y, Djiokep RO, Roberts CT, et al. Mid-trimester maternal ADAM12 levels differ according to fetal gender in pregnancies complicated by preeclampsia. Reprod Sci. 2015 Feb;22(2):235-41.
65. Faupel-Badger JM, Wang Y, Karumanchi SA, Stanczyk F, Pollak M, McElrath T, et al. Associations of pregnancy characteristics with maternal and cord steroid hormones, angiogenic factors, and insulin-like growth factor axis. Cancer Causes Control. 2011 Nov;22(11):1587-95.



Part II

Clinical markers associated with adverse pregnancy outcomes and maternal cardiovascular health

6

Maternal blood pressure to vascular resistance ratio is positively associated with embryonic and fetal growth: The Rotterdam Periconception Cohort

Lotte W. Voskamp, Koen Verdonk, Régine P.M. Steegers-Theunissen, A.H. Jan Danser, Melek Rousian

Submitted

ABSTRACT

Objective: To investigate whether the ratios of mean arterial pressure (MAP) to uterine artery pulsatility (UtA-PI) or resistance index (UtA-RI) are associated with prenatal growth.

Design: Prospective cohort study.

Setting: Tertiary hospital, the Netherlands.

Population or Sample: 500 singleton pregnancies included between 2010-2022.

Methods: Maternal characteristics and pregnancy outcomes were obtained from clinical records and questionnaires. MAP, UtA-PI and UtA-RI were measured at 7, 9, 11, 21–24 and 30–33 weeks' gestation. MAP/PI and MAP/RI ratios were calculated per visit. Associations with Crown–rump length and embryonic volume were analysed using linear mixed models and adjusted for confounders. Associations with 20-week fetal biometry and birthweight Z-scores were assessed using linear regression. Ratio levels were compared across birthweight categories—small-for-gestational-age (SGA, <10th percentile), appropriate-for-gestational-age (AGA, 10th–90th percentile) and large-for-gestational-age (LGA, >90th percentile).

Main Outcome Measures: Crown-rump length, embryonic volume, fetal size, and birthweight.

Results: Higher MAP/PI and MAP/RI ratios at 9 weeks were associated with greater embryonic growth between 9 and 11 weeks (e.g. MAP/PI and EV: adjusted β=0.001 cm^3 [95%CI 0.000-0.003], p=0.041). First-trimester ratios were positively associated with estimated fetal weight at 20 weeks (e.g. MAP/PI: adjusted β=0.011 SD [95%CI 0.005-0.018], p=0.001). SGA pregnancies showed lower ratios than AGA and LGA pregnancies from mid-pregnancy onward (e.g., MAP/PI at 21–24 weeks: 85 [75-94] vs. 99 [95-103] vs. 121 [101-141], p=0.002).

Conclusions: MAP/PI and MAP/RI ratios are positively associated with embryonic growth, mid-pregnancy fetal size and birthweight. These ratios may serve as early markers of prenatal growth potential.

INTRODUCTION

Fetal growth abnormalities, such as fetal growth restriction (FGR) and small- (SGA) or large-for-gestational-age (LGA) neonates, are important predictors of perinatal outcomes, with FGR being the primary cause of stillbirth and neonatal mortality [1-5]. Monitoring fetal growth is therefore a central objective in obstetric care, yet ultrasound assessment has limited predictive value, and other early predictive markers remain scarce.

Given that fetal growth largely depends on the maternal supply of nutrients via the placenta, physiologically relevant markers may include parameters reflecting uteroplacental perfusion, such as mean arterial pressure (MAP) and uterine artery (UtA) Doppler indices. MAP represents the driving pressure of maternal blood flow, while Doppler indices quantify downstream resistance within the uteroplacental circulation. Alterations in either may compromise placental perfusion and impair fetal growth. However, previous studies have focused on these aspects separately in relation to embryonic and fetal growth trajectories [6-10]. In a previous study conducted within the Rotterdam Periconception Cohort, first-trimester MAP and UtA-PI trajectories showed no consistent associations with embryonic or fetal growth, except for a positive association between MAP and birthweight in pregnancies without placenta-related disorders [6]. Additionally, UtA-PI has been extensively investigated and shown to be a valuable predictor of adverse pregnancy outcomes, particularly preeclampsia, FGR or SGA neonates [11-14]. Current UK guidelines recommend second-trimester UtA-PI screening for women at risk for a pregnancy with fetal growth restriction [15]. Other studies have evaluated UtA-PI and MAP together in multivariable models with maternal characteristics and fetal biometry, showing that MAP improved prediction only in the third trimester [16, 17]. However, none of these studies have evaluated the combined predictive value of MAP and UtA-PI independent of clinical characteristics, leaving a gap in our understanding of their joint physiological contribution to uteroplacental perfusion and subsequent fetal growth.

We hypothesized that a ratio of MAP and UtA Doppler indices would more accurately reflect uteroplacental perfusion than each parameter separately. For instance, fetal growth restriction in preeclampsia typically occurs only when elevated MAP is accompanied by increased vascular resistance [18]. Conversely, low MAP could compromise uteroplacental perfusion even when vascular resistance is normal. Therefore, our objective was to investigate whether the ratios MAP/UtA-PI and MAP/UtA-RI are associated with embryonic growth, fetal size or birthweight.

METHODS

Study design

This study was conducted within the ongoing Rotterdam Periconceptional Cohort (Predict Study), an ongoing prospective observational study conducted at the Department of Obstetrics and Gynaecology of the Erasmus MC, University Medical Centre, a tertiary hospital in Rotterdam, the Netherlands (Dutch Trial Register NTR6854) [19, 20]. This study was performed with permission from the local Medical Ethics Review Committee (MEC-2004-227). Women aged ≥18 years with a singleton pregnancy were enrolled before 10 weeks of gestation between November 2010 and December 2022 and were followed through pregnancy until 1 year postpartum. For this study, exclusion criteria included miscarriage, termination of pregnancy, and intrauterine or neonatal death. For women with multiple pregnancies in the cohort, only the first was included.

Data collection

Participants completed a questionnaire at enrolment to record demographic, lifestyle, and medical information. Body mass index (BMI) was calculated from height and weight measured during the initial study visit. Blood pressure was measured on the upper left arm in a seated position by a trained physician using a manual cuff and stethoscope. Furthermore, all blood pressure (BP) measurements recorded during routine outpatient visits were extracted from the electronic patient records. MAP was calculated as: systolic BP + 2 times the diastolic BP, divided by 3. When no blood pressure measurement was performed on the same day, the closest measurement to the date of ultrasound examination was taken, with a maximum of 7 days difference in the first trimester and 28 days in the second and third trimester.

Ultrasound examinations were performed at 9 and 11 weeks GA (transvaginal) and between 21-24 and between 30-33 weeks GA (transabdominal) using Voluson Expert E8/E10 systems (GE, Zipf, Austria). At each visit, trained sonographers obtained bilateral UtA-PI and UtA-RI measurements using standard power Doppler settings[21]. Three measurements per side were recorded, and the mean per side was used for analysis[21].

For the Crown-Rump length (CRL) and embryonic volume (EV) measurements, 3D ultrasound datasets acquired using a transvaginal approach were used. These datasets were transferred automatically to a Virtual Reality setting, in which the CRL was measured manually, while the EV was measured semi-automatically, using the V-Scope software program, as described previously [19, 22, 23]. Fetal growth was assessed using biometric measurements from the routine 20-week anomaly scan, performed via 2D transabdominal ultrasound and offered to all pregnant women in the Netherlands [24]. Measurements included head circumference (HC), biparietal diameter (BPD), trans cerebellar diameter (TCD), abdominal circumference (AC), and femur length (FL), from which estimated fetal

weight (EFW) was calculated using the Hadlock formula [19, 25]. Gestational age (GA) was determined from the first day of the last menstrual period (LMP) in cases of regular menstrual cycles (21-35 days) or calculated by adding 14 days to the date of oocyte retrieval or 19 days to the cryopreserved embryo transfer date for pregnancies conceived via assisted reproductive technology [20]. In cases of irregular cycles, GA was assigned based on the CRL; however since the CRL is one of the outcomes within our study these cases were excluded from embryonic growth analyses. To account for retrospective dating and variation in clinical scheduling, GA-specific windows were applied to each study visit: 6+6 to 8+0 weeks for the 7-week visit, 8+6 to 10+0 weeks for the 9-week visit, 10+6 to 12+0 weeks for the 11-week visit, 21+0 to 24+6 weeks for the second-trimester visit, and 30+0 to 33+6 weeks for the third-trimester visit.

Pregnancy outcomes were obtained from medical records and crosschecked using postpartum questionnaires. Gestational hypertension was defined as systolic BP ≥140 mmHg or diastolic BP ≥90 mmHg after 20 weeks of gestation, without preexisting hypertension [26]. Preeclampsia was defined as gestational hypertension with proteinuria (>300 mg/24 h) or evidence of maternal organ or utero-placental dysfunction[26]. Preterm birth was defined as birth before 37 weeks GA. Birthweight groups were defined as: small-for-gestational age (SGA, <10th percentile), large-for-gestational age (LGA, >90th percentile), and appropriate-for-gestational age (AGA, 10th–90th percentiles) [27].

Statistical analysis

The primary exposure variable was the ratio of MAP to UtA-PI or UtA-RI, computed for each visit. To assess baseline characteristics, we used t-tests or Mann–Whitney U-tests for continuous variables and Chi-square or Fisher's exact test for categorical variables. For growth analyses, we used ratios measured before each outcome: 9 weeks for embryonic growth, 9 or 11 weeks for fetal size at 20 weeks (adjusted for timing), and 21–24 or 30–33 weeks for birthweight. Associations with CRL and EV were analyzed using multiple linear mixed regression, adjusting for confounders identified through a directed acyclic graph informed by biological plausibility and prior literature. CRL and EV were square- and cube-root transformed for normality. Fetal biometry and birthweight were expressed as Z-scores relative to a reference population and analyzed using linear regression. Categorical outcomes based on birthweight groups (SGA, AGA and LGA) were compared using group means and standard errors. To further explore patterns within the SGA subgroup, we conducted a post-hoc analysis approximating fetal growth restriction (FGR) using birthweight <3rd centile, as fetal biometric measurements beyond 20 weeks' gestation were not available. We then compared mean ratios and standard errors between this approximated FGR subgroup, the remaining SGA infants (birthweight 3rd–10th centile), and the other birthweight groups. Analyses were performed on participants with complete data for each specific outcome. Cases with missing outcome data were excluded from

that analysis; the number of included observations is reported per outcome. Statistical significance was set at $p<0.05$.

RESULTS

Baseline and pregnancy characteristics

Of the 3,170 pregnancies enrolled in the Rotterdam Periconception Cohort between 2010 and 2022, 500 met the inclusion criteria after sequential exclusions for missing blood pressure data, absence of uterine artery Doppler measurements, and pregnancy outcomes not eligible for analysis (Supplementary Figure 1). Of these, 498 (99.6%) were followed until delivery. Two were lost to follow-up after the first trimester. Baseline maternal characteristics are summarized in Table 1. The median maternal age at conception was 32.0 years (interquartile range (IQR) 29.1–35.4), and median BMI at the first study visit was 25.3 kg/m^2 (IQR 22.7–29.7). Fifty-eight percent of pregnancies were conceived naturally, with the remainder resulting from in vitro fertilization (IVF), and most participants were nulliparous (58%). Median GA at delivery was 272 days (IQR 265–279), median birthweight was 3270 grams (IQR 2900-3590), and median birthweight percentile was 46 (IQR 20–74). SGA neonates (n=69) accounted for 14% of the cohort, while 8% (n=41) were LGA.

Ratio Trends Across Gestation

MAP, UtA Doppler indices, and calculated ratios for each study visit are detailed in Supplemental Table 1. In total, 1,225 ratios were calculated: 984 based on same-day measurements and 241 with a median interval of 1 day between measurements. Overall, MAP followed a non-linear pattern during pregnancy, rising slightly in the first trimester, decreasing by mid-pregnancy, and peaking at 30–33 weeks GA (85 ± 9 mmHg). Both UtA indices decreased progressively with advancing gestation. Correspondingly, MAP/PI and MAP/RI ratios increased markedly from early pregnancy to 30–33 weeks GA.

Embryonic growth

Embryonic growth data were available for 301 women. In both unadjusted and adjusted models, a higher MAP/PI ratio at 9 weeks was positively associated with embryonic volume between 9 and 11 weeks of gestation (EV: adjusted β (aβ) = 0.001 cm^3; 95% CI: 0.000; 0.003), but not with CRL (√CRL: aβ = 0.002 mm; 95% CI: -0.000; 0.004) (Figure 1, Table 2). For example, at the mean MAP/PI ratio measured at 9 weeks, the predicted embryonic volume at 11 weeks was 8.5 cm^3 (95% CI 6.7; 10.6), compared with 8.0 cm^3 (6.2; 10.1) and 9.0 cm^3 (7.0; 11.4) at −2 SD and +2 SD of the ratio, respectively (Supplementary Table 2). The MAP/RI ratio displayed the same overall associations as observed for the MAP/PI ratio (Table 2).

Table 1. Baseline and pregnancy characteristics.

	Total population (n=500)	N Missing
Maternal characteristics		
BMI at first study visit in first trimester , kg/m^2	25.3[22.7, 29.6]	
Maternal age at conception, years	32.0 [29.1, 35.4]	
Geographic origin, n (%)		22 (4.4)
1 – Netherlands	371 (74.2)	
2 – Europe	20 (4.0)	
3 – Outside of Europe	22 (17.4)	
Periconception period lifestyle factors:		
Smoking, n (%)	52 (10.4)	21 (4.2)
Alcohol use, n (%)	122 (24.4)	21 (4.2)
Folic acid supplement use, n (%)	471 (94.2)	21 (4.2)
Pre-existing hypertension, n (%)	27 (5.4)	6 (1.2)
Pregnancy characteristics		
Maternal		
Mode of conception, n (%)		
Natural	292 (58.4)	
IVF/ICSI	208 (41.6)	
Nulliparous, n (%)	288 (57.6)	2 (0.4)
Gestational age at delivery, days	272 [265, 279]	11 (2.2)
Preterm birth, n (%)	59 (11.8)	11 (2.2)
Preeclampsia, n (%)	27 (5.4)	5 (1.0)
Gestational hypertension, n (%)	42 (8.4)	35 (7.0)
Gestational diabetes, n (%)	75 (15.0)	8 (1.6)
Fetal/neonatal		
Birthweight, grams	3270 [2900, 3590]	6 (1.2)
Birthweight, percentile[32]	46.0 [19.8, 73.6]	14 (2.8)
SGA, n (%)	69 (13.8)	14 (2.8)
LGA, n (%)	41 (8.2)	14 (2.8)
Fetal sex, n (%)		2 (0.4)
Female	257 (51.4)	
Male	241 (48.2)	

Continuous variables are presented as median [Q1, Q3]. Natural conceptions include spontaneous pregnancies and pregnancies conceived through ovulation induction or intra-uterine insemination. IVF = in-vitro fertilization, ICSI = intra-cytoplasmatic sperm injection. BMI = body mass index.

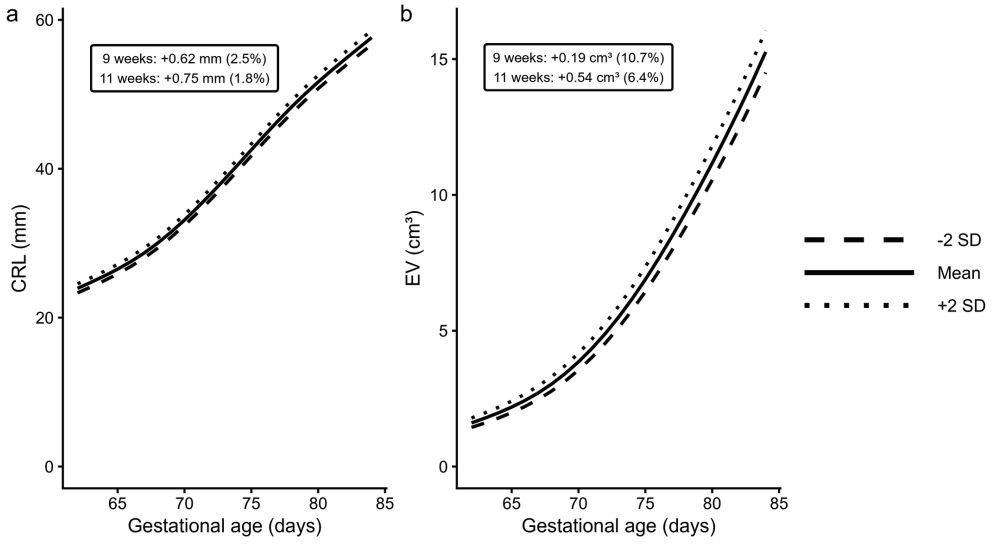


Figure 1. First-trimester embryonic growth trajectories according to the ratio of mean arterial pressure to uterine artery pulsatility index (MAP/PI) at 9 weeks. Lines represent the mean, −2 standard deviations (SD), and +2 SD. CRL, crown–rump length; EV, embryonic volume. Differences shown in the annotation boxes reflect the estimated difference between the mean and +2 SD MAP/PI ratio group.

Table 2. Effect estimates of linear mixed models for ratio of MAP/PI at 9 weeks and trajectory of CRL and EV between weeks 9 and 11 weeks GA.

		√ CRL (mm)			∛EV (cm³)		
		N	Beta (95% CI)	P-value	N	Beta (95% CI)	P-value
MAP/PI	Model 1	301	0.002 (-0.000; 0.004)	0.092	244	0.001 (0.000; 0.003)	**0.029**
	Model 2	287	0.002 (-0.000; 0.004)	0.075	231	0.001 (0.000; 0.003)	**0.041**
MAP/RI	Model 1	301	0.002 (-0.000; 0.003)	0.133	244	0.001 (0.000; 0.002)	**0.026**
	Model 2	287	0.002 (0.000; 0.004)	**0.027**	231	0.001 (0.000; 0.003)	**0.013**

Model 1: adjusted for gestational age. Model 2: adjusted for gestational age, maternal age, body-mass index, parity, smoking in periconception period, folic acid supplement use in periconception period, fetal sex and conception mode (natural or IVF/ICSI).

Fetal size

To explore the associations between MAP/PI and MAP/RI ratios and fetal size, we analysed data from the 20-week anomaly scan, available for 348 pregnancies (Table 3). A higher MAP/PI ratio at 9–11 weeks was significantly associated with larger EFW, AC, and FL Z-scores. A 10-unit increase in MAP/PI ratio corresponded to a 0.10 SD increase in EFW (approximately 2.9 grams). These associations remained significant after adjusting for confounders. MAP/RI ratio showed similar but slightly weaker associations with EFW, AC, and FL, also reaching statistical significance in both crude and adjusted models. No consistent associations were observed between either ratio and HC or BPD Z-scores, although the direction of the associations was positive.

Table 3. Effect estimates of linear models for ratio of MAP/PI and MAP/RI at 9-11 weeks GA and second trimester fetal growth parameters Z-scores compared with reference population.

		EFW (n=337)		**AC (n=348)**		**FL (n=347)**	
Model		Beta (95% CI)	P	Bèta (95% CI)	P	Beta (95% CI)	P
MAP/PI	1	0.010 (0.002;0.017)	**0.005**	0.008 (0.002;0.013)	**0.007**	0.008 (0.002;0.013)	**0.009**
	2	0.011 (0.004;0.018)	**0.001**	0.008 (0.003;0.014)	**0.003**	0.009 (0.003;0.014)	**0.002**
MAP/RI	1	0.008 (0.002;0.014)	**0.008**	0.007 (0.002;0.011)	**0.006**	0.006 (0.001;0.010)	**0.026**
	2	0.010 (0.005;0.016)	**<0.001**	0.008 (0.003;0.013)	**0.003**	0.008 (0.003;0.012)	**0.002**
		HC (n=347)		**BPD (n=343)**			
		Beta (95% CI)	P	Beta (95% CI)	P		
MAP/PI	1	0.004 (-0.003;0.011)	0.235	0.005 (-0.002; 0.013)	0.163		
	2	0.006 (-0.001;0.012)	0.089	0.006 (-0.001;0.014)	0.084		
MAP/RI	1	0.001 (-0.005;0.007)	0.719	0.003 (-0.004;0.009)	0.383		
	2	0.004 (-0.001;0.010)	0.141	0.006 (-0.001;0.012)	0.072		

Model 1: adjusted for gestational age. Model 2: adjusted for gestational age, maternal age, body-mass index, parity, smoking in periconception period, folic acid supplement use in periconception period, fetal sex and conception mode (natural or IVF/ICSI). P = p-value; PI = Pulsatility Index; RI = Resistance Index; EFW = Estimated Fetal Weight; AC = Abdominal Circumference; FL = Femur Length; HC = Head Circumference; BPD = Biparietal Diameter.

Birthweight

Using linear regression models, we observed positive associations between ratios of MAP/PI and MAP/RI between 21-24 weeks and between 30-33 weeks with birthweight Z-scores (Table 4). In the second trimester, each 1-unit increase in MAP/PI ratio was associated with a 0.007 SD increase in birthweight (p=0.004), equivalent to approximately 5.6 grams. Across the interquartile range of the MAP/PI ratio (42), this corresponds to an estimated increase of 160 grams (95% CI: 72-248 grams) or 11.2 percentile points (95% CI: 5.1-17.1). Similarly, the difference in MAP/PI ratio between the 25th and 75th percentile at 32 weeks (IQR=41) corresponded to a 0.19 SD increase in birthweight (95% CI: 0.05-0.33), translating to an increase of 100 gram (95% CI: 25-175 grams) or 7.6 percentile points (95% CI: 1.9-13.1).

Table 4. Effect estimates of linear models for ratio of MAP/PI and MAP/RI at 21-24 and 30-33 weeks GA and birthweight Z-scores (calculated with reference population).

				Birthweight Z-scores	
			n	Beta (95% CI)	P-value
22 weeks	Ratio PI	Model 1	258	0.007 (0.003; 0.011)	**<0.001**
		Model 2	238	0.008 (0.004; 0.012)	**<0.001**
22 weeks	Ratio RI	Model 1	258	0.006 (0.003; 0.010)	**0.001**
		Model 2	238	0.006 (0.003; 0.010)	**0.001**
32 weeks	Ratio PI	Model 1	324	0.005 (0.001; 0.008)	**0.009**
		Model 2	306	0.005 (0.002; 0.009)	**0.003**
32 weeks	Ratio RI	Model 1	324	0.004 (0.001; 0.007)	**0.023**
		Model 2	306	0.004 (0.001; 0.008)	**0.011**

Model 1: unadjusted model. Model 2: adjusted for maternal age, body-mass index, smoking in periconception period, folic acid supplement use in periconception period, fetal sex and conception mode (natural or IVF/ICSI).

Associations of MAP and UtA with growth outcomes

In unadjusted models, MAP alone showed no associations with any outcomes, and UtA indices alone were not associated with CRL or fetal size at 20 weeks. A borderline negative association was observed with EV (p=0.05), and UtA indices at 22 and 30 weeks were negatively associated with birthweight (data not shown).

SGA and LGA

Figure 2 illustrates the MAP/PI ratio trajectories for pregnancies resulting in SGA, AGA, and LGA neonates. At all timepoints, SGA pregnancies demonstrated lower MAP/PI and MAP/RI values compared to AGA and LGA pregnancies, reaching significance in the second and third trimester. For example, at 21-24 weeks GA, the mean MAP/PI was significantly lower in SGA pregnancies (84.7±28.6) compared to AGA (99.1±32.1) and LGA pregnancies (120.7±37.3, p=0.002). These differences in ratios can be attributed to disparities in both UtA resistance and MAP. At 21–24 weeks GA, mean UtA-PI was highest in SGA pregnancies, while LGA pregnancies exhibited slightly higher MAP values, particularly during the second-trimester visit. MAP, UtA indices, and ratios by birthweight group are provided in Supplementary Table 3 and Supplementary Figure 2. A sensitivity analysis excluding cases with iatrogenic and spontaneous preterm birth (<37 weeks GA) did not significantly alter the observed differences (data not shown). In the post-hoc analysis approximating FGR using birthweight <3rd centile, we also observed lower MAP/PI and MAP/RI ratios at 11 weeks and 21-24 weeks compared with AGA infants (Supplementary Figure 3). At 11 weeks, MAP/PI ratio was significantly lower in the FGR group compared with infants born with a birthweight percentile between the 3rd and 10th percentile: 39.8 ±6.7 vs. 50.3 ±17.0, p = 0.042.

Prediction of SGA and LGA

As a proof-of-principle analysis, we explored the potential additive value of MAP/PI and MAP/RI ratios in predicting SGA and LGA neonates, based on the observed differences in these ratios during the second trimester. In the cohort with available growth data from the 20-week anomaly scan (n=452), EFW percentile alone predicted SGA with an AUC of 0.73, sensitivity of 0.67, and specificity of 0.75 (Supplementary Table 4). Among a smaller subset of this cohort with complete data including ratios at both 9 and 22 weeks GA (n=167), the same model showed higher performance (AUC=0.82, sensitivity=0.82, specificity=0.72), yielding a detection rate (DR) of 50% for SGA at a 10% false-positive rate (Figure 3). Adding either ratio at 9 weeks only did not improve predictive performance. Including MAP/RI ratios from both 9 and 22 weeks GA increased the DR to 59% and slightly increased the AUC to 0.84, although not significantly (p=0.70). This performance was comparable to that of a model including only UtA-RI at both timepoints (DR 59%, AUC 0.83). For LGA neonates, the predictive performance of EFW alone was low (DR 22%, AUC 0.46) and although the AUC increased by adding MAP/RI ratio, DR at a false-positive rate of 10% did not improve (11%).

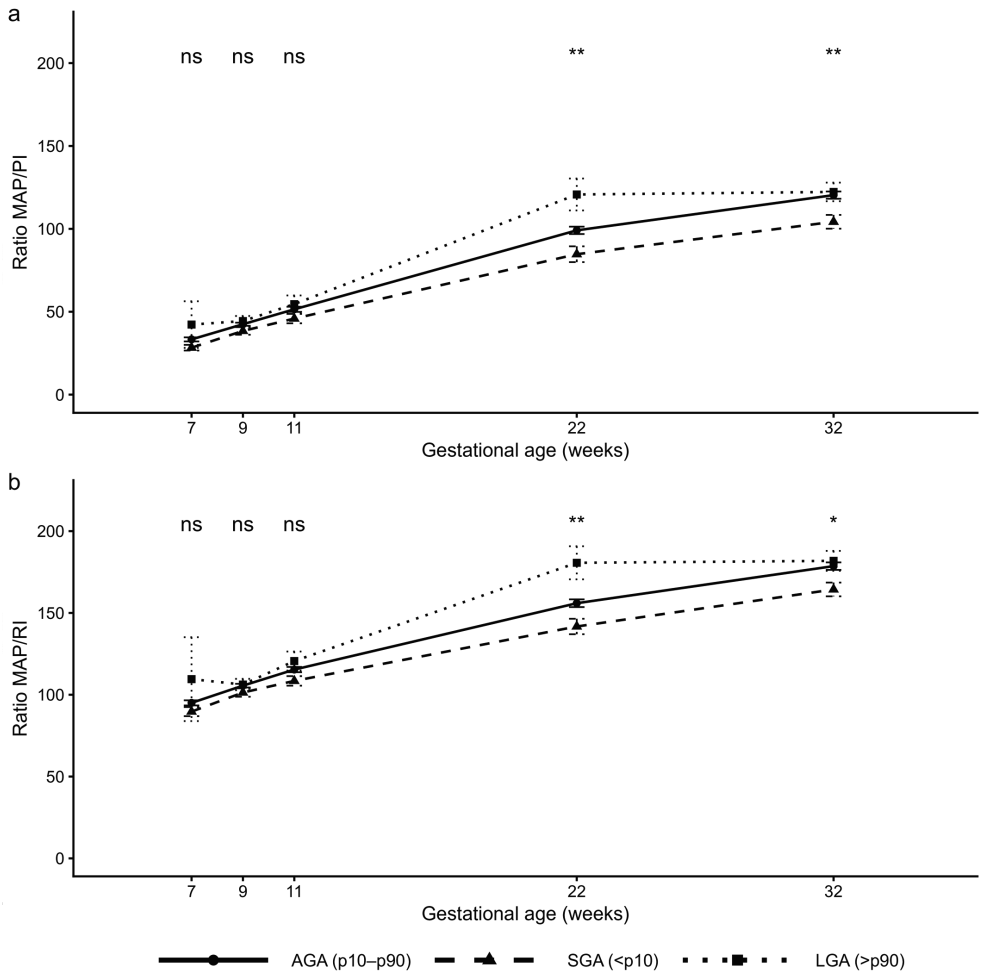


Figure 2. Ratio of mean arterial pressure to uterine artery pulsatility index (MAP/PI) and ratio of mean arterial pressure to uterine artery resistance index (MAP/RI) across study visits during pregnancy. For pregnancies with small-for-gestational-age (SGA, dashed), appropriate-for-gestational-age (AGA, solid) and large-for-gestational-age (LGA, dotted) neonates. Ns =$p > 0.05$; *= $p \leq 0.05$; ** = $p \leq 0.01$.

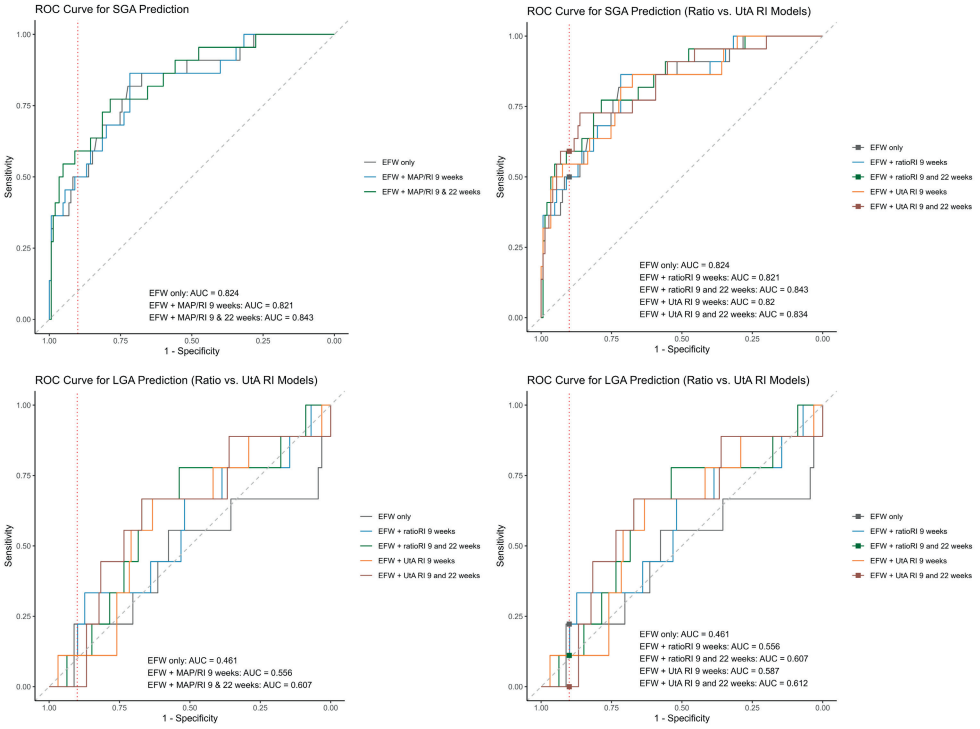


Figure 3. ROC curves for prediction of small-for-gestational-age (SGA) and large-for-gestational-age (LGA) neonates.

DISCUSSION

Main findings

In this study, we observed positive associations of the ratios of MAP to UtA-PI and MAP to UtA-RI with prenatal growth across pregnancy. Higher ratios at 9 weeks GA were positively associated with embryonic volume trajectory, and with fetal growth parameters (EFW, AC and FL) at 20 weeks GA. Moreover, MAP/PI and MAP/RI ratios in the second and third trimester were positively associated with birthweight. SGA pregnancies exhibited significantly lower ratios in the second and third trimester, while LGA pregnancies showed significantly higher ratios in the second trimester. In a proof-of-principle analysis, adding the MAP/RI ratio at 9 and 22 weeks GA to the EFW at 20 weeks GA for the prediction of SGA increased the detection rate from 50% to 59% at a 10% false-positive-rate (AUC from 0.824 to 0.843, p=0.70).

Strengths and limitations

A major strength of this study is the availability of detailed embryonic growth data—using CRL and embryonic volume as markers—together with longitudinal prenatal growth

measurements across gestation. Moreover, the rich clinical information available in this high-risk tertiary care cohort enabled comprehensive adjustment for relevant maternal characteristics. Nevertheless, several limitations should be acknowledged. Inherent to the high-risk population, its external generalizability is probably limited. The high proportion of assisted reproductive technologies was addressed by adjusting the analyses, which did not alter the findings. Furthermore, the modest sample size limited predictive modelling for SGA and LGA. Within the subset with available EFW and ratios at 9 and 22 weeks GA, the AUC for EFW alone was already 0.82, compared to 0.73 in the full cohort, which may reflect selection bias or overfitting in the subset, potentially overestimating model performance. Although adding MAP/RI ratio slightly improved prediction within this subset, it remains unclear whether similar improvements would be observed in the full cohort. Lastly, fetal biometric measures after 20 weeks GA were not available, limiting comparisons with current screening standards. Consequently, we were also unable to identify FGR cases using the Delphi consensus definition (AC or EFW <3rd centile) [28]. Nevertheless, the observed associations—both in the proof-of-principle analysis and in the post-hoc approximation of FGR using birthweight <3rd centile—warrant further investigation.

Interpretation

Previous research on blood pressure and UtA resistance and embryonic growth is limited to earlier work within our cohort, which reported no individual associations between these parameters and CRL or EV [6]. Our unadjusted analyses in the present study similarly showed no consistent associations of MAP or UtA indices alone with CRL or fetal size at 20 weeks, although UtA resistance at later gestational ages was negatively associated with birthweight (data not shown). These findings suggest that the ratio may capture haemodynamic imbalance more effectively than the individual parameters. In contrast, several studies have reported a higher blood pressure at baseline or in the third trimester to be associated with lower birthweight [9, 10]. In case of unaffected UtA indices, this increase in MAP would result in higher ratios, contrasting with our findings. Unfortunately, UtA indices were not measured in these studies.

Studies that did incorporate both MAP and UtA indices together with maternal characteristics to predict SGA observed that while second-trimester MAP was not associated with birthweight in uncomplicated pregnancies, a higher MAP was associated with lower birthweight in SGA pregnancies [16]. Moreover, higher third-trimester MAP was consistently associated with lower birthweight or increased SGA risk, particularly when combined with elevated UtA resistance [17]. In both studies, higher UtA-PI was associated with lower birthweight Z-scores, consistent with our findings. To our knowledge, associations between MAP or UtA indices and LGA risk have not been directly studied and warrant further exploration.

Future perspectives

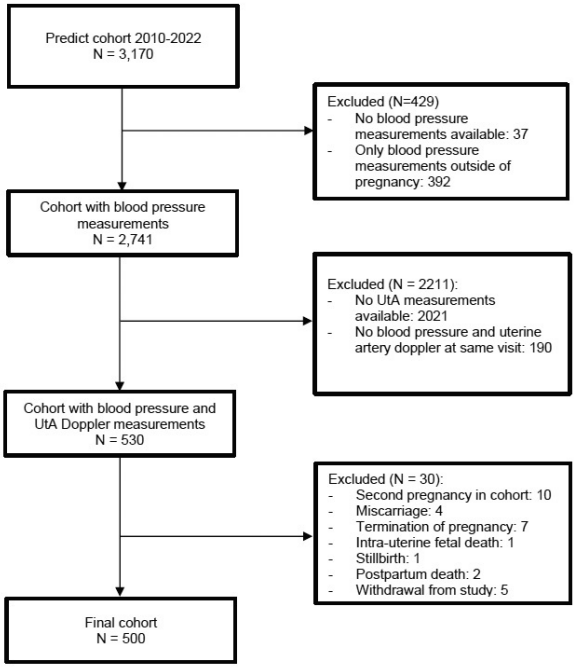
This study suggests that combining MAP and PI or RI into a ratio provides a more physiologically integrated marker of uteroplacental perfusion than either parameter alone. Both components are already accessible in routine obstetric care, and their combined use could potentially improve early identification of pregnancies at risk of SGA or LGA neonates. While UtA Doppler screening around 20 weeks GA is currently recommended by the RCOG to enhance the detection of SGA and plan ultrasound follow-up in pregnancy, MAP is not routinely included in this assessment [15]. MAP could serve as a proxy for a more elaborate set of maternal characteristics, as it is associated with a higher maternal BMI, lifestyle factors including smoking and diet, and history of cardiometabolic or hypertensive disorders of pregnancy [29-33]. If future studies show that these ratios have superior predictive performance compared to EFW or UtA indices alone for predicting SGA or LGA neonates, the integration of these ratios into standard mid-pregnancy screening protocols can pave the way to more personalized prenatal care trajectories.

From a research perspective, further studies are required to validate the predictive value of the MAP/PI and MAP/RI ratios for SGA and LGA pregnancies, and to establish clinically relevant cut-offs. Moreover, future research could assess whether incorporating these ratios alongside second- and third-trimester biometry enhances prediction of SGA or LGA. While this study used the simplest ratio to test a physiological hypothesis of associations with prenatal growth, alternative approaches such as expressing values as multiples of the median or percentiles, may further improve prediction and could be explored in future research. Another research direction is to examine how different antihypertensive treatment strategies in hypertensive disorders of pregnancy affect these ratios. As different drugs may influence MAP and UtA resistance differently, measuring these ratios could potentially guide therapy choices to balance maternal blood pressure control with prenatal growth preservation.

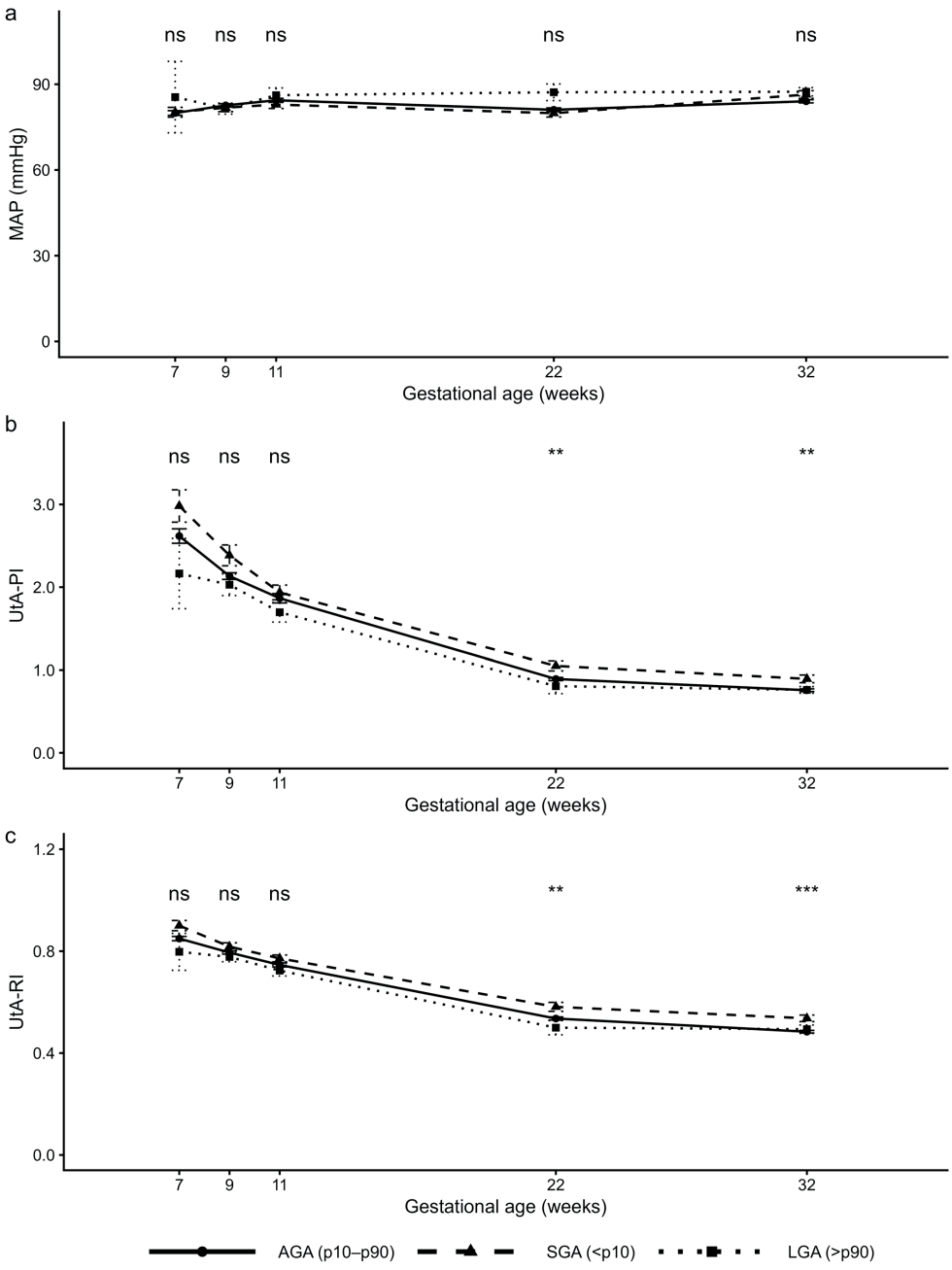
Conclusion

The ratio of mean arterial pressure to UtA-PI and UtA-RI is positively associated with embryonic growth, fetal growth, and birthweight. Measured between 21 and 24 weeks GA, both ratios are significantly lower in SGA pregnancies, and higher in LGA pregnancies, compared to normotensive pregnancies.

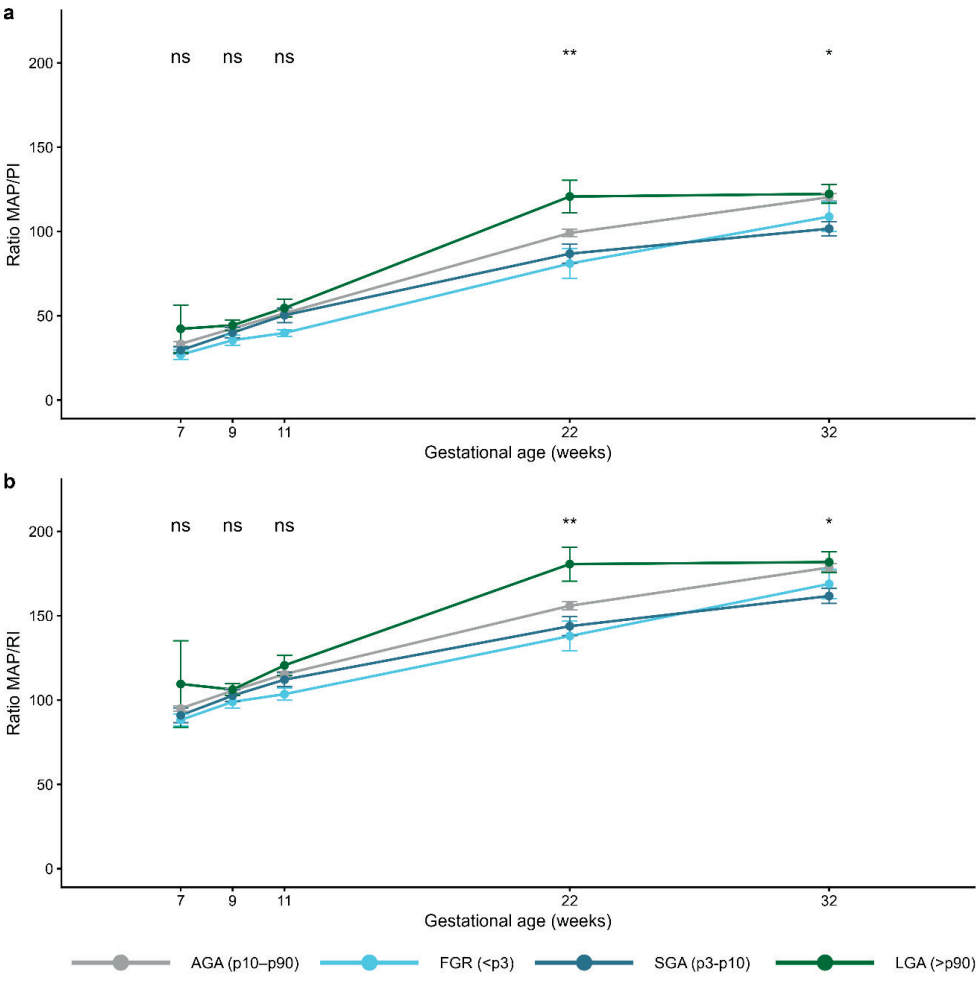
SUPPLEMENTARY MATERIAL



Supplementary Figure 1. Flowchart of patient inclusion and exclusion.



Supplementary Figure 2. Mean arterial pressure and uterine artery pulsatility index and resistance index across study visits during pregnancy. For pregnancies with small-for-gestational-age (SGA, blue), appropriate-for-gestational-age (AGA, grey) and large-for-gestational-age (LGA, green) neonates.



Supplementary Figure 3. Ratio of mean arterial pressure to uterine artery pulsatility index (MAP/PI) and ratio of mean arterial pressure to uterine artery resistance index (MAP/RI) across study visits during pregnancy. For pregnancies with fetal growth restriction (FGR, light blue), small-for-gestational-age (SGA, dark blue), appropriate-for-gestational-age (AGA, grey) and large-for-gestational-age (LGA, green) neonates. Ns =p > 0.05; *= p ≤ 0.05; ** = p ≤ 0.01.

REFERENCES

1. Chavkin U, Wainstock T, Sheiner E, Sergienko R, Walfisch A. Perinatal outcome of pregnancies complicated with extreme birth weights at term. J Matern Fetal Neonatal Med. 2019 Jan;32(2):198-202.
2. Carter EB, Stockburger J, Tuuli MG, Macones GA, Odibo AO, Trudell AS. Large-for-gestational age and stillbirth: is there a role for antenatal testing? Ultrasound Obstet Gynecol. 2019 Sep;54(3):334-7.
3. Bukowski R, Hansen NI, Willinger M, Reddy UM, Parker CB, Pinar H, et al. Fetal growth and risk of stillbirth: a population-based case-control study. PLoS Med. 2014 Apr;11(4):e1001633.
4. Gardosi J, Madurasinghe V, Williams M, Malik A, Francis A. Maternal and fetal risk factors for stillbirth: population based study. BMJ. 2013 Jan 24;346:f108.
5. Flenady V, Koopmans L, Middleton P, Froen JF, Smith GC, Gibbons K, et al. Major risk factors for stillbirth in high-income countries: a systematic review and meta-analysis. Lancet. 2011 Apr 16;377(9774):1331-40.
6. Reijnders IF, Mulders A, Koster M, Kropman A, Koning A, Willemsen SP, et al. First-trimester maternal haemodynamic adaptation to pregnancy and placental, embryonic and fetal development: the prospective observational Rotterdam Periconception cohort. BJOG. 2022 Apr;129(5):785-95.
7. Melchiorre K, Leslie K, Prefumo F, Bhide A, Thilaganathan B. First-trimester uterine artery Doppler indices in the prediction of small-for-gestational age pregnancy and intrauterine growth restriction. Ultrasound Obstet Gynecol. 2009 May;33(5):524-9.
8. Velauthar L, Plana MN, Kalidindi M, Zamora J, Thilaganathan B, Illanes SE, et al. First-trimester uterine artery Doppler and adverse pregnancy outcome: a meta-analysis involving 55,974 women. Ultrasound Obstet Gynecol. 2014 May;43(5):500-7.
9. Macdonald-Wallis C, Tilling K, Fraser A, Nelson SM, Lawlor DA. Associations of blood pressure change in pregnancy with fetal growth and gestational age at delivery: findings from a prospective cohort. Hypertension. 2014 Jul;64(1):36-44.
10. Bakker R, Steegers EA, Hofman A, Jaddoe VW. Blood pressure in different gestational trimesters, fetal growth, and the risk of adverse birth outcomes: the generation R study. Am J Epidemiol. 2011 Oct 1;174(7):797-806.
11. Cnossen JS, Morris RK, ter Riet G, Mol BW, van der Post JA, Coomarasamy A, et al. Use of uterine artery Doppler ultrasonography to predict pre-eclampsia and intrauterine growth restriction: a systematic review and bivariable meta-analysis. CMAJ. 2008 Mar 11;178(6):701-11.
12. Poon LC, Lesmes C, Gallo DM, Akolekar R, Nicolaides KH. Prediction of small-for-gestational-age neonates: screening by biophysical and biochemical markers at 19-24 weeks. Ultrasound Obstet Gynecol. 2015 Oct;46(4):437-45.
13. Familiari A, Bhide A, Morlando M, Scala C, Khalil A, Thilaganathan B. Mid-pregnancy fetal biometry, uterine artery Doppler indices and maternal demographic characteristics: role in prediction of small-for-gestational-age birth. Acta Obstet Gynecol Scand. 2016 Feb;95(2):238-44.
14. Parry S, Sciscione A, Haas DM, Grobman WA, Iams JD, Mercer BM, et al. Role of early second-trimester uterine artery Doppler screening to predict small-for-gestational-age babies in nulliparous women. Am J Obstet Gynecol. 2017 Nov;217(5):594 e1- e10.
15. Morris RK, Johnstone E, Lees C, Morton V, Smith G, Royal College of O, Gynaecologists. Investigation and Care of a Small-for-Gestational-Age Fetus and a Growth

Restricted Fetus (Green-top Guideline No. 31). BJOG. 2024 Aug;131(9):e31-e80.
16. Lesmes C, Gallo DM, Saiid Y, Poon LC, Nicolaides KH. Prediction of small-for-gestational-age neonates: screening by uterine artery Doppler and mean arterial pressure at 19-24 weeks. Ultrasound Obstet Gynecol. 2015 Sep;46(3):332-40.
17. Bakalis S, Stoilov B, Akolekar R, Poon LC, Nicolaides KH. Prediction of small-for-gestational-age neonates: screening by uterine artery Doppler and mean arterial pressure at 30-34 weeks. Ultrasound Obstet Gynecol. 2015 Jun;45(6):707-14.
18. Perry H, Binder J, Gutierrez J, Thilaganathan B, Khalil A. Maternal haemodynamic function differs in pre-eclampsia when it is associated with a small-for-gestational-age newborn: a prospective cohort study. BJOG. 2021 Jan;128(2):167-75.
19. Rousian M, Schoenmakers S, Eggink AJ, Gootjes DV, Koning AHJ, Koster MPH, et al. Cohort Profile Update: the Rotterdam Periconceptional Cohort and embryonic and fetal measurements using 3D ultrasound and virtual reality techniques. Int J Epidemiol. 2021 Nov 10;50(5):1426-7l.
20. Steegers-Theunissen RP, Verheijden-Paulissen JJ, van Uitert EM, Wildhagen MF, Exalto N, Koning AH, et al. Cohort Profile: The Rotterdam Periconceptional Cohort (Predict Study). Int J Epidemiol. 2016 Apr;45(2):374-81.
21. Reijnders IF, Mulders A, Koster MPH, Koning AHJ, Frudiger A, Willemsen SP, et al. New imaging markers for preconceptional and first-trimester uteroplacental vascularization. Placenta. 2018 Jan;61:96-102.
22. Rousian M, Koster MPH, Mulders A, Koning AHJ, Steegers-Theunissen RPM, Steegers EAP. Virtual reality imaging techniques in the study of embryonic and early placental health. Placenta. 2018 Apr;64 Suppl 1:S29-S35.
23. Rousian M, Koning AH, van Oppenraaij RH, Hop WC, Verwoerd-Dikkeboom CM, van der Spek PJ, et al. An innovative virtual reality technique for automated human embryonic volume measurements. Hum Reprod. 2010 Sep;25(9):2210-6.
24. Salomon LJ, Alfirevic Z, Berghella V, Bilardo CM, Chalouhi GE, Da Silva Costa F, et al. ISUOG Practice Guidelines (updated): performance of the routine mid-trimester fetal ultrasound scan. Ultrasound Obstet Gynecol. 2022 Jun;59(6):840-56.
25. Hadlock FP, Harrist RB, Sharman RS, Deter RL, Park SK. Estimation of fetal weight with the use of head, body, and femur measurements--a prospective study. Am J Obstet Gynecol. 1985 Feb 1;151(3):333-7.
26. Magee LA, Brown MA, Hall DR, Gupte S, Hennessy A, Karumanchi SA, et al. The 2021 International Society for the Study of Hypertension in Pregnancy classification, diagnosis & management recommendations for international practice. Pregnancy hypertension. 2022 2022/3//;27:148-69.
27. Hoftiezer L, Hof MHP, Dijs-Elsinga J, Hogeveen M, Hukkelhoven C, van Lingen RA. From population reference to national standard: new and improved birthweight charts. Am J Obstet Gynecol. 2019 Apr;220(4):383 e1- e17.
28. Gordijn SJ, Beune IM, Thilaganathan B, Papageorghiou A, Baschat AA, Baker PN, et al. Consensus definition of fetal growth restriction: a Delphi procedure. Ultrasound Obstet Gynecol. 2016 Sep;48(3):333-9.
29. Guedes-Martins L, Carvalho M, Silva C, Cunha A, Saraiva J, Macedo F, et al. Relationship between body mass index and mean arterial pressure in normotensive and chronic hypertensive pregnant women: a prospective, longitudinal study. BMC Pregnancy Childbirth. 2015 Oct 30;15:281.
30. Pathirana MM, Lassi Z, Ali A, Arstall M, Roberts CT, Andraweera PH. Cardiovascular risk factors in women with previous gestational diabetes mellitus: A system-

atic review and meta-analysis. Rev Endocr Metab Disord. 2021 Dec;22(4):729-61.
31. Andriani H, Kosasih RI, Putri S, Kuo HW. Effects of changes in smoking status on blood pressure among adult males and females in Indonesia: a 15-year population-based cohort study. BMJ Open. 2020 Apr 30;10(4):e038021.
32. Filippou CD, Tsioufis CP, Thomopoulos CG, Mihas CC, Dimitriadis KS, Sotiropoulou LI, et al. Dietary Approaches to Stop Hypertension (DASH) Diet and Blood Pressure Reduction in Adults with and without Hypertension: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Adv Nutr. 2020 Sep 1;11(5):1150-60.
33. Behrens I, Basit S, Melbye M, Lykke JA, Wohlfahrt J, Bundgaard H, et al. Risk of post-pregnancy hypertension in women with a history of hypertensive disorders of pregnancy: nationwide cohort study. BMJ. 2017 Jul 12;358:j3078.

7

Risk factors for chronic hypertension 5 years after a pregnancy complicated by preeclampsia: a systematic review and meta-analysis

Lotte W. Voskamp, Melek Rousian, Joni J. Koerts,
Régine P.M. Steegers-Theunissen, A.H. Jan Danser, Koen Verdonk

Journal of Hypertension. 2025 Jun 1;43(6):939-948.

ABSTRACT

Approximately 30% of women with a history of pre-eclampsia develop chronic hypertension within ten years of pregnancy. This systematic review summarizes risk factors before, during, and immediately after pregnancy for the development of chronic hypertension five years after preeclampsia. Databases were searched with terms "preeclampsia" and "postpartum hypertension" or "cardiovascular disease" up to October 30th, 2023. Observational studies reporting chronic hypertension more than 5 years after preeclampsia were included. Quality was assessed using the Newcastle-Ottawa scale. Where possible, a meta-analysis was conducted. Twenty-one cohort and five case-control studies, with a median quality score of 8/10, were included, involving 197,793 patients and reporting 32 risk factors. Preeclampsia in a subsequent pregnancy is associated with chronic hypertension (RR 2.26, 95%CI 1.59-3.22, n=45,626). Other significant risk factors include early-onset of preeclampsia (<34 weeks gestation), maternal body mass index, blood pressure, diabetes, and family history of hypertension.

INTRODUCTION

The incidence of preeclampsia has increased over the past decade, and it is now widely acknowledged that the morbidity and mortality associated with preeclampsia are not limited to pregnancy [1]. Women with a history of preeclampsia are at an increased risk of developing cardiovascular disease later in life, possibly mediated by chronic hypertension, the most important modifiable risk factor for cardiovascular disease [1]. Approximately 30% of women with preeclampsia develop chronic hypertension within ten years of pregnancy, which is six times higher than that observed in normotensive pregnancies [2, 3]. However, how to identify women at the highest risk of these long-term complications and who would benefit most from post-preeclampsia surveillance remains unknown. It is postulated that acute vascular damage and atherosclerosis, triggered by endothelial dysfunction, inflammation, and oxidative stress in preeclampsia, contribute to the development of chronic hypertension [1, 4]. Additionally, the link between preeclampsia and chronic hypertension could be attributed to genetic predisposition and shared risk factors such as obesity, diabetes, and renal disease [1, 4, 5]. This association is further emphasized by the increased risk of preeclampsia in individuals with prepregnancy chronic hypertension. To reduce the impact of preeclampsia on cardiovascular health later in life, organizations such as the American Heart Association and the Lancet Women and Cardiovascular Disease Commission have stressed the significance of enhancing post-pregnancy surveillance to prevent cardiovascular events [6, 7]. However, standardization of surveillance programs, follow-up frequency and topics covered during visits, remains a subject of debate. A tailored approach to post-pregnancy care, rather than a one-size-fits-all approach, may be preferable, especially in the context of healthcare costs and the burden on patients from unnecessary follow-up visits. Ideally, early postpartum risk assessment can offer insights into chronic hypertension risk, educate patients on health and lifestyle, and help tailor follow-up care for those at higher risk. To achieve this goal, it is essential to acquire knowledge of all (non)modifiable risk factors that contribute to chronic hypertension after pregnancies complicated by preeclampsia. Previous systematic reviews have largely focused on determining the short-term incidence of chronic hypertension following preeclampsia. Additionally, two reviews reported one risk factor for the development of chronic hypertension, namely, experiencing preeclampsia in more than one pregnancy compared to only in the first pregnancy [1, 8]. However, to the best of our knowledge, no reviews are currently available on risk factors for chronic hypertension as primary outcome. Several studies have demonstrated that the cumulative incidence and hazard ratios for chronic hypertension increase over the first decade following preeclampsia [2, 9, 10]. Consequently, to capture this prolonged period of increased risk, we extended our follow-up period to at least five years after birth for a more comprehensive assessment. Hence, this systematic review aimed to provide a comprehensive summary of factors associated with chronic hypertension at least five years after pregnancies complicated by preeclampsia, with the aim of guiding decisions on which women require post-pregnancy surveillance.

METHODS

Design

This systematic review was conducted at the Erasmus MC, University Medical Center, Rotterdam, the Netherlands, according to the guidelines outlined in the Meta-analysis of Observational Studies in Epidemiology (MOOSE) [11]. The literature search was performed on October 30, 2023, and included the following databases: Embase, Medline (Ovid), Cochrane Central, Web-of-Science, and Google Scholar. The search terms used were "preeclampsia" and "postpartum hypertension" or "cardiovascular risk" (Appendix 1). This review was restricted to studies published in English, with no limitations on the date of publication. The protocol for this review was registered in the PROSPERO International Prospective Register of Systematic Reviews with ID CRD42023412134.

This research focused on observational studies, including cohort and case-control designs, involving women with preeclampsia. Preeclampsia was defined as the presence of new-onset hypertension (systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg) after 20 weeks of gestation and proteinuria (24-hour urine protein excretion exceeding 300 mg or a spot protein-to-creatinine ratio greater than 0.3 mg/mg), fetal growth restriction or maternal end-organ dysfunction [12]. The primary outcome was the incidence of hypertension 5 years postpartum, defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg, or the use of antihypertensive drugs [13]. As the definitions of preeclampsia and hypertension can differ between countries and over time, these were compared between the included studies. Studies were excluded if they lacked sufficient outcome data, were non-peer-reviewed, were found to have a high risk of bias following assessment, or included women with diagnosed pre-existing hypertension (systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg, or use of antihypertensive drugs) and did not adjust for this in the analyses. We did not exclude articles that included multiple pregnancies to avoid limiting the available literature. We sought to identify the potential risk factors associated with the development of hypertension after preeclampsia. Therefore, pregnancy-related clinical data were collected, including maternal characteristics such as age, body mass index (BMI), ethnicity, and smoking status, as well as relevant obstetric and medical history. Descriptive statistics and effect measurements, that is, hazard ratios, odds ratios, and RR, were collected from the included studies. When possible, risk ratios were calculated to gain insight into the relative risks associated with specific exposures. When crude patient data were not available in the included studies, we attempted to contact the authors via email. Studies were identified and screened according to the Preferred Reporting Items for Systematic Reviews (PRISMA) 2020 statement for systematic reviews [14]. The initial screening of the title and abstract was performed independently by two researchers (LWV and JJK). Both researchers independently extracted data, and their extractions were compared to identify inconsistencies. Any discrepancies in full-text screening or data extraction were

resolved through discussion, with involvement of a third author (MR). The extracted data included authors, year, country, number of patients included, population characteristics (e.g., women with preeclampsia or subgroups), study aim, type and methodology (e.g., cohort or case-control), exclusion criteria, confounders, patient characteristics, clinical outcomes (e.g., hypertension risk, cardiovascular/metabolic complications), and blood pressure measurements. We used the Covidence systematic review software Veritas Health Innovation (Melbourne, Australia) for full-text screening and data extraction.

Quality assessment

The quality of the included studies was evaluated using the Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomized studies in meta-analyses [15]. This tool assesses cohort or case-control study quality in three areas: group selection, group comparability, and ascertainment of exposure or outcome. Studies could earn up to nine stars, with 7-9 stars rated as Good Quality, 4-6 as Fair Quality, and 0-3 as Poor Quality. Two reviewers (LWV and JJK) conducted the assessments, and any disagreements were resolved with a third author (MR) until consensus was reached.

Statistical analysis

Risk and hazard ratios are presented in the original article. If no summary effect measures were reported, the risk ratios were synthesized using the crude numbers reported in the article. A meta-analysis was performed using a random-effects model if exposure was described in at least three individual studies and suitable data were available. When multiple studies described patients from the same study population, we included only the largest study in the meta-analysis. For narrative synthesis, we noted all relevant studies, including those with overlapping populations, to provide a comprehensive overview of the risk factors. I^2 was calculated to assess the heterogeneity. Publication bias was evaluated by visually inspecting funnel plots for asymmetry. Analyses were conducted using R version 4.3.0, and the package "meta" version 6.5-0.

RESULTS

Study selection process

Figure 1 presents the PRISMA flow diagram illustrating the initial identification of studies, screening process, and reasons for exclusion. Finally, 26 studies were included in this systematic review.

Characteristics of Included Studies

The characteristics of the included studies are shown in Table 1. Most studies were retrospective cohorts (N =18). Preeclampsia case numbers varied widely, ranging from 20 to 52,691 women. Follow-up duration, reported as mean, median, or range, spanned from

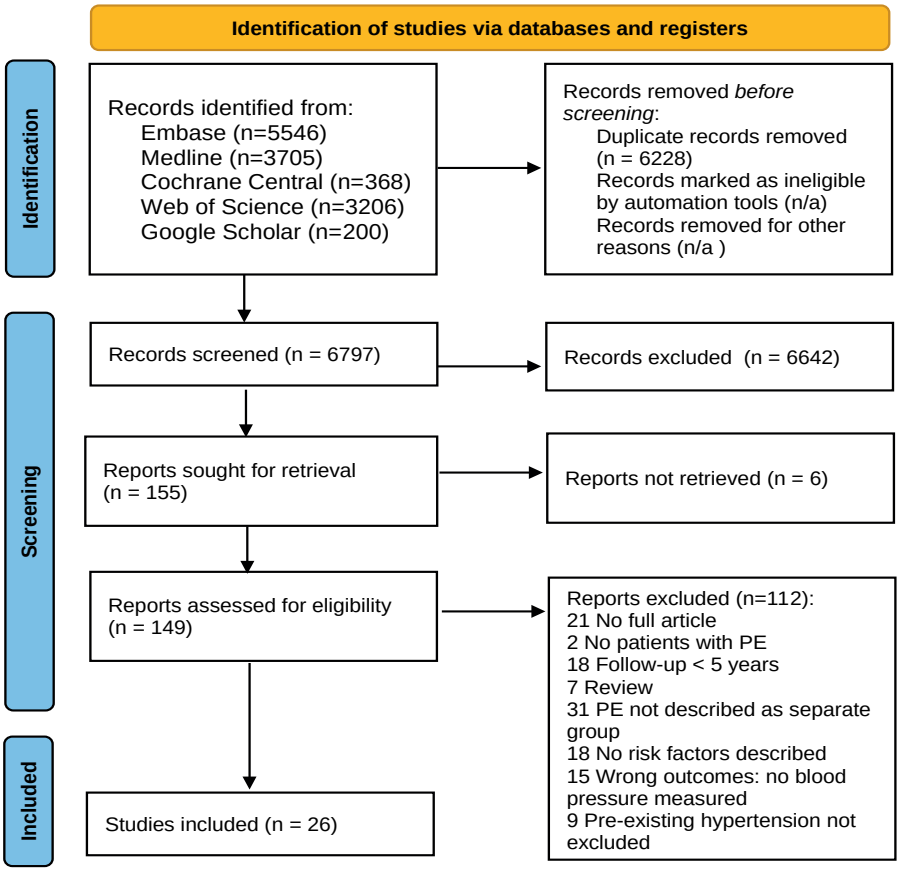


Figure 1. PRISMA Flow Chart

5 to 46 years, with an overall average of 11.1 years across all studies. The diagnosis of preeclampsia was derived from medical records in 19 studies, all of which defined preeclampsia as the presence of hypertension and proteinuria. Six studies used ICD codes to identify women with preeclampsia, and one study relied on self-report. The incidence or prevalence of chronic hypertension was derived from diagnosis codes (in six studies), drug registry data (in one study), medical records (in two studies), or blood pressure measurements at the study follow-up (12 studies). Five studies used surveys and self-reported chronic hypertension or antihypertensive medication use as the outcome measurements. All studies defined hypertension as SBP ≥ 140 mmHg or DBP ≥ 90 mmHg, or use of antihypertensive drugs, except for one study which used a lower cut-off (SBP ≥ 130 mmHg or DBP ≥ 80 mmHg, or use of antihypertensive drugs) [16]. We contacted authors via email when crude patient data were not available. Despite our attempts, none of the authors responded to our requests for patient data. The quality scores of the included studies ranged from 5 to 9, with a median score of 8. No studies were rated as poor quality. The

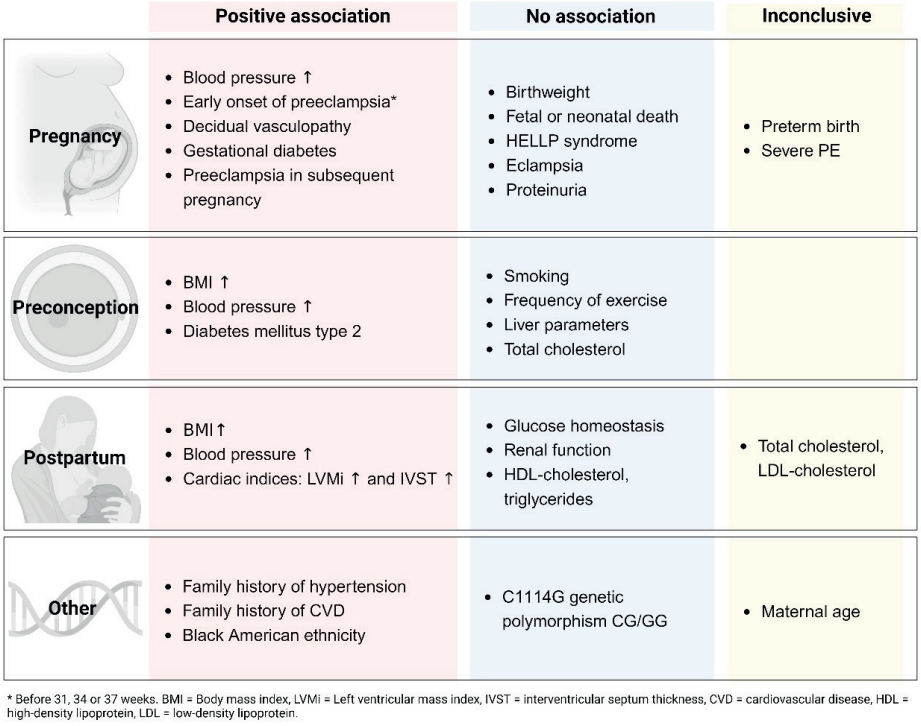


Figure 2. Risk factors and associations with chronic hypertension after preeclampsia.

NOS quality score evaluation for all included studies is presented in Supplementary Table S1. A total of 32 risk factors were identified. Figure 2 displays all the reported risk factors and effect directions.

Risk factors

Maternal Characteristics

Maternal age in relation to chronic hypertension was described in five studies [16-20]. Two studies reported that women who developed chronic hypertension after pregnancy were on average 2-4 years older at the time of conception, or at delivery compared to those who did not develop chronic hypertension [17, 19]. In the other three studies, of which two included women from the same hospital population, age at onset of preeclampsia or at postpartum visit was not different in women who did or did not develop chronic hypertension [16, 18, 20]. One study reported chronic hypertension according to clinician-assigned ethnicity, showing that Black Americans have a higher incidence of chronic hypertension than non-Black Americans after preeclampsia [21].

Table 1. Characteristics of included studies.

Nr.	First author last name	Year of publication	Study design	Country	Risk factors (n)	Pre-eclampsia cases (n)	Follow-up (years)	NOS Quality Score
1	Auger	2017	PCS	Canada	1	39559	15.5	8
2	Berends	2008	CCS	Netherlands	1	45	7	8
3	Cho	2019	RCS	South Korea	6	1910	8	6
4	Clemmensen	2020	CCS	Denmark	1	49	12	8
5	Engeland	2015	RCS	Norway	2	52.691	46	9
6	Gaugler	2008	CCS	Netherlands	1	20	5.5	8
7	Ghossein-Doha	2013	RCS	Netherlands	13	349	6	6
8	Gronning-saeter	2022	RCS	Norway	1	69	7	8
9	Haßden-teufel	2023	RCS	Germany	1	22744	4.7	8
10	Haug	2018	RCS	Norway	1	1092	7	8
11	Hooijschuur	2023	RCS	Netherlands	11	185	11	8
12	Kivela	2023	PCS	Finland	11	1357	10	8
13	Kvehaugen	2014	CCS	Norway	1	934	15.1	7
14	Lazdam	2012	RCS	United Kingdom	1	90	9.75	8
15	Lykke	2009	RCS	Denmark	4	33826	12.9	9
16	Magnussen	2009	PCS	Norway	1	737	16.5	8
17	Marín	2000	CCS	Spain	1	94	13.6	8
18	Portelinha	2009	RCS	Portugal	1	65	6	6
19	Shahbazian	2011	RCS	Iran	1	35	5.7	8
20	Sibai	1991	RCS	United States	1	125	5.4	8
21	Sibai	1992	RCS	United States	1	223	7.2	8
22	Sibai	1986	RCS	United States	3	406	6.6	7
23	Simon	2023	RCS	France	2	37043	5 to 10	8
24	Spaan	2012	RCS	Netherlands	9	339	6	8
25	Stevens	2015	RCS	Netherlands	1	119	9.5	5
26	Stuart	2018	RCS	United States	1	3687	26.9	6

NOS = Newcastle-Ottawa Scale; PCS = prospective cohort study; CCS = case-control study; RCS = retrospective cohort study.

Genetic factors

Two studies showed that for women with preeclampsia, having a first-degree family member with hypertension is associated with an increased risk of chronic hypertension, even after correcting for age, smoking, and BMI [17, 22]. In addition, having a first-degree family member with other cardiovascular diseases is associated with an increased risk of developing chronic hypertension [22]. No significant association was found between a family history of preeclampsia in mothers and sisters and chronic hypertension five years post-pregnancy [19]. Moreover, the polymorphism rs4606 in the Regulator of G protein signaling 2 (RGS2) gene was unrelated to the development of chronic hypertension [23].

Maternal lifestyle factors

One study found no association between pre-pregnancy smoking or exercise frequency, and the incidence of chronic hypertension after pregnancy [17]. A higher pre-pregnancy BMI in women with later chronic hypertension was reported in two studies [17, 19]. Additionally, the association between maternal postpartum BMI and the incidence of postpartum hypertension was described in three studies with overlapping study populations [16, 18, 22]. In the largest of these three overlapping studies, including 349 women, a higher BMI at postpartum screening yielded a hazard ratio of 1.11 (1.04-1.18, $p<0.01$) for the development of hypertension [18].

Blood pressure

Five studies investigated blood pressure as a risk factor for the development of chronic hypertension [16-19, 22]. High pre-pregnancy blood pressure (systolic blood pressure (SBP) 130-139 and diastolic blood pressure (DBP) 86-89) was found to be associated with chronic hypertension [17]. During pregnancy, higher blood pressure at the first antenatal visit was significantly associated with later chronic hypertension [19].

Ghossein et al. reported that a higher blood pressure, as well as prehypertension (defined as SBP 120-139 and DBP 80-89) at 8 months postpartum, were predictive of chronic hypertension during a mean follow-up of 6 years [18]. This result was confirmed by two other studies conducted at the same hospital [16, 22].

Laboratory parameters

Laboratory parameters for metabolic, hepatic, and renal functions were assessed in four studies [16-18, 22]. One study measured total cholesterol (TC), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT) levels during the preconception period and found no association with chronic hypertension [17]. Glomerular filtration rate, microalbuminuria, triglycerides, glucose, insulin, and homeostatic model insulin resistance (HOMA-IR), assessed in normotensive women during postpartum screening, were also not associated with chronic hypertension [16, 18, 22]. Spaan et al. initially did not find significant differences in cholesterol (total, low-density

lipoprotein (LDL), and high-density lipoprotein (HDL)) levels between women with and without chronic hypertension >5 years after pregnancy, but the same research group later reported higher postpartum LDL and total cholesterol levels in women who later developed hypertension [16]. Finally, Kivela et al. reported no significant difference in maximum proteinuria in g/24 h during index pregnancy with preeclampsia between women with and without hypertension [19].

Cardiac function

In two studies, extensive cardiac function tests were performed on women who were normotensive at the time of assessment during postpartum screening, occurring an average of 8 months postpartum. Both studies were conducted at the same hospital [16, 18]. A higher postpartum left ventricular mass index (LVMi) and interventricular septum thickness (IVST) were positively associated with the development of chronic hypertension.

Gestational Age at Diagnosis of Preeclampsia

Ten studies described gestational age (GA) at the diagnosis of preeclampsia as a potential risk factor for chronic hypertension. Early onset preeclampsia (eoPE), with onset at <34 weeks GA, was evaluated in 9 studies [9, 16, 18, 19, 24-28]. Seven of these studies showed that eoPE resulted in increased risks or incidence of chronic hypertension, one study mentioned that there was no increased risk, and one study observed a non-significant lower relative risk of chronic hypertension [26]. Of these studies, the two that partly assessed the same study population showed a positive association [16, 18]. When combining individual patient data reported in five studies and performing a meta-analysis, a higher risk ratio for chronic hypertension was found in women with eoPE, although not significant (RR 1.48, 95%CI 0.82-2.64) (Figure 3A) [18, 19, 25-27].

In three studies, different cut-offs for GA at the onset of preeclampsia were assessed as risk factors for chronic hypertension. Sibai et al. classified onset <31 weeks GA as eoPE [21] and observed an increased risk of developing chronic hypertension in this group versus women with onset at >31 weeks (OR = 2.3, p=0.01). Two studies compared onset at <37 weeks GA versus onset at >37 weeks GA. The largest of these two studies (n=37,043) observed a higher hazard ratio for chronic hypertension in the <37 weeks group after adjusting for several confounders, including maternal age, obesity, smoking, and family history (4.53, 95%CI 3.73-5.51) [9]. No significant association was found in another study (involving 349 women) [18]. In conclusion, an earlier onset of preeclampsia is associated with a higher risk of chronic hypertension >5 years after pregnancy.

Gestational Age at Birth

Seven studies evaluated GA at birth as a potential risk factor [16, 18, 19, 29-32]. Among these, four studies reported an increased risk of chronic hypertension in women who delivered <37 weeks GA compared to those who delivered >37 weeks GA [16, 29-31]. Conversely, three other

studies did not observe an altered risk of chronic hypertension [18, 19, 32], whereas one study found a significantly lower GA at birth in women who developed chronic hypertension, although the calculated hazard ratio was not significantly different from 1.0 [18]. Hence, it remains inconclusive whether preterm birth, defined as delivery before 37 weeks of GA, is significantly associated with an elevated risk of chronic hypertension later in life.

Preeclampsia, eclampsia and HELLP

Two studies investigated severe preeclampsia and/or hemolysis, elevated liver enzymes, and low platelet syndrome (HELLP) as a risk factor for chronic hypertension [16, 31]. The first study defined severe preeclampsia as having SBP >160 mmHg, DPB >110 mmHg, proteinuria >5 gram/24 hours, or the presence of cerebral disturbances or pulmonary edema, or presence of HELLP syndrome or eclampsia, and found that severe preeclampsia was associated with a significantly increased risk of developing chronic hypertension later in life compared to mild preeclampsia (aHR 3.61, 95%CI 3.43–3.80), [31].

Another study observed no difference in hypertension at follow-up between women with preeclampsia with and without HELLP syndrome [16]. Finally, women with eclampsia tend to exhibit a lower incidence of chronic hypertension (6%) than preeclamptic women without eclampsia (38%), but this was not statistically significant [33]. Additionally, in women with eclampsia, a lower GA at diagnosis (<31 weeks) was associated with an increased risk of chronic hypertension compared to those diagnosed at 37-41 weeks GA (17.9% vs. 4.8%) [34]. In conclusion, the relationship between severe preeclampsia and chronic hypertension is inconclusive, with studies showing mixed results regarding the association with HELLP and eclampsia.

Other pregnancy-related outcomes

Birthweight was investigated in five studies, of which three had an overlap in the study population [16, 18, 19, 22, 31]. In all of these studies, both birth weight and/or small-for-gestational age, defined as birth weight either below the 10th percentile or 2 SD below the mean, were not associated with the incidence of chronic hypertension. Fetal and perinatal deaths were not associated with the risk of chronic hypertension in one study [18]. One study observed a higher prevalence of gestational diabetes mellitus (GDM) (31% vs. 12%, p=0.002) and pre-existing diabetes type 2 (3.4% vs. 0.3%, p=0.006) before pregnancy in women with preeclampsia who developed chronic hypertension [19]. Lastly, decidual vasculopathy during placental pathological assessment was associated with an increased risk of chronic hypertension in one study [35].

Subsequent pregnancy with HDP

Twelve studies described risk of chronic hypertension after the first pregnancy with preeclampsia based on the presence of preeclampsia or gestational hypertension in a subsequent pregnancy [10, 16, 18, 21, 22, 30-32, 36-39]. Except for one small study (including 20

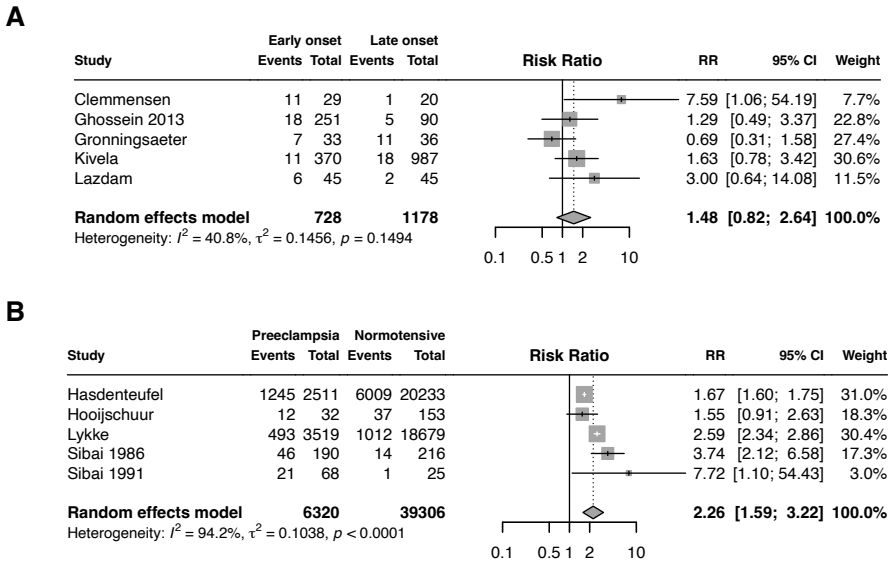


Figure 3. Meta-analyses of risk factors A) Early onset preeclampsia (<34 weeks GA) and B) Preeclampsia in a subsequent pregnancy. Events is having chronic hypertension after a follow-up period of 5 years.

women) [38], all effect measures reported in these studies point to an increased risk of chronic hypertension when a second pregnancy is also complicated by preeclampsia compared to women with a normotensive subsequent pregnancy.

When performing a meta-analysis including 45,626 women from five studies, we observed a risk ratio of RR 2.26 [1.59-3.22] for the development of chronic hypertension in women with a second pregnancy complicated by preeclampsia (Figure 3B) [10, 16, 21, 31, 36]. In contrast, Kivela et al. reported that for women with preeclampsia included in their study, the risk of chronic hypertension at follow-up was similar between women with and without prior pregnancy with preeclampsia [19].

DISCUSSION

Summary of findings

In this review, we describe the risk factors associated with the development of chronic hypertension in women with preeclampsia five years after pregnancy. Our comprehensive search and analysis revealed 32 possible risk factors, of which 14 showed a positive association with increased risk for the development of chronic hypertension: a higher

body mass index or blood pressure during the preconception or postpartum period, increased left ventricular mass index or interventricular septum thickness at postpartum assessment, early onset preeclampsia (<34 weeks GA), preterm preeclampsia (<37 weeks), GDM, presence of decidual vasculopathy, and subsequent pregnancies affected by preeclampsia. No associations were found for preconceptional smoking and exercise, fetal birth weight, perinatal death, HELLP syndrome, eclampsia, liver parameters, and total cholesterol measured before pregnancy, or measures for glucose homeostasis, renal function, and HDL cholesterol or triglycerides postpartum. The literature is inconclusive regarding the association between maternal age, preterm birth, severe PE, and postpartum measured total and LDL cholesterol levels with chronic hypertension after pregnancy.

Interpretation and comparison with existing literature

Established risk factors for chronic hypertension and cardiovascular disease

Our findings align with the broader literature on established cardiovascular and metabolic risk factors that identifies obesity and type 2 diabetes as significant risk factors for chronic hypertension [40]. Although our study found no association between smoking and lack of physical exercise with chronic hypertension after preeclampsia, the well-documented effects of these behaviors on overall cardiovascular health suggest a latent risk that may not surface within the immediate postpartum period but could contribute cumulatively over time [40, 41]. Dyslipidemia is traditionally associated with an increased risk of chronic hypertension [40]. Our review yielded inconclusive results regarding LDL and total cholesterol in patients with a history of preeclampsia and risk of later chronic hypertension and no associations for HDL cholesterol and triglycerides. Additionally, the increased risk of chronic hypertension after preeclampsia observed in individuals with familial predispositions underscores the genetic component of chronic hypertension [42]. In our review, we included one study that investigated a specific genetic polymorphism; however, it found no association with development of chronic hypertension after preeclampsia [23]. As a result, of the established risk factors for cardiovascular disease, maternal body mass index, pre-existing diabetes, and familial cardiovascular disease showed the greatest potential for inclusion in risk assessment models.

Pregnancy related risk factors

Previous studies have established associations between early onset preeclampsia and subsequent preeclampsia with the development of hypertension, but these have typically also included follow-up periods as short as 12 months postpartum [8, 43]. Our study extends this knowledge by confirming that these risks persist for at least five years postpregnancy, highlighting the importance of these risk factors for risk stratification. Our study found no associations between HELLP syndrome or eclampsia and the development of chronic hypertension, suggesting that these conditions may not independently influence long-term hypertensive risk after preeclampsia. Based on the current literature,

the association between severe preeclampsia and later hypertension remains inconclusive, and we speculate that this might be due to the lack of a standardized definition and use in clinical practice.

Our results further indicate that GDM is a risk factor for chronic hypertension, a relationship that holds even in pregnant women without preeclampsia [44]. While this might be attributed to shared risk factors already present before pregnancy, causal pathways might include insulin resistance, which exacerbates GDM, leading to elevated blood pressure by promoting vascular smooth muscle proliferation and sympathetic nervous system activity [45]. Moreover, GDM may trigger inflammatory responses and endothelial dysfunction, further contributing to hypertension by increasing systemic vascular resistance and impairing vascular tone [45].

Additionally, decidual vasculopathy in placental pathology is associated with chronic hypertension. The lesions in decidual vasculopathy, also known as acute atherosis, are characterized by fibrinoid deposits, thickened vessel walls, narrowed lumens, and inflammatory cell infiltration, and resemble those in atherosclerosis, potentially explaining the underlying mechanism of this association [46]. If a pathological assessment is conducted, identifying this condition could provide significant additional value for assessing the risk of chronic hypertension.

Our study identified no significant associations between fetal outcomes, including birth weight, fetal or neonatal death, and the development of chronic hypertension after preeclampsia. The relationship between preterm birth and chronic hypertension remains inconclusive, and to our knowledge, this aspect has not been extensively reviewed.

Physiological and Biochemical Markers

Our systematic review identified a consistent association across multiple studies between elevated blood pressure measurements at various stages, preconceptionally, at the first prenatal visit, during an eclamptic insult, and 8 months postpartum, and an increased risk of chronic hypertension more than five years post-pregnancy. This association likely reflects a pre-existing trend of rising blood pressure preceding the development of hypertension.

Moreover, an increased left ventricular mass index (LVMi) and increased interventricular septum thickness on echocardiography performed in normotensive women during postpartum screening at 8 months postpartum are associated with the development of chronic hypertension. The observed increases in the left ventricular mass index and interventricular septum thickness could be a residual effect of hypertension experienced during preeclampsia, despite a return to normotensive levels postpartum. These cardiac measurements, which are also typically elevated in individuals with existing hypertension [47], suggest their potential utility as risk stratification tools for postpartum women.

In the reviewed studies, laboratory parameters relating to metabolic, hepatic, and renal functions measured before or after pregnancy generally showed no association with chronic hypertension more than five years after pregnancy. Consequently, incorporating laboratory parameters into risk stratification models for predicting long-term chronic hypertension post-pregnancy does not seem to offer a significant utility. Notably, our search did not identify studies exploring the association between angiogenic markers, such as soluble fms-like tyrosine-kinase 1 (sFlt-1) and placental growth factor (PlGF), with chronic hypertension five years after pregnancy, although one study showed no association with chronic hypertension 1 year after pregnancy [48]. Lastly, aspirin use was reported in only one study, in which the association with the development of chronic hypertension has not been investigated [35]. This lack of information can be due to the fact that aspirin use is only recently part of common clinical practice, with many included studies published several years ago or using data before introduction of aspirin as a preventative medicine.

Strengths and limitations

Our systematic review makes a unique contribution by identifying all known risk factors for chronic hypertension following preeclampsia, a gap not explicitly addressed in previous reviews. Unlike prior studies, such as Groenhof et al. (2017) and Hutchesson et al. (2022), we take a broader and more comprehensive approach to risk factors [49, 50]. This overview is essential for developing targeted screening programs and risk models. Moreover, by calculating relative risks and conducting a meta-analysis, we enhanced the clinical applicability of the data.

However, the meta-analysis revealed substantial heterogeneity (I^2 = 92% and 94% for subsequent pregnancy), which primarily stemmed from differences in incidence of chronic hypertension after preeclampsia between the included studies. This might be explained by differences in study populations, such as specialized hospital cohorts versus population-based cohorts in high-income countries. Meta-regression analyses suggested that older studies reported a higher relative risk for chronic hypertension, which may reflect changes in diagnostic criteria or management practices over time. Despite these variations, excluding studies published before 2000 did not significantly change our overall findings. Furthermore, visual inspection of funnel plots (data not shown) did not indicate significant publication bias. However, it is important to note that the number of studies included in the meta-analysis is quite low, which may limit the ability to detect publication bias.

Other acknowledged limitations of this review arise from the quality of the included studies, particularly owing to their retrospective design and insufficient adjustment for confounders such as maternal age or BMI [25, 37]. Additionally, our review was unable to perform subgroup analyses for women with pre-existing type I chronic hypertension (prenatal systolic blood pressure 130–139 mmHg or diastolic blood pressure 80–89 mmHg) due

to insufficient data in the included studies. Women in this subgroup may exhibit distinct risk factors for chronic hypertension later in life, warranting further investigation. Despite these limitations, this review successfully identified several promising risk factors for chronic hypertension five years after preeclampsia.

Future perspectives and clinical application

An increased risk of cardiovascular disease, including heart failure, stroke, and coronary artery disease, has been reported in women with a history of preeclampsia [39, 51] and therefore, preeclampsia has been added as a risk factor for cardiovascular disease by the American Heart Association [52]. In addition to being a risk factor, the diagnosis of preeclampsia may also be regarded as an early warning sign and an opportunity to address other risk factors for cardiovascular disease, including chronic hypertension. The development of a screening tool for calculating chronic hypertension risk for use in postpartum surveillance could improve follow-up care after preeclampsia, promote better cardiovascular disease prevention, and contribute to a healthier population. A meaningful screening tool should go beyond an exhaustive list of potential risk factors. We suggest selecting factors with substantial evidence or those that can be easily and affordably implemented in healthcare settings. Based on this systematic review, the strongest evidence suggests that preeclampsia in subsequent pregnancies is a risk factor for chronic hypertension. However, the use of this risk factor during postpartum visits is not possible. Therefore, we propose to use maternal anthropometrics related to cardiovascular disease, such as BMI and maternal blood pressure, in combination with onset of preeclampsia at <34 weeks GA, since these have shown consistent associations with chronic hypertension after preeclampsia and are easily available in clinical practice. This approach is further supported by the results of a prospective cohort study, which found that blood pressure and BMI accounted for up to 77% of the excess risk of cardiovascular disease in women with a history of hypertensive disorders of pregnancy, such as preeclampsia [53]. In a more extensive risk assessment, the results of placental pathologic investigation (specifically, the presence of decidual vasculopathy) and cardiac function parameters (e.g., LVMI) could provide additional insights. Naturally, a new integrated risk score should be prospectively investigated in the clinical setting before implementation.

Conclusion

Although current practice has become aware of the increased risk of cardiovascular disease after preeclampsia, and there is more and more attention for monitoring cardiovascular health after preeclampsia, we have yet to identify patients that could benefit most from the developed surveillance programs. In this systematic review, we identified 14 different risk factors that affect the risk of developing chronic hypertension five years after preeclampsia. Early onset preeclampsia and preeclampsia in more than one pregnancy seem to be the most important risk factors for clinical practice.

SUPPLEMENTARY MATERIAL

Supplementary Table 1. Quality assessment Newcastle-Ottawa Scale.

Nr.	First Author	Year of publication	Selection (max 4)	Comparability (max 2)	Outcome (max 3)	Total score (max 9)	Quality Rating (Good, Fair, Poor)
1	Auger	2017	***	**	***	8	Good
2	Berends	2008	****	*	***	8	Good
3	Cho	2019	****	*	***	7	Good
4	Clemmensen	2020	****	*	***	8	Good
5	Engeland	2015	****	**	***	9	Good
6	Gaugler	2008	****	*	***	8	Good
7	Ghossein-Doha	2013	****	*	**	7	Good
8	Gronningsaeter	2022	****	*	***	8	Good
9	Haßdenteufel	2023	****	*	***	8	Good
10	Haug	2018	***	**	***	8	Good
11	Hooijschuur	2023	****	*	***	8	Good
12	Kivela	2023	****	*	***	8	Good
13	Kvehaugen	2014	***	**	**	7	Good
14	Lazdam	2012	****	*	***	8	Good
15	Lykke	2009	****	**	***	9	Good
16	Magnussen	2009	***	**	***	8	Good
17	Marín	2000	****	*	***	8	Good
18	Portelinha	2009	****	*	*	6	Fair
19	Shahbazian	2011	****	*	***	8	Good
20	Sibai	1986	****	*	***	8	Good
21	Sibai	1991	****	*	***	8	Good
22	Sibai	1992	***	*	***	7	Good
23	Simon	2023	****	*	***	8	Good
24	Spaan	2012	****	**	**	8	Good
25	Stevens	2015	***	*	*	5	Fair
26	Stuart	2018	**	**	**	6	Fair

Appendix 1: Search strategy

Embase 5546

('eclampsia and preeclampsia'/exp OR 'HELLP syndrome'/de OR (eclamp* OR preeclamp* OR pre-eclamp* OR preclamp* OR ((toxemia*) NEAR/3 (pregnan*)) OR hellp):ab,ti,kw) AND (((puerperium/de OR 'longitudinal study'/de OR 'follow up'/de) AND (hypertension/de OR 'elevated blood pressure'/de OR 'antihypertensive agent'/exp OR 'cardiovascular risk'/de)) OR (((postpart* OR post-part* OR puerperium* OR puerperal* OR chronic OR persist* OR 1-year* OR 2-year* OR 3-year OR 4-year* OR 5-year* OR 12-month* OR 24-month* OR 36-month* OR 48-month* OR 60-month* OR longitudinal OR long-term* OR longterm OR longer OR sustain* OR after-preeclamp* OR post-preeclamp* OR history-of-preeclam* OR formerly-preeclam* OR subsequent* OR late* OR year* OR follow-up* OR followup*) NEAR/3 (hypertens* OR high-blood-press* OR elevat*-blood-press* OR increas*-blood-press* OR antihypertens* OR anti-hypertens* OR cardiovascular*))):ab,ti,kw) NOT (([Conference Abstract]/lim OR [Conference Review]/lim) AND [1800-2020]/py) NOT ('case report'/de OR (case-report):ti)

Medline

(exp Hypertension, Pregnancy-Induced/ OR (eclamp* OR preeclamp* OR pre-eclamp* OR preclamp* OR ((toxemia*) ADJ3 (pregnan*)) OR hellp).ab,ti,kf.) AND (((Postpartum Period/ OR Longitudinal Studies/ OR Follow-Up Studies/) AND (Hypertension/ OR exp Antihypertensive Agents/ OR exp Heart Disease Risk Factors/)) OR (((postpart* OR post-part* OR puerperium* OR puerperal* OR chronic OR persist* OR 1-year* OR 2-year* OR 3-year OR 4-year* OR 5-year* OR 12-mont.h* OR 24-month* OR 36-month* OR 48-month* OR 60-month* OR longitudinal OR long-term* OR longterm OR longer OR sustain* OR after-preeclamp* OR post-preeclamp* OR history-of-preeclam* OR formerly-preeclam* OR subsequent* OR late* OR year* OR follow-up* OR followup*) ADJ3 (hypertens* OR high-blood-press* OR elevat*-blood-press* OR increas*-blood-press* OR antihypertens* OR anti-hypertens* OR cardiovascular*))).ab,ti,kf.) NOT ((news OR congres* OR abstract* OR book* OR chapter* OR dissertation abstract*).pt. AND 1800:2020.(sa_year).) NOT (Case Reports/ OR (case-report).ti.)

Cochrane

((eclamp* OR preeclamp* OR pre NEXT eclamp* OR preclamp* OR ((toxemia*) NEAR/3 (pregnan*)) OR hellp):ab,ti) AND ((((postpart* OR post NEXT part* OR puerperium* OR puerperal* OR chronic OR persist* OR 1 NEXT year* OR 2 NEXT year* OR 3 NEXT year OR 4 NEXT year* OR 5 NEXT year* OR 12 NEXT month* OR 24 NEXT month* OR 36 NEXT month* OR 48 NEXT month* OR 60 NEXT month* OR longitudinal OR long NEXT term* OR longterm OR longer OR sustain* OR after NEXT preeclamp* OR post NEXT preeclamp* OR history NEXT of NEXT preeclam* OR formerly NEXT preeclam* OR subsequent* OR late* OR year* OR follow-up* OR followup*) NEAR/3 (hypertens* OR high NEXT blood NEXT press* OR elevated NEXT blood NEXT press* OR increased NEXT blood NEXT press* OR antihypertens* OR anti-hypertens* OR cardiovascular*))):ab,ti) NOT "conference abstract":kw

Database searched	Platform	Years of coverage	Records	Records after duplicates removed
Medline ALL	Ovid	1946 - Present	3705	3686
Embase	Embase.com	1971 - Present	5546	2438
Web of Science Core Collection*	Web of Knowledge	1975 - Present	3206	395
Cochrane Central Register of Controlled Trials	Wiley	1992 - Present	368	223
Additional Search Engines: Google Scholar*** (200 top-ranked)			200	55
Total			13025	6797

*Science Citation Index Expanded (1975-present) ; Social Sciences Citation Index (1975-present) ; Arts & Humanities Citation Index (1975-present) ; Conference Proceedings Citation Index–Science (1990-present) ; Conference Proceedings Citation Index–Social Science & Humanities (1990-present) ; Emerging Sources Citation Index (2005-present)

***Google Scholar was searched via "Publish or Perish" to download the results in EndNote.

No other database limits were used than those specified in the search strategies

exclude conference abstracts from 2020 and older

exclude case reports

Web of Science

TS=(((eclamp* OR preeclamp* OR pre-eclamp* OR preclamp* OR ((toxemia*) NEAR/2 (pregnan*)) OR hellp)) AND ((((postpart* OR post-part* OR puerperium* OR puerperal* OR chronic OR persist* OR 1-year* OR 2-year* OR 3-year OR 4-year* OR 5-year* OR 12-month* OR 24-month* OR 36-month* OR 48-month* OR 60-month* OR longitudinal OR long-term* OR longterm OR longer OR sustain* OR after-preeclamp* OR post-preeclamp* OR history-of-preeclam* OR formerly-preeclam* OR subsequent* OR late* OR year* OR follow-up* OR followup*) NEAR/2 (hypertens* OR high-blood-press* OR elevated-blood-press* OR increased-blood-press* OR antihypertens* OR anti-hypertens* OR cardiovascular*))))) NOT (DT=(Meeting Abstract OR Meeting Summary) AND py=(1800-2020)) NOT TI=(case-report)

Google Scholar

eclampsia|preeclampsia "postpartum|puerperium|puerperal|persistent|sustained|chronic|subsequent hypertension|hypertensive|antihypertensives"

eclampsia|preeclampsia 'postpartum|puerperium|puerperal|persistent|sustained|chronic|subsequent hypertension|hypertensive|antihypertensives'

REFERENCES

1. Bellamy L, Casas JP, Hingorani AD, Williams DJ. Pre-eclampsia and risk of cardiovascular disease and cancer in later life: systematic review and meta-analysis. BMJ. 2007 Nov 10;335(7627):974.
2. Behrens I, Basit S, Melbye M, Lykke JA, Wohlfahrt J, Bundgaard H, et al. Risk of post-pregnancy hypertension in women with a history of hypertensive disorders of pregnancy: nationwide cohort study. BMJ. 2017 Jul 12;358:j3078.
3. Giorgione V, Ridder A, Kalafat E, Khalil A, Thilaganathan B. Incidence of postpartum hypertension within 2 years of a pregnancy complicated by pre-eclampsia: a systematic review and meta-analysis. BJOG. 2021 Feb;128(3):495-503.
4. Yang C, Baker PN, Granger JP, Davidge ST, Tong C. Long-Term Impacts of Preeclampsia on the Cardiovascular System of Mother and Offspring. Hypertension. 2023 Sep;80(9):1821-33.
5. Bartsch E, Medcalf KE, Park AL, Ray JG, High Risk of Pre-eclampsia Identification G. Clinical risk factors for pre-eclampsia determined in early pregnancy: systematic review and meta-analysis of large cohort studies. BMJ. 2016 Apr 19;353:i1753.
6. Vogel B, Acevedo M, Appelman Y, Bairey Merz CN, Chieffo A, Figtree GA, et al. The Lancet women and cardiovascular disease Commission: reducing the global burden by 2030. Lancet. 2021 Jun 19;397(10292):2385-438.
7. Brown HL, Warner JJ, Gianos E, Gulati M, Hill AJ, Hollier LM, et al. Promoting Risk Identification and Reduction of Cardiovascular Disease in Women Through Collaboration With Obstetricians and Gynecologists: A Presidential Advisory From the American Heart Association and the American College of Obstetricians and Gynecologists. Circulation. 2018 Jun 12;137(24):e843-e52.
8. Brouwers L, van der Meiden-van Roest AJ, Savelkoul C, Vogelvang TE, Lely AT, Franx A, van Rijn BB. Recurrence of pre-eclampsia and the risk of future hypertension and cardiovascular disease: a systematic review and meta-analysis. BJOG. 2018 Dec;125(13):1642-54.
9. Simon E, Bechraoui-Quantin S, Tapia S, Cottenet J, Mariet AS, Cottin Y, et al. Time to onset of cardiovascular and cerebrovascular outcomes after hypertensive disorders of pregnancy: a nationwide, population-based retrospective cohort study. Am J Obstet Gynecol. 2023 09;229(3):296.e1-.e22.
10. Hasdenteufel K, Muller M, Gutsfeld R, Goetz M, Bauer A, Wallwiener M, et al. Long-term effects of preeclampsia on maternal cardiovascular health and postpartum utilization of primary care: an observational claims data study. Arch Gynecol Obstet. 2023;307(1):275-84.
11. Brooke BS, Schwartz TA, Pawlik TM. MOOSE Reporting Guidelines for Meta-analyses of Observational Studies. JAMA Surg. 2021 Aug 1;156(8):787-8.
12. Gestational Hypertension and Preeclampsia: ACOG Practice Bulletin, Number 222. Obstet Gynecol. 2020 Jun;135(6):e237-e60.
13. Unger T, Borghi C, Charchar F, Khan NA, Poulter NR, Prabhakaran D, et al. 2020 International Society of Hypertension global hypertension practice guidelines. J Hypertens. 2020 Jun;38(6):982-1004.
14. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021 Mar 29;372:n71.
15. Wells GA, Shea, B., O'Connell, D., Peterson, J., Welch, V., Losos, M., & Tugwell, P. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomized studies in meta-analyses. . [updated May 3, 2021; cited 2024 July 8]; Available from: https://www.ohri.ca/programs/clinical_epidemiology/oxford.asp.

16. Hooijschuur MCE, Janssen E, Mulder EG, Kroon AA, Meijers JMJ, Brugts JJ, et al. Prediction model for hypertension in first decade after pre-eclampsia in initially normotensive women. Ultrasound Obstet Gynecol. 2023 Oct;62(4):531-9.
17. Cho GJ, Kim HY, Park JH, Ahn KH, Hong SC, Kim HJ, et al. Prepregnancy Factors Are Associated with Development of Hypertension Later in Life in Women with Pre-Eclampsia. J Womens Health (Larchmt). 2019 Jul;28(7):984-9.
18. Ghossein-Doha C, Peeters L, van Heijster S, van Kuijk S, Spaan J, Delhaas T, Spaanderman M. Hypertension after preeclampsia is preceded by changes in cardiac structure and function. Hypertension. 2013;62(2):382-90.
19. Kivela A, Heinonen S, Kivinen K, Kere J, Kajantie E, Jaaskelainen T, Laivuori H. Hypertensive pregnancy complications and maternal characteristics as predictors of cardiovascular health within ten years after delivery. Pregnancy Hypertens. 2023 Sep 12;34:5-12.
20. Shahbazian N, Shahbazian H, Ehsanpour A, Aref A, Gharibzadeh S. Hypertension and microalbuminuria 5 years after pregnancies complicated by pre-eclampsia. Iran J Kidney Dis. 2011;5(5):324-7.
21. Sibai BM, el-Nazer A, Gonzalez-Ruiz A. Severe preeclampsia-eclampsia in young primigravid women: subsequent pregnancy outcome and remote prognosis. Am J Obstet Gynecol. 1986;155(5):1011-6.
22. Spaan JJ, Sep SJ, van Balen VL, Spaanderman ME, Peeters LL. Metabolic syndrome as a risk factor for hypertension after preeclampsia. Obstet Gynecol. 2012;120(2 Pt 1):311-7.
23. Kvehaugen AS, Melien O, Holmen OL, Laivuori H, Dechend R, Staff AC. Hypertension after preeclampsia and relation to the C1114G polymorphism (rs4606) in RGS2: data from the Norwegian HUNT2 study. BMC Med Genet. 2014;15:28.
24. Berends AL, de Groot CJ, Sijbrands EJ, Sie MP, Benneheij SH, Pal R, et al. Shared constitutional risks for maternal vascular-related pregnancy complications and future cardiovascular disease. Hypertension. 2008;51(4):1034-41.
25. Clemmensen TS, Christensen M, Logstrup BB, Kronborg CJS, Knudsen UB. Reduced coronary flow velocity reserve in women with previous pre-eclampsia: link to increased cardiovascular disease risk. Ultrasound Obstet Gynecol. 2020 06;55(6):786-92.
26. Gronningsaeter L, Skulstad H, Quattrone A, Langesaeter E, Estensen ME. Reduced left ventricular function and sustained hypertension in women seven years after severe preeclampsia. Scand Cardiovasc J. 2022;56(1):292-301.
27. Lazdam M, de la Horra A, Diesch J, Kenworthy Y, Davis E, Lewandowski AJ, et al. Unique blood pressure characteristics in mother and offspring after early onset preeclampsia. Hypertension. 2012 Nov;60(5):1338-45.
28. Portelinha A, Cerdeira AS, Belo L, Braga J, Tejera E, Pinto A, et al. Haemostatic factors in women with history of preeclampsia. Thromb Res. 2009 May;124(1):52-6.
29. Stuart JJ, Tanz LJ, Missmer SA, Rimm EB, Spiegelman D, James-Todd TM, Rich-Edwards JW. Hypertensive Disorders of Pregnancy and Maternal Cardiovascular Disease Risk Factor Development: An Observational Cohort Study. Ann Intern Med. 2018;169(4):224-32.
30. Engeland A, Bjørge T, Klungsøyr K, Skjærven R, Skurtveit S, Furu K. Preeclampsia in pregnancy and later use of antihypertensive drugs. European Journal of Epidemiology. 2015 2015/6//;30(6):501-8.
31. Lykke JA, Langhoff-Roos J, Sibai BM, Funai EF, Triche EW, Paidas MJ. Hypertensive pregnancy disorders and subsequent cardiovascular morbidity and type 2 diabetes mellitus in the mother. Hypertension. 2009 Jun;53(6):944-51.

32. Magnussen EB, Vatten LJ, Smith GD, Romundstad PR, Romundstad PR. Hypertensive Disorders in Pregnancy and Subsequently Measured Cardiovascular Risk Factors2009. Report No.: 00297844/09.
33. Marin R, Gorostidi M, Portal CG, Sanchez M, Sanchez E, Alvarez J. Long-term prognosis of hypertension in pregnancy. HYPERTENS PREGNANCY. 2000;19(2):199-209.
34. Sibai BM, Sarinoglu C, Mercer BM. Eclampsia. VII. Pregnancy outcome after eclampsia and long-term prognosis. Am J Obstet Gynecol. 1992;166(6 Pt 1):1757-61; discussion 61.
35. Stevens DU, Smits MP, Bulten J, Spaanderman ME, van Vugt JM, Al-Nasiry S. Prevalence of hypertensive disorders in women after preeclamptic pregnancy associated with decidual vasculopathy. HYPERTENS PREGNANCY. 2015;34(3):332-41.
36. Sibai BM, Mercer B, Sarinoglu C. Severe preeclampsia in the second trimester: recurrence risk and long-term prognosis. Am J Obstet Gynecol. 1991;165(5 Pt 1):1408-12.
37. Auger N, Fraser WD, Schnitzer M, Leduc L, Healy-Profitos J, Paradis G. Recurrent pre-eclampsia and subsequent cardiovascular risk. Heart. 2017 Feb;103(3):235-43.
38. Gaugler-Senden IPM, Berends AL, de Groot CJM, Steegers EAP. Severe, very early onset preeclampsia: Subsequent pregnancies and future parental cardiovascular health. European Journal of Obstetrics and Gynecology and Reproductive Biology. 2008;140(2):171-7.
39. Haug EB, Horn J, Markovitz AR, Fraser A, Vatten LJ, Macdonald-Wallis C, et al. Life Course Trajectories of Cardiovascular Risk Factors in Women With and Without Hypertensive Disorders in First Pregnancy: The HUNT Study in Norway. J Am Heart Assoc. 2018 08 07;7(15):e009250.
40. van Oort S, Beulens JWJ, van Ballegooijen AJ, Grobbee DE, Larsson SC. Association of Cardiovascular Risk Factors and Lifestyle Behaviors With Hypertension: A Mendelian Randomization Study. Hypertension. 2020 Dec;76(6):1971-9.
41. Huai P, Xun H, Reilly KH, Wang Y, Ma W, Xi B. Physical activity and risk of hypertension: a meta-analysis of prospective cohort studies. Hypertension. 2013 Dec;62(6):1021-6.
42. Kashani M, Eliasson A, Vernalis M, Costa L, Terhaar M. Improving assessment of cardiovascular disease risk by using family history: an integrative literature review. J Cardiovasc Nurs. 2013 Nov-Dec;28(6):E18-27.
43. Dall'Asta A, D'Antonio F, Saccone G, Buca D, Mastantuoni E, Liberati M, et al. Cardiovascular events following pregnancy complicated by pre-eclampsia with emphasis on comparison between early- and late-onset forms: systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2021 May;57(5):698-709.
44. Tobias DK, Hu FB, Forman JP, Chavarro J, Zhang C. Increased risk of hypertension after gestational diabetes mellitus: findings from a large prospective cohort study. Diabetes Care. 2011 Jul;34(7):1582-4.
45. McElwain CJ, Tuboly E, McCarthy FP, McCarthy CM. Mechanisms of Endothelial Dysfunction in Pre-eclampsia and Gestational Diabetes Mellitus: Windows Into Future Cardiometabolic Health? Front Endocrinol (Lausanne). 2020;11:655.
46. Stevens DU, de Nobrega Teixeira JA, Spaanderman MEA, Bulten J, van Vugt JMG, Al-Nasiry S. Understanding decidual vasculopathy and the link to preeclampsia: A review. Placenta. 2020 Aug;97:95-100.
47. Lauer MS, Anderson KM, Levy D. Influence of contemporary versus 30-year blood pressure levels on left ventricular mass and geometry: the Framingham Heart Study. J Am Coll Cardiol. 1991 Nov 1;18(5):1287-94.

48. Neuman RI, Figaroa AMJ, Nieboer D, Saleh L, Verdonk K, Danser AHJ, et al. Angiogenic markers during preeclampsia: Are they associated with hypertension 1 year postpartum? Pregnancy Hypertens. 2021 Mar;23:116-22.
49. Groenhof TKJ, van Rijn BB, Franx A, Roeters van Lennep JE, Bots ML, Lely AT. Preventing cardiovascular disease after hypertensive disorders of pregnancy: Searching for the how and when. Eur J Prev Cardiol. 2017 Nov;24(16):1735-45.
50. Hutchesson M, Campbell L, Leonard A, Vincze L, Shrewsbury V, Collins C, Taylor R. Do modifiable risk factors for cardiovascular disease post-pregnancy influence the association between hypertensive disorders of pregnancy and cardiovascular health outcomes? A systematic review of observational studies. Pregnancy Hypertens. 2022 Mar;27:138-47.
51. Wu P, Haththotuwa R, Kwok CS, Babu A, Kotronias RA, Rushton C, et al. Preeclampsia and Future Cardiovascular Health: A Systematic Review and Meta-Analysis. Circ Cardiovasc Qual Outcomes. 2017 Feb;10(2).
52. Mosca L, Benjamin EJ, Berra K, Bezanson JL, Dolor RJ, Lloyd-Jones DM, et al. Effectiveness-based guidelines for the prevention of cardiovascular disease in women--2011 update: a guideline from the american heart association. Circulation. 2011 Mar 22;123(11):1243-62.
53. Haug EB, Horn J, Markovitz AR, Fraser A, Klykken B, Dalen H, et al. Association of Conventional Cardiovascular Risk Factors With Cardiovascular Disease After Hypertensive Disorders of Pregnancy: Analysis of the Nord-Trondelag Health Study. JAMA Cardiol. 2019 Jul 1;4(7):628-35.

8

Blood pressure patterns during pregnancy and early postpartum in preeclampsia with severe features: predictors of chronic hypertension

Lotte W. Voskamp, Koen Verdonk, Jeanine E. Roeters van Lennep, Johannes J. Duvekot, Sten P. Willemsen, A.H. Jan Danser, Régine P.M. Steegers-Theunissen, Melek Rousian

Pregnancy Hypertension. 2025 Sep;41:101240.

ABSTRACT

Rationale

Women with a history of preeclampsia are at increased risk of chronic hypertension and subsequently cardiovascular disease. Early identification of high-risk patients facilitates personalized interventions to mitigate these risks. Reliable predictors for chronic hypertension after preeclampsia are lacking. This study analyzed blood pressure patterns during pregnancies with preeclampsia in relation to development of chronic hypertension.

Methods

This prospective cohort study included only women with preeclampsia with severe features, treated at the Erasmus Medical Center between 2010 and 2022, who attended a follow-up visit 1 to 3 years postpartum. Maternal characteristics, pregnancy outcomes and blood pressure measurements were collected from hospital records. Blood pressure patterns were analyzed using linear mixed regression models and antihypertensive medication usage was evaluated with standardized defined daily doses.

Results

Among 72 participants, 29 (40%) developed chronic hypertension, defined as blood pressure >140/>90 mmHg or antihypertensive medication use at follow-up (mean 1.78 years postpartum). Significant blood pressure differences were observed between weeks 8 and 24 of pregnancy peaking at week 18, where systolic blood pressure averaged 131 mmHg [95% CI 127-134] in women with chronic hypertension at follow-up, versus 117 mmHg [114-120] in those without. Furthermore, women who developed chronic hypertension used higher antihypertensive dosages during pregnancy (3.8 [95% CI 2.9–5.6] vs. 2.6 [1.5–3.7]).

Conclusion

A higher blood pressure before 24 weeks of pregnancy is significantly associated with chronic hypertension after preeclampsia. These findings suggest a role for blood pressure patterns in postpartum risk assessment, enabling personalized follow-up for women at increased risk for chronic hypertension.

INTRODUCTION

Preeclampsia is a relatively common and often severe condition occurring in 2-8% of pregnancies [1]. The syndrome is characterized by hypertension accompanied by either proteinuria, fetal growth restriction or evidence of maternal organ injury, during the second half of pregnancy [2]. Although preeclampsia typically resolves after delivery, women who have had preeclampsia have a six-fold higher risk of developing chronic hypertension 2 years postpartum, which is a significant risk factor for cardiovascular disease [3, 4].

The precise pathophysiological mechanisms underlying the association between preeclampsia and subsequent chronic hypertension remain unclear. Risk factors and genetic predisposition for cardiovascular diseases may contribute to the development of chronic hypertension [5]. Furthermore, chronic hypertension might also arise as a consequence of vascular damage and atherosclerosis mediated by the endothelial dysfunction, inflammation, and oxidative stress associated with preeclampsia.

Although follow-up programs focused on cardiovascular health may mitigate the risk of chronic hypertension in women with a history of preeclampsia, it is crucial to accurately identify those at highest risk. Universal surveillance is not only costly but also imposes an unnecessary burden on patients, particularly given that the absolute risk of cardiovascular events in this population remains relatively low. Over-monitoring may result in overtreatment and strain healthcare resources without yielding significant benefits. Therefore, the development of reliable early risk stratification tools is essential to guide targeted interventions and ensure that long-term surveillance is reserved for those most likely to benefit.

Blood pressure is routinely measured throughout pregnancy, ensuring the availability of longitudinal data for all patients. Recent evidence suggests that elevated blood pressure during uncomplicated pregnancies is associated with an increased risk of chronic hypertension in later life [6]. We hypothesize that blood pressure measurements in pregnancy may also serve as a predictive factor for chronic hypertension following preeclampsia. This study aimed to investigate blood pressure trajectories during pregnancy in women with preeclampsia with severe features and their association with the development of hypertension 1-3 years postpartum. Additionally, we aimed to determine whether these trajectories or other pregnancy characteristics differed between women who did and did not develop chronic hypertension after pregnancy.

METHODS

Study design and population

We conducted a retrospective analysis on combined data from two ongoing cohort studies at the Erasmus University Medical Center, Rotterdam, The Netherlands. The Rotterdam Periconception Cohort (Predict study) is a large prospective cohort study that aims to investigate the interplay between (epi)genetics, lifestyle and environmental factors in early pregnancy and their impact on reproductive performance, embryonic and fetal development and pregnancy complications [7, 8]. The study has included 2,876 pregnant women who were recruited at the outpatient clinic during their first prenatal visit or preconceptionally from October 2010 until December 2022. Participants attend 2-3 visits in the first trimester, 1 in the second, and 1 in the third trimester. Data on maternal lifestyle and environmental factors, medical history, and dietary intake were collected through questionnaires and hospital records. The Follow-up Preeclampsia Outpatient Clinic (FUPEC) cohort study includes women attending a multidisciplinary clinic for comprehensive care and follow-up after preeclampsia with severe features [9]. The FUPEC aims to improve cardiovascular health through blood pressure (BP) monitoring, laboratory testing, lifestyle checks and cardiovascular risk assessments. Ethical approval by the local Medical Ethics Committee was present for both studies (MEC-2004-227, MEC-2012-311) and separately obtained for combining these studies (MEC-2023-0493). This study adhered to the 1975 Declaration of Helsinki and informed consent was obtained from all participants.

Our study population consisted of women ≥18 years old who participated in both the Predict study and FUPEC from the start of both studies (2010 and 2011) until December 2022 and were diagnosed with preeclampsia with severe features. Exclusion criteria were an incomplete follow-up less than 1 year and twin pregnancies. If a patient had more than one pregnancy complicated by preeclampsia during the study period, only data from the first pregnancy were used. Patients using antihypertensive drugs at the first visit were excluded from the main analyses and included in a sensitivity analysis for medication use.

Exposure and outcomes

Chronic hypertension was defined as systolic blood pressure (SBP) ≥140 mmHg, diastolic blood pressure (DBP) ≥90 mmHg or the use of antihypertensive medication at one or both follow-up visits. For preeclampsia we used the definition of hypertension (de novo or preexisting) together with proteinuria, maternal end-organ injury or signs of uteroplacental dysfunction [10]. Severe features include SBP ≥160 mmHg and DBP ≥110 mmHg on separate measurements, thrombocytopenia, neurological or visual impairments, compromised kidney function, liver dysfunction and pulmonary edema [11]. BP measurements were obtained from electronic medical records. During pregnancy and the first week postpartum, all BP measurements taken at the outpatient clinic, during hospitalization and from referral letters from peripheral hospitals were documented. At the follow-up

visit 1 and/or 3 years after delivery, office BP was measured at the outpatient clinic using a validated automated BP monitoring device from Hillrom. Maternal demographics and pregnancy outcomes were collected from health records and, if incomplete, supplemented with Predict Study questionnaires.

Antihypertensive drug use was documented in mg/day from treatment initiation onward. To improve comparability of drug use for patients prescribed various drugs at different dosages, medication use was quantified by adding up the number of different drugs, as well as by assessing the defined daily doses (DDD) per drug according to the World Health Organization Anatomical Therapeutic Chemical index [12].

Statistical analysis

Statistical analyses included descriptive measures and linear regression. Data were summarized using mean/median, interquartile range (IQR), and 95% confidence intervals (CI). T-tests, Mann-Whitney U, and Chi-square/Fisher's exact tests were used as appropriate. A p-value of <0.05 was considered significant. Women using antihypertensive medication at the first prenatal visit were excluded from the main analyses. Linear mixed regression models were used for repeated BP measurements and in this analysis, all measurements up to the initiation of antihypertensive treatment were included. The model-based estimations of SBP and DBP values were reported, as these values represent the expected average BP trajectories for each group (women with and without chronic hypertension) throughout gestation. Multiple BP measurements taken on the same day were averaged. For comparisons in antihypertensive drug use, we conducted a sensitivity analysis including patients already on antihypertensives at first prenatal visit. Data analysis was conducted using R version 4.3.0 and IBM SPSS Statistics version 28.0.1.0.

RESULTS

Between 2011 and 2022, 86 patients participated in both studies. After excluding multiple pregnancies (n=4), molar pregnancy (n=1) and patients with missing BP data (n=9), a total of 72 patients were eligible for inclusion in our analyses. At follow-up (mean 1.78 [range 0.98-3.51] years), chronic hypertension was present in 39 (54.2%) patients. Ten women were already using antihypertensive medication at the first prenatal visit; all of them had chronic hypertension at follow-up and were excluded from the main analyses. Details of these women are provided in Supplementary Table 1.

The women who did not use antihypertensive medication at the first prenatal visit (n=62) were included in the main analyses. Among them, 29 (46.8%) developed chronic hypertension and 33 (53.2%) remained normotensive. Maternal characteristics in early pregnancy were comparable between these two groups (Table 1). In both groups, most

Table 1. Baseline demographic and clinical characteristics, stratified by presence of chronic hypertension at follow-up.

	All patients	Chronic hypertension	No chronic hypertension
	n=62	n=29	n=33
Demographic factors			
Maternal age at conception, years	30.2 +- 4.7	30.1 ±4.7	30.2 ±4.8
Maternal BMI at first study visit, kg/m^2 (n=60,29,32)	24.7 [22.1-27.7]	24.9 [23.0-27.7]	24.5 [21.2-28.0]
Paternal age at conception, years (n=52, 25, 27)	31.8 [29.0-35.6]	32.0 [28.3-37.0]	31.6 [29.5-35.1]
Smoking during periconception period, n (%) (n=44, 21, 23)	11 (25.0)	6 (28.6)	5 (21.7)
Geographic origin, n (%) (n=42, 21, 21)			
Dutch	30 (71.4)	14 (66.7)	16 (69.6)
Western, other	2 (4.8)	1 (4.8)	1 (4.8)
Non-Western, other	10 (23.8)	6 (28.6)	4 (19.0)
Medical history			
Preexisting diabetes, n (%)	0	0	0
Pregnancy			
Nulliparous, n (%)	42 (67.7)	18 (62.1)	24 (72.7)
Multiparous, n (%)	20 (32.3)	11 (37.9)	9 (24.2)
with history of preeclampsia (% of multiparous)	8 (40.0)	6 (54.5)	2 (22.2)
Mode of conception, n (%)			
spontaneous	55 (88.7)	24 (82.8)	31 (93.9)
ovulation induction	2 (3.2)	1 (3.4)	1 (3.0)
in vitro fertilisation	4 (6.5)	3 (10.3)	1 (3.0)
in vitro fertilisation with oocyte donation	1 (1.6)	1 (3.4)	0

Continuous data are presented as mean ± standard deviation (SD) or median [Q1-Q3]. BMI = body mass index.

women were nulliparous (62.1%,72.7%) and median body mass index (BMI) was just within normal range (24.9 and 24.5 respectively). Forty-six patients (74.2%) developed preeclampsia before 34 weeks and mean gestational age (GA) at delivery was 32 weeks and 0 days. Pregnancy and fetal outcomes did not differ significantly between the two groups (Table 2). Low birthweight below the 10th percentile and extremely low birthweight below the 3rd percentile were frequent in both groups (67.7% and 43.5%).

Table 2. Maternal and fetal pregnancy outcomes, stratified by presence of chronic hypertension at follow-up.

	All patients n=62	Chronic hypertension n=29	No chronic hypertension n=33	p-value
Pregnancy outcomes				
Gestational age at delivery, days	224 +- 28.4	228 +- 28.8	222 +- 27.2	0.387
Gestational age at diagnosis of pre-eclampsia, days (n=61, 28, 33)	213 [192-238]	218 [194-244]	210 [190-237]	0.496
Early onset preeclampsia (<34 weeks), n (%)	46 (74.2)	21 (72.4)	25 (75.8)	0.993
Days between diagnosis and delivery (n=61, 28, 33)	6 [3-11]	8 [3-20]	6 [4-10]	0.446
HELLP syndrome, n (%)	17 (27.4)	7 (24.1)	10 (30.3)	0.797
Gestational diabetes, n (%)	2 (3.2)	1 (3.4)	1 (3.0)	1.000
Fetal and neonatal outcomes				
Birthweight, grams	1345 [932-2273]	1400 [950-2435]	1300 [913-2215]	0.425
Birthweight percentile < 10, n (%)	42 (67.7)	18 (62.1)	24 (72.7)	0.533
Birthweight percentile < 3, n (%)	27 (43.5)	12 (41.4)	15 (45.5)	0.947
Termination of pregnancy, n (%)	1 (1.6)	1 (3.4)	0	1.000
Intra-uterine fetal death, n (%)	3 (4.8)	2 (6.9)	1 (3.0)	1.000
Neonatal death (<28 days), n (%)	2 (3.2)	1 (3.4)	1 (3.0)	1.000

Continuous data are presented as mean ± standard deviation (SD) or median [Q1-Q3] . HELLP syndrome = Hemolysis, Elevated Liver enzymes and Low Platelet syndrome.

Blood pressure patterns

Figure 1 shows the effect plots with modelled BP patterns over the course of pregnancy for patients with and without chronic hypertension at follow-up. For patients with chronic hypertension at follow-up, a near linear increase of SBP from start of pregnancy onwards was observed (Figure 1A). In comparison, women without chronic hypertension at follow-up showed a constant SBP level until 16 weeks GA, followed by an increase of SBP from 16 weeks GA onwards. Between weeks 11 and 25, SBP was significantly higher in women with compared to those without chronic hypertension at follow-up, although mean SBP remains below 140 mmHg, the cutoff value for diagnosis of hypertension in pregnancy. The largest difference in SBP was observed at week 18 of gestation: 131 mmHg (95%CI 127-134) vs. 117 mmHg (95%CI 114-120).

DBP trajectories are shown in Figure 1B. Again, women without chronic hypertension at follow-up have lower DBP during late first and early second trimester, compared to women with chronic hypertension, with the largest difference at 16 weeks: 77 mmHg (95% CI 74-80) vs. 68 mmHg (95% CI 64-71). However, unlike SBP trajectories, the pattern of increase of DBP throughout pregnancy is comparable between women with and without later chronic hypertension.

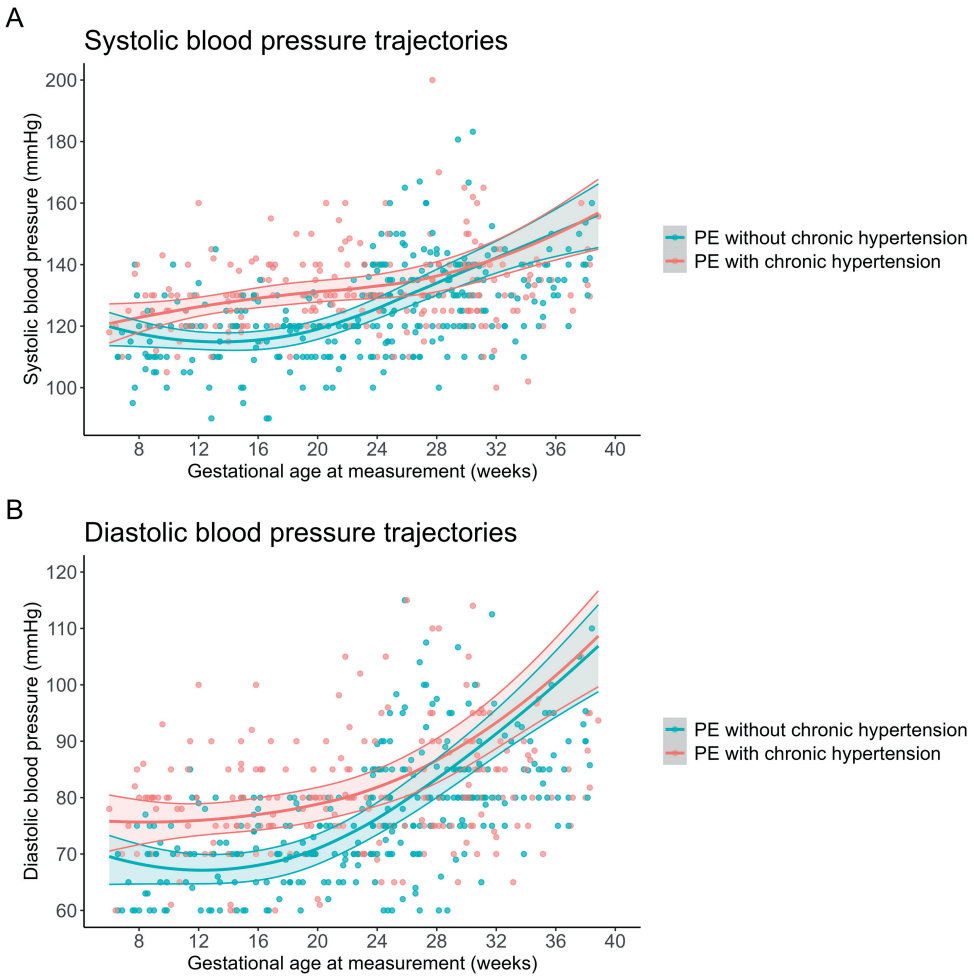


Figure 1. Effect plots of blood pressure during gestation using unadjusted linear mixed models comparing women with and without chronic hypertension after preeclampsia. A) Systolic blood pressure, B) Diastolic blood pressure.

Medication in pregnancy

Details on antihypertensive drug use are summarized in Table 3. The gestational age at initiation of antihypertensive therapy was comparable between women who developed chronic hypertension and those who remained normotensive. However, all women who started with antihypertensive drugs before 24 weeks were hypertensive at follow-up (Figure 2A).

Table 3. Medication use in pregnancy, stratified by presence of chronic hypertension at follow-up.

	Chronic hypertension	No chronic hypertension	p-value
	n=29	n=33	
Gestational age at start of antihypertensive drug use, days (n=28;30)	214 [173-219]	208 [191-227]	0.484
Defined daily doses (DDD)			
Maximum DDD in first trimester (n=2;0)	1.1 [0.9-1.3]	NA	NA
Maximum DDD in second trimester (n=10;10)	3.8 [1.9-6.7]	2.3 [1.5-4.8]	0.403
Maximum DDD in third trimester (n=24;25)	3.5 [2.7-5.0]	2.6 [1.5-3.4]	0.052
Maximum DDD in pregnancy (n=28;30)	3.8 [2.9-5.6]	2.6 [1.5-3.7]	**0.025**
Number of different antihypertensive drugs (ND)			
Maximum ND in first trimester (n=2;0)	1.0 [1.0-1.0]	NA	NA
Maximum ND in second trimester (n=10;10)	2.5 [2.0-3.0]	1.5 [1.0-2.0]	0.103
Maximum ND in third trimester (n=24;25)	2.0 [1.8-3.0]	2.0 [1.0-3.0]	0.569
Maximum ND in pregnancy (n=28;30)	2.5 [2.0-3.0]	2.0 [1.0-3.0]	0.149
Use of MgSO4, n (%)	26 (89.7)	25 (75.8)	0.273
Use of nicardipine, n (%)	19 (65.5)	20 (60.6)	0.892

Continuous data are presented as median [Q1-Q3] calculated for the women using antihypertensive drugs, or number of individuals (%) of the total of women in the groups.

Women who later developed chronic hypertension required significantly higher maximum DDD during pregnancy (3.8 [95% CI 2.9–5.6] vs. 2.6 [1.5–3.7], p=0.025; Figure 2B). While the number of different antihypertensive drugs used was not significantly higher, all women who required four different agents at any point during pregnancy were hypertensive at follow-up (Figure 2C). The use of intravenous medication (nicardipine and magnesium sulfate) was comparable between the two groups.

During the first week postpartum, antihypertensive medication use was not significantly different, except for a higher number of different drugs used at day 5 in women with later chronic hypertension (Figure 2D and 2E). Notably, many women were discharged at day 3 or 4 postpartum, limiting the number of patients available for analyses beyond day 4.

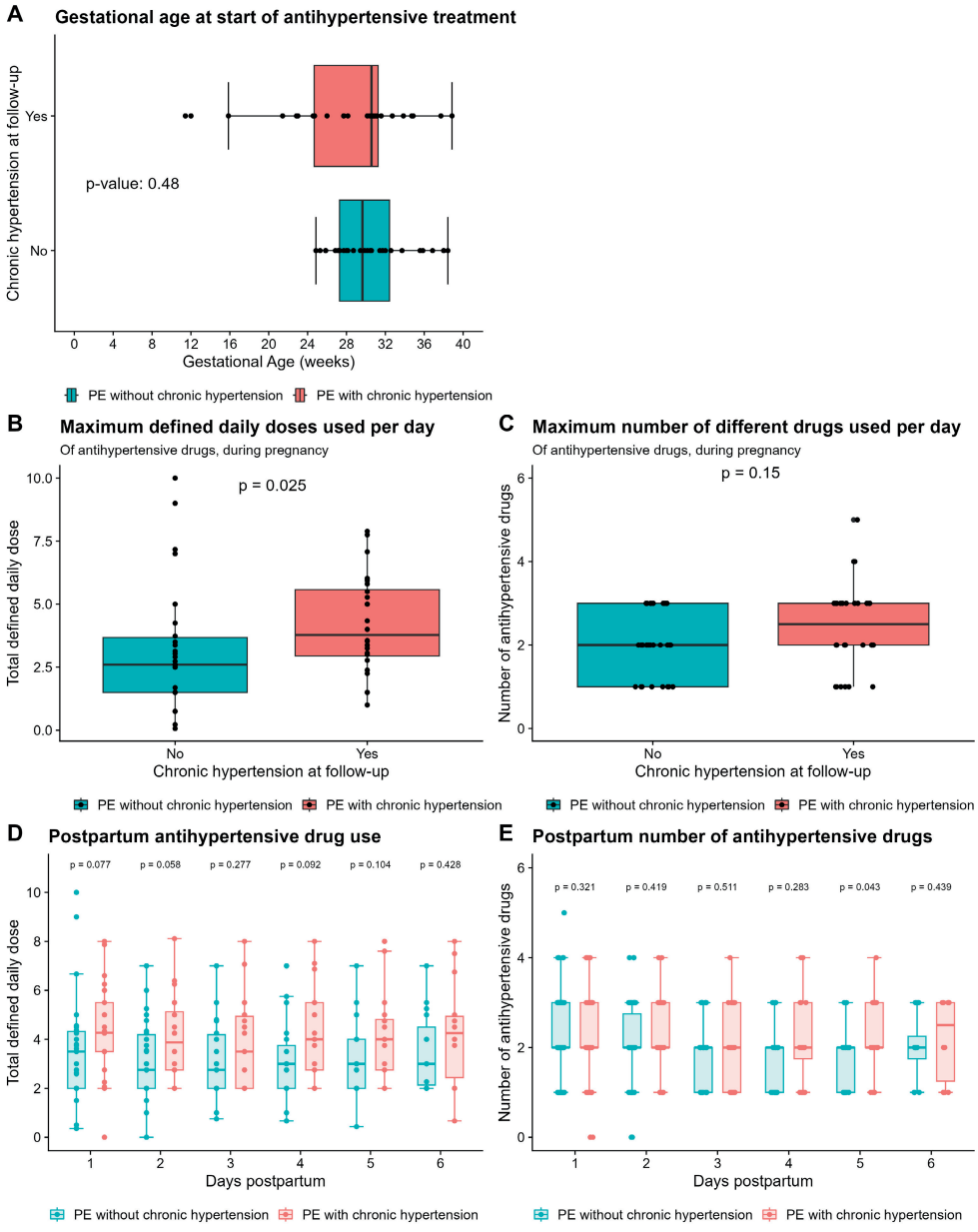


Figure 2. Antihypertensive drug use. A) Gestational age at start of antihypertensive therapy, B) maximum antihypertensive drug use in pregnancy in Defined Daily Doses, C) maximum antihypertensive drug use in pregnancy in number of drugs, D) postpartum antihypertensive drug use in Defined Daily Doses, E) postpartum antihypertensive drug use in number of drugs.

Sensitivity analysis

After including the 10 women who used antihypertensive medication at first prenatal visit, differences in DDD across trimesters became more pronounced. Women with chronic hypertension at follow-up continued to have a higher maximum DDD during pregnancy (Table 4, Figure 3B). Moreover, the number of different antihypertensive drugs used was higher in this group (median 3 vs. 2, p=0.019; Figure 3C). During the first week postpartum, women with chronic hypertension at follow-up received higher DDDs compared to those normotensive at follow-up (mean difference 1.44 [1.25-1.63], p<0.001; Figure 3D).

Table 4. Medication use in pregnancy, stratified by presence of chronic hypertension at follow-up, including patients that used medication at first visit.

	Chronic hypertension	No chronic hypertension	p-value
	n=39	n=33	
Gestational age at start of antihypertensive drug use, days (n=38;30)	188 [91-217]	208 [191-227]	**0.016**
Defined daily doses (DDD)			
Maximum DDD in first trimester (n=10;0)	1.2 [0.8-1.5]	NA	NA
Maximum DDD in second trimester (n=20;10)	4.2 [2.0-5.6]	2.3 [1.5-4.8]	0.354
Maximum DDD in third trimester (n=32;25)	4.2 [3.0-5.8]	2.6 [1.5-3.4]	**0.006**
Maximum DDD in pregnancy (n=38;30)	4.8 [3.0-6.0]	2.6 [1.5-3.7]	**0.002**
Number of different antihypertensive drugs (ND)			
Maximum ND in first trimester (n=10;0)	1.0 [1.0 -1.0]	NA	NA
Maximum ND in second trimester (n=20;10)	2.5 [1.8-3.0]	1.5 [1.0-2.0]	0.074
Maximum ND in third trimester (n=32;25)	3.0 [2.0-3.0]	2.0 [1.0-3.0]	0.142
Maximum ND in pregnancy (n=38;30)	3.0 [2.0-3.0]	2.0 [1.0-3.0]	**0.019**
Use of MgSO4, n (%)	36 (92.3)	25 (75.8)	0.106
Use of nicardipine, n (%)	28 (71.8)	20 (60.6)	0.452

Continuous data are presented as median [Q1-Q3] calculated for the women using antihypertensive drugs, or number of individuals (%) of the total of women in the groups.

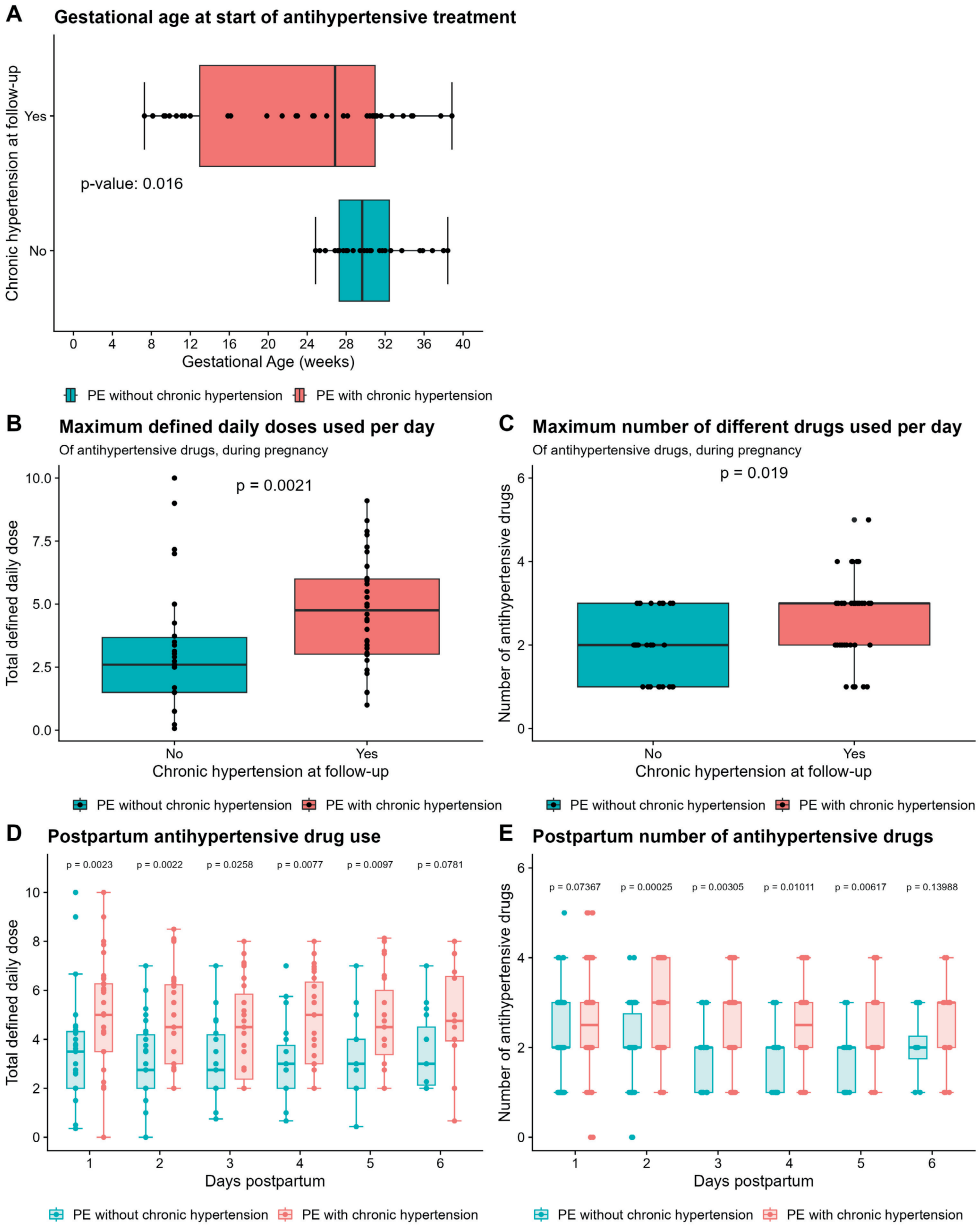


Figure 3. Sensitivity analysis of antihypertensive drug use, including patients that used antihypertensive drugs at first visit. A) Gestational age at start of antihypertensive therapy, B) maximum antihypertensive drug use in pregnancy in Defined Daily Doses, C) maximum antihypertensive drug use in pregnancy in number of drugs, D) postpartum antihypertensive drug use in Defined Daily Doses, E) postpartum antihypertensive drug use in number of drugs.

DISCUSSION

In this study, we found that women who develop chronic hypertension 1-3 years after a pregnancy complicated by preeclampsia with severe features have distinct BP patterns compared to similar women who did not develop chronic hypertension. Both SBP and DBP at 8 and 24 weeks of gestation were higher in women with compared to those without chronic hypertension. Notably, during this period, both groups have BP levels that do not exceed the threshold for diagnosis of hypertension.

Interpretation and comparison with existing literature

Blood pressure

The observation of higher BP levels during the first half of pregnancy in women who later developed chronic hypertension aligns with previous studies, although these studies typically reported BP only at the first prenatal visit or upon admission for preeclampsia, rather than longitudinally [13, 14]. One other study, the POUCHmoms study, investigated longitudinal BP in relation to later chronic hypertension, and observed that moderately elevated BP (SBP ≥120 and DBP ≥80 mmHg) at any point during pregnancy was associated with an increased risk of future hypertension [6]. However, this study population mostly consisted of women without hypertensive disorders during pregnancy. To our knowledge, our study is the first to analyze BP patterns specifically in women with severe preeclampsia.

In addition to higher BP levels, we observed a distinct linear increase in SBP throughout pregnancy in women who later developed chronic hypertension. An explanation for our findings could be that pre-existing subclinical cardiovascular disease impairs cardiovascular adaptation to pregnancy in women who develop chronic hypertension [15]. In pregnancies uncomplicated by hypertensive disorders, BP typically decreases in the first trimester due to reduced vascular resistance [15, 16]. Consequently, the renin-angiotensin-aldosterone system is activated, stimulating sodium and water retention and increasing plasma volume, which is followed by an increase in cardiac output. It is already known that in women with hypertensive disorders of pregnancy, SBP does not decrease during early pregnancy [15]. Our study adds to this knowledge by showing that within women with severe preeclampsia, those who later develop chronic hypertension exhibit an early increase in SBP, possibly indicating an even worse cardiovascular adaptation. Moreover, while DBP followed a similar trajectory in both groups, women who developed chronic hypertension already had higher DBP values in early pregnancy. The HUNT study, which investigated BP trajectories before and after pregnancy, found no decline in DBP postpartum among women with preeclampsia [17]. Together, this supports the potential role of early-pregnancy DBP as a marker for persistent hypertension risk, even within a uniformly high-risk group. The differences in SBP and DBP patterns translate to a higher pulse pressure in women with hypertension at follow-up, potentially indicating increased arterial stiffness. Previous

research has established that pre-existing cardiovascular disease risk factors, including pre-pregnancy hypertension and diabetes, are associated with increased arterial stiffness, which on its own is a risk factor for the development of chronic hypertension [18].

Antihypertensive drug use

Women who were hypertensive at follow-up used significantly higher maximum DDD any time during pregnancy. This aligns with our findings of higher BP during pregnancy, as requiring more medication for adequate BP control might suggest a more severe form of hypertension. We did not observe differences in the use of magnesium sulfate, which is often prescribed for prevention of eclamptic insults in women with preeclampsia [2, 11], or intravenous antihypertensive drugs. To better reflect real-world scenarios where early medication use may indicate a higher risk of future hypertension, particularly when untreated BP values are unavailable, we conducted a sensitivity analysis including women already using antihypertensive medication at first prenatal visit. In this analysis, the association between higher maximum DDD during pregnancy or postpartum and later chronic hypertension became more pronounced. To our knowledge, no other studies have described the relationship between antihypertensive drug use during pregnancy and chronic hypertension following preeclampsia. Although prescription preferences may introduce some bias (e.g., some physicians might prefer prescribing multiple types of drugs, while others prefer first maximizing the dose of one drug), we believe that counting the maximum number of drugs needed during pregnancy could still be useful for risk stratification. Knowledge of the number of antihypertensive drugs combined with clinical and anthropometric factors might help to predict chronic hypertension. This needs to be investigated in a larger, prospective cohort setting.

Baseline and pregnancy characteristics

Baseline characteristics were comparable between women with and without chronic hypertension at follow-up. While previous studies have identified an association between pre-pregnancy BMI and the development of hypertension following preeclampsia, we observed no significant difference in BMI between groups [13, 19]. The majority had a normal BMI, suggesting BMI may not be a key driver of hypertension in this population. Another known risk factor for chronic hypertension is having subsequent pregnancies complicated by preeclampsia [20]. In our study, approximately one-third of the women were multiparous, with a slightly higher, though non-significant, proportion among those who developed chronic hypertension.

Pregnancy outcomes, including early-onset preeclampsia, HELLP syndrome, and small-for-gestational-age births, were similar between groups. This contrasts with existing literature suggesting early-onset and preterm birth as risk factors for chronic hypertension after preeclampsia. This discrepancy may be due to our study population predominantly comprising women with severe preeclampsia and early-onset cases, making differences less discernible.

Strengths and limitations

Our study is the first to present longitudinal BP data during pregnancy combined with detailed antihypertensive drug use. Additionally, our focus on preeclampsia with severe features provides critical insights into managing this specific population. We encountered several limitations. Primarily, antihypertensive treatment during pregnancy obscures BP patterns. To address this limitation, we investigated whether antihypertensive drug use could function as a proxy for hypertension severity, which indeed showed an association with future chronic hypertension. Another limitation pertains to postpartum discharge practices. Patients are typically discharged on day three or four, which could introduce a selection bias towards those who remain hospitalized until day seven. Consequently, we propose the data from the first three days postpartum as the most appropriate for our analysis. Additionally, the absence of preconception BP measurements and the variability in measurement protocols across different healthcare providers introduce some heterogeneity. However, this variability reflects the real-world clinical environment and presents a challenge to our analysis rather than a limitation. It underscores the need for adaptable, real-world solutions in managing and predicting hypertension after pregnancies complicated by preeclampsia.

Future perspectives

With our aim to personalize follow-up care for women at increased risk of chronic hypertension after preeclampsia, identification of those women at the highest risk is essential. Recent trials have highlighted the critical role of early postpartum BP management in reducing long-term hypertension, particularly in women with preeclampsia [21, 22]. By using BP measurements from early pregnancy to identify women at greatest risk of developing hypertension, we can more effectively implement these management strategies, ensuring that adequate monitoring and, if necessary, antihypertensive therapies are initiated promptly postpartum. However, within our relatively small sample size, we were yet unable to validate the predictive values of the observed BP patterns. Therefore, the first step involves prospectively testing the predictive quality of our BP model. For now, the most clinically applicable risk factors identified are the use of antihypertensive drugs before 24 weeks of GA and the use of four or more antihypertensive drugs at any point during pregnancy.

Conclusion

To conclude, our findings demonstrate a significant association between higher BP during the first half of pregnancy and the development of chronic hypertension 1-3 years after preeclampsia with severe features. Women at the highest risk of chronic hypertension are those who exhibit an increasing trend in SBP throughout pregnancy, initiate antihypertensive treatment before 24 weeks GA, and use four or more different antihypertensive drugs at any point during their pregnancy.

SUPPLEMENTARY MATERIAL

Supplementary Table 1. Baseline characteristics and pregnancy outcomes for the 10 women with pre-existing hypertension, that were included in the sensitivity analysis on medication use.

	Patients using medication at first prenatal visit (n=10)
Demographic factors and clinical characteristics	
Maternal age at conception, years	31.7 +- 4.3
Maternal BMI at first study visit, kg/m^2 (n=9)	24.9 [23.0-27.4]
Paternal age at conception, years (n=9)	28.4 [26.9-42.3]
Smoking during periconception period, n (%) (n=6)	0
Geographic origin, n (%) (n=8)	
Dutch	4 (66.7)
Western, other	0
Non-Western, other	2 (33.3)
Preexisting diabetes, n (%)	0
Antihypertensive medication at first prenatal visit, n (%)	10 (100)
Pregnancy	
Nulliparous, n (%)	5 (50.0)
Multiparous, n (%)	5 (50.0)
with history of preeclampsia (% of multiparous)	3 (60.0)
Mode of conception, n (%)	
spontaneous	9 (90.0)
Intra-uterine insemination	1 (10.0)
Maternal pregnancy outcomes	
Gestational age at delivery, days	219 +- 32
Gestational age at diagnosis of preeclampsia, days (n=9)	192 [187-222]
Early onset preeclampsia (<34 weeks), n (%)	7 (77.8)
Days between diagnosis and delivery (n=9)	8 [5-19]
HELLP syndrome, n (%)	1 (10)
Gestational diabetes, n (%)	0
Fetal and neonatal outcomes	
Birthweight, grams	1375 [961-2046]
Birthweight percentile < 10, n (%)	8 (80.0)
Birthweight percentile < 3, n (%)	3 (30.0)
Termination of pregnancy, n (%)	0
Intra-uterine fetal death, n (%)	0
Neonatal death (<28 days), n (%)	0

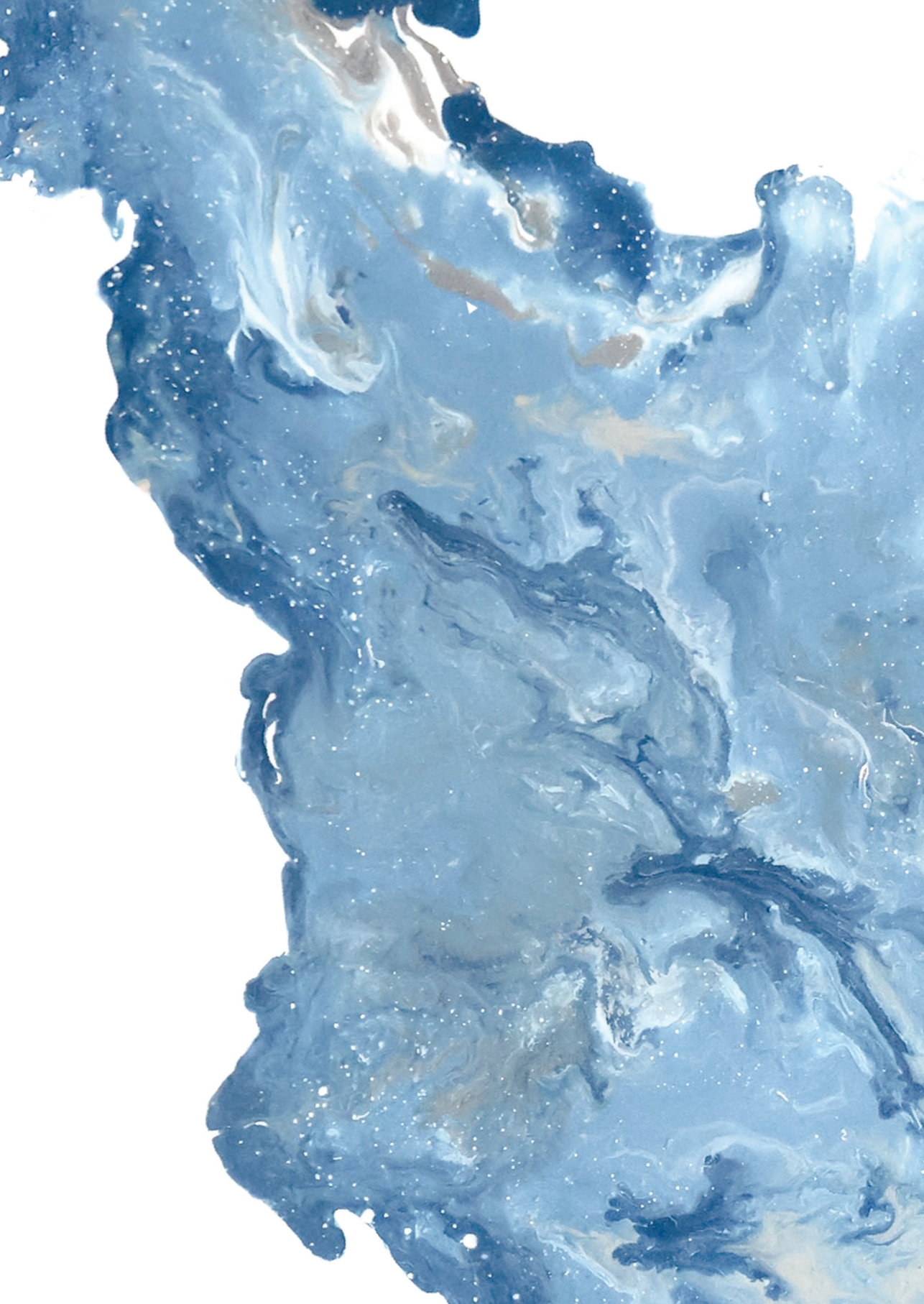
Continuous data are presented as mean ± standard deviation (SD) or median [Q1-Q3] . BMI = body mass index. HELLP syndrome = Hemolysis, Elevated Liver enzymes and Low Platelet syndrome.

REFERENCES

1. Abalos E, Cuesta C, Grosso AL, Chou D, Say L. Global and regional estimates of preeclampsia and eclampsia: a systematic review. Eur J Obstet Gynecol Reprod Biol. 2013 Sep;170(1):1-7.
2. Brown MA, Magee LA, Kenny LC, Karumanchi SA, McCarthy FP, Saito S, et al. Hypertensive Disorders of Pregnancy: ISSHP Classification, Diagnosis, and Management Recommendations for International Practice. Hypertension. 2018 Jul;72(1):24-43.
3. Giorgione V, Ridder A, Kalafat E, Khalil A, Thilaganathan B. Incidence of postpartum hypertension within 2 years of a pregnancy complicated by pre-eclampsia: a systematic review and meta-analysis. BJOG. 2021 Feb;128(3):495-503.
4. Flint AC, Conell C, Ren X, Banki NM, Chan SL, Rao VA, et al. Effect of Systolic and Diastolic Blood Pressure on Cardiovascular Outcomes. N Engl J Med. 2019 Jul 18;381(3):243-51.
5. Bellamy L, Casas JP, Hingorani AD, Williams DJ. Pre-eclampsia and risk of cardiovascular disease and cancer in later life: systematic review and meta-analysis. BMJ. 2007 Nov 10;335(7627):974.
6. Dunietz GL, Strutz KL, Holzman C, Tian Y, Todem D, Bullen BL, Catov JM. Moderately elevated blood pressure during pregnancy and odds of hypertension later in life: the POUCHmoms longitudinal study. BJOG. 2017 Sep;124(10):1606-13.
7. Rousian M, Schoenmakers S, Eggink AJ, Gootjes DV, Koning AHJ, Koster MPH, et al. Cohort Profile Update: the Rotterdam Periconceptional Cohort and embryonic and fetal measurements using 3D ultrasound and virtual reality techniques. Int J Epidemiol. 2021 Nov 10;50(5):1426-7l.
8. Steegers-Theunissen RP, Verheijden-Paulissen JJ, van Uitert EM, Wildhagen MF, Exalto N, Koning AH, et al. Cohort Profile: The Rotterdam Periconceptional Cohort (Predict Study). Int J Epidemiol. 2016 Apr;45(2):374-81.
9. van der Bijl MF, Al-Hassany L, Wijtzes AI, Verdonk K, Duvekot JJ, Roeters van Lennep J. FUPEC study, a prospective open-cohort on severe pre-eclampsia and cardiovascular risk factors based in the Netherlands. BMJ Open. 2024 Nov 12;14(11):e093423.
10. Magee LA, Brown MA, Hall DR, Gupte S, Hennessy A, Karumanchi SA, et al. The 2021 International Society for the Study of Hypertension in Pregnancy classification, diagnosis & management recommendations for international practice. Pregnancy hypertension. 2022 2022/3//;27:148-69.
11. Gestational Hypertension and Preeclampsia: ACOG Practice Bulletin, Number 222. Obstet Gynecol. 2020 Jun;135(6):e237-e60.
12. ATC/DDD Index [database on the Internet]2023. Available from: https://www.whocc.no/atc_ddd_index/.
13. Kivela A, Heinonen S, Kivinen K, Kere J, Kajantie E, Jaaskelainen T, Laivuori H. Hypertensive pregnancy complications and maternal characteristics as predictors of cardiovascular health within ten years after delivery. Pregnancy Hypertens. 2023 Sep 12;34:5-12.
14. Aberg H, Karlsson L, Melander S. Studies on toxaemia of pregnancy with special reference to blood pressure. II. Results after 6-11 years' follow-up. Ups J Med Sci. 1978;83(2):97-102.
15. de Haas S, Mulder E, Schartmann N, Mohseni Z, Abo Hasson F, Alsadah F, et al. Blood pressure adjustments throughout healthy and hypertensive pregnancy: A systematic review and meta-analysis. Pregnancy Hypertens. 2022 Mar;27:51-8.
16. Chapman AB, Abraham WT, Zamudio S, Coffin C, Merouani A, Young D, et al. Temporal relationships between hormonal and hemodynamic changes in

early human pregnancy. Kidney Int. 1998 Dec;54(6):2056-63.

17. Haug EB, Horn J, Markovitz AR, Fraser A, Vatten LJ, Macdonald-Wallis C, et al. Life Course Trajectories of Cardiovascular Risk Factors in Women With and Without Hypertensive Disorders in First Pregnancy: The HUNT Study in Norway. J Am Heart Assoc. 2018 08 07;7(15):e009250.
18. Nakano H, Shiina K, Takahashi T, Fujii M, Iwasaki Y, Matsumoto C, et al. Bidirectional Longitudinal Relationships Between Arterial Stiffness and Hypertension Are Independent of Those Between Arterial Stiffness and Diabetes: A Large-Scale Prospective Observational Study in Employees of a Japanese Company. J Am Heart Assoc. 2022 Jul 5;11(13):e025924.
19. Cho GJ, Kim HY, Park JH, Ahn KH, Hong SC, Kim HJ, et al. Prepregnancy Factors Are Associated with Development of Hypertension Later in Life in Women with Pre-Eclampsia. J Womens Health (Larchmt). 2019 Jul;28(7):984-9.
20. Brouwers L, van der Meiden-van Roest AJ, Savelkoul C, Vogelvang TE, Lely AT, Franx A, van Rijn BB. Recurrence of pre-eclampsia and the risk of future hypertension and cardiovascular disease: a systematic review and meta-analysis. BJOG. 2018 Dec;125(13):1642-54.
21. Kitt J, Fox R, Frost A, Shanyinde M, Tucker K, Bateman PA, et al. Long-Term Blood Pressure Control After Hypertensive Pregnancy Following Physician-Optimized Self-Management: The POP-HT Randomized Clinical Trial. JAMA. 2023 Nov 28;330(20):1991-9.
22. Kitt JA, Fox RL, Cairns AE, Mollison J, Burchert HH, Kenworthy Y, et al. Short-Term Postpartum Blood Pressure Self-Management and Long-Term Blood Pressure Control: A Randomized Controlled Trial. Hypertension. 2021 Aug;78(2):469-79.





Part III

Discussion and appendices

9

General discussion

GENERAL DISCUSSION

Summary of key findings

This thesis investigated (patho)physiological mechanisms and risk factors associated with the development of placenta-related pregnancy complications, and with chronic hypertension after pregnancies complicated by preeclampsia (Figure 1). **Part I** focused on periconception immunological and hormonal alterations in association with adverse pregnancy outcomes. Longitudinal glycosylation patterns of maternal immunoglobulins IgG and IgA varied according to maternal characteristics, including a more anti-inflammatory profile in pregnancies conceived after IVF/ICSI, and a more pro-inflammatory profile in pregnancies with a higher maternal BMI and those complicated by preeclampsia, pregnancy-induced hypertension, preterm birth and small-for-gestational age. These findings suggest that immune-mediated mechanisms play a role in the development of placental dysfunction.

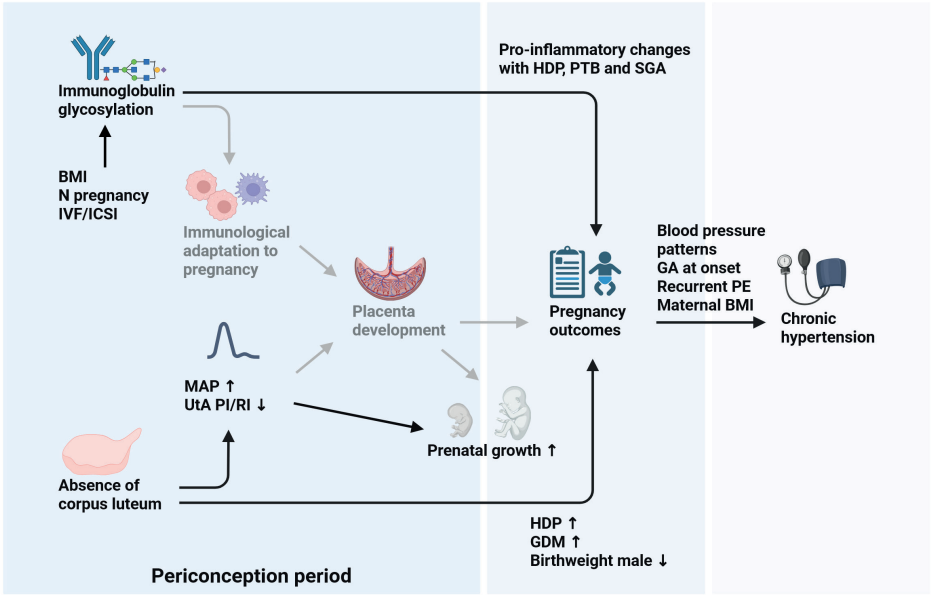


Figure 1. Summary of findings from this thesis. Black arrows represent associations explored in this thesis, while grey arrows represent hypothetical pathways. BMI = body mass index; IVF = in-vitro fertilization; ICSI = intra-cytoplasmatic sperm injection; MAP = mean arterial pressure; UtA PI/RI = uterine artery pulsatility index and resistance index; HDP = hypertensive disorders of pregnancy; PTB = preterm birth; SGA = small for gestational age; GA = gestational age; PE = preeclampsia; GDM = gestational diabetes mellitus.

Furthermore, the number of corpora lutea, known to affect the levels of prorenin and relaxin during the first trimester of pregnancy, influenced maternal cardiovascular adaptation: the absence of a corpus luteum was associated with a higher mean arterial pressure and lower uterine artery resistance indices. After investigating associations with pregnancy outcomes, we observed increased risks of gestational diabetes, hypertensive disorders, and a lower birthweight in male neonates in pregnancies conceived in the absence of a corpus luteum.

Part II investigated maternal hemodynamic markers and other risk factors for the development of chronic hypertension after pregnancies with preeclampsia. We found that a higher ratio between mean arterial pressure and uterine Doppler indices was positively associated with prenatal growth and may contribute to the early prediction of fetal growth restriction. Additionally, distinct blood pressure trajectories during pregnancy and the initiation of antihypertensive therapy before 24 weeks gestational age were associated with an elevated risk of developing chronic hypertension after preeclampsia. To contextualize these findings, we conducted a systematic review of the literature on risk factors for hypertension more than five years after pregnancies complicated by preeclampsia. The most consistently reported predictors included pre-existing cardiovascular risk factors, such as elevated baseline blood pressure and a higher BMI, as well as the recurrence of preeclampsia in subsequent pregnancies.

Together, these findings demonstrate that the cardiovascular adaptations during pregnancy reveal underlying vulnerabilities in maternal health that persist into later life. They offer novel insights into the interplay between periconceptional immune modulation, hormonal signaling, and cardiovascular adaptation in shaping both pregnancy outcomes and long-term maternal health. In the following sections of this general discussion, we reflect on the methodological considerations of the studies presented in this thesis, explore their implications for the scientific field and clinical practice, and consider the broader societal impact.

Methodological considerations

The studies in this thesis are embedded within the prospective, tertiary hospital-based Rotterdam Periconceptional Cohort (Predict Study). This offers a unique opportunity to combine detailed periconception and longitudinal follow-up data [1, 2]. The comprehensive data on maternal characteristics, cardiovascular parameters, and maternal and neonatal outcomes allowed for adjustment for a wide range of potential confounders. This adjustment is essential for isolating the true associations between the studied variables, minimizing the risk of bias, and enhancing the reliability of the conclusions drawn. The integration of data from the Follow-Up After Preeclampsia Cohort (FUPEC) further strengthened the thesis by enabling long-term cardiovascular follow-up of the mothers [3]. A variety of statistical approaches was used, tailored to the specific research questions

and data structures, including longitudinal modelling, subgroup comparisons, predictive modelling and meta-analyses, enhancing the robustness and interpretability of the findings. While these methods strengthen internal validity, the observational nature of the studies limits causal inference. Residual confounding cannot be entirely ruled out, despite careful adjustment for known variables. The study on the number of corpora lutea is particularly prone to indication and selection bias, as all pregnancies without a corpus luteum were, by definition, conceived through programmed-cycle frozen embryo transfer. We addressed this by treating the number or corpora lutea as a continuous variable, comparing natural cycle frozen embryo transfers with natural conceptions, and adjusting for relevant confounders. Still, disentangling biological from treatment-related effects remains a significant methodological challenge. Although both the Predict study and the FUPEC study are large prospective cohorts based on patients from the same hospital, the overlap of participants between the two was small, reducing the sample size for long-term follow-up analyses. This limitation underscores the importance of developing integrated pregnancy cohorts with harmonized postpartum surveillance, and improved data availability across studies.

Internal validity was supported by standardized data collection protocols within the Predict study and the use of validated analytical techniques, such as LC-MS for glycosylation profiling. However, measurement bias may have influenced certain outcomes, particularly blood pressure readings extracted from electronic medical records and the documentation of medication use during pregnancy. For these variables, clinical variability in measurement techniques and inconsistencies in documentation practices may have affected data accuracy. External validity is limited by the cohort composition. The study population predominantly includes Dutch-speaking women receiving tertiary care, with a relatively high proportion of pregnancies conceived after IVF/ICSI treatment (30–40%) [1]. While this setting allowed for detailed phenotyping and periconception assessments, it may limit extrapolation to broader or more diverse populations. Replication in population-based cohorts and inclusion of underrepresented groups is needed to validate and extend these findings.

Interpretation and Integration of Findings

Scientific Implications

A key contribution of this thesis' research to the scientific field lies in the longitudinal characterization of immunoglobulin glycosylation during early pregnancy. Building on existing knowledge that pregnancy shifts IgG and IgA glycosylation toward a more anti-inflammatory profile [4, 5], this thesis adds evidence that these changes are already present in the first trimester. Moreover, these glycosylation patterns were shown to vary with maternal and pregnancy characteristics, such as the number of previous pregnancies and mode of conception. Most notably, pregnancies complicated by placenta-related

disorders exhibited less pronounced anti-inflammatory glycosylation shifts. These findings suggest that altered glycosylation may not only reflect immune dysregulation but could possibly contribute mechanistically to placental dysfunction. The implications extend beyond pregnancy: the glycosylation profiles observed in placenta-related complications, in particular reduced galactosylation and sialylation, mirror those associated with endothelial dysfunction and cardiovascular disease in non-pregnant populations [6, 7]. This raises the question of whether aberrant glycosylation might contribute to both impaired vascular adaptation during pregnancy and the increased long-term cardiovascular risk observed after adverse pregnancy outcomes. An alternative explanation for the observed associations between altered glycosylation profiles and pregnancy outcomes is that both glycosylation changes and adverse pregnancy outcomes could stem from an underlying factor, such as changes in pregnancy hormones or lifestyle factors. In this case, the association may not reflect a causal or mechanistic relationship, but rather a mere correlation due to shared underlying influences. However, it is also possible that these factors act upstream within the same causal pathway. Future research should focus on disentangling whether glycosylation changes are causal or consequential, validating findings in other cohorts, and exploring whether glycan-based scores, which are already under investigation in cardiovascular risk prediction [8], could eventually be adapted for pregnancy-related risk stratification.

This thesis also reinforces the importance of the periconceptional hormonal environment, particularly the role of the corpus luteum, in shaping maternal cardiovascular adaptation to pregnancy. While prior studies had already established the association between absence of a corpus luteum in programmed frozen embryo transfer cycles with hypertensive disorders of pregnancy [9], our work adds value by validating these associations in a larger cohort. Moreover, we identified sex-specific differences in birthweight: in male neonates, the presence of more than one corpus luteum was associated with lower birthweight, while this was not observed in female neonates. Additionally, the observation that the lower uterine artery resistance observed in pregnancies without a corpus luteum is independent of blood pressure, suggests a direct vascular effect of corpus luteum hormones such as relaxin and progesterone. Although these findings do not fundamentally alter existing knowledge, they reinforce the association between absence of a corpus luteum and adverse maternal pregnancy outcomes, including hypertensive disorders and gestational diabetes. Given the consistency of observational findings across studies, further observational research may not be necessary to guide clinical practice. Instead, the field now awaits results from randomized trials, such as the NatPro trial [10], to determine whether and how assisted reproduction protocols should be adapted.

Building on these mechanistic insights, this thesis also proposes a broader view of maternal circulatory adaptation: not only as a physiological process, but as a potential clinical marker across what can be conceptualized as the pregnancy-to-postpartum continuum.

A proposed marker, the ratio of mean arterial pressure to uterine artery resistance indices, offers a physiologically grounded, integrative measure of both systemic and local vascular adaptation. Its positive association with embryonic volume, fetal growth, and birthweight suggests that it reflects the efficiency of early cardiovascular adaptation and placental perfusion. This builds on prior studies that show that uterine artery flow is associated with fetal growth [11-13], while adding a systemic maternal component that could enhance predictive accuracy. Given its physiological basis and ease of measurement, this marker may warrant further investigation in prospective clinical studies. In another study we showed that distinct blood pressure trajectories during pregnancy, particularly rising systolic blood pressure and the early need for antihypertensive treatment, were associated with the development of chronic hypertension after preeclampsia. These findings support a shift from viewing blood pressure as a static threshold to considering it as a dynamic, longitudinal marker of maternal cardiovascular adaptation. From a research perspective, both studies underscore the importance of systematically documenting and standardizing hemodynamic measurements throughout pregnancy and postpartum. Doing so would not only facilitate more robust risk prediction models but also enable the application of advanced analytical techniques, such as machine learning, to large-scale datasets. These approaches could help uncover subtle patterns and trajectories that may otherwise go undetected.

Moreover, the observed associations between blood pressure patterns, medication use, and later hypertension raise an important question: while blood pressure measurements during pregnancy can serve as a risk factor for the development of hypertension after preeclampsia, it remains unclear whether managing blood pressure during pregnancy could actually reduce the long-term risk of chronic hypertension. Furthermore, the level of blood pressure control during pregnancy may also influence the risk of developing preeclampsia. In an ongoing study, we aim to assess the association between blood pressure control throughout pregnancy and the development of preeclampsia in women at increased risk. Using clinical markers, future research could also explore whether identifying hypo- or hyperdynamic states during pregnancy, for example by using the ratio of blood pressure to uterine artery resistance, can inform more individualized antihypertensive strategies aimed at optimizing both fetal growth and development of chronic hypertension.

Clinical Implications

The findings presented in this thesis have several implications for clinical practice, including preconception care, pregnancy monitoring, and postpartum follow-up. While some insights reinforce existing knowledge, others offer new perspectives that may inform future guidelines.

Programmed versus natural frozen embryo transfer in IVF/ICSI protocols

While the number of corpora lutea is not always modifiable, it becomes a relevant consideration in the context of assisted reproductive technologies. Programmed-cycle frozen embryo transfers, which lack a corpus luteum, have been associated with increased risks of hypertensive disorders [9]. If ongoing randomized controlled trials, such as the one currently underway in the United States (NCT04551807), confirm these associations, the choice between programmed and natural cycle frozen embryo transfer should be explicitly addressed during patient counselling [10]. Although definitive evidence is still pending, current data support considering natural cycle frozen embryo transfer when feasible, especially in women already at elevated risk for hypertensive complications.

Measuring uterine artery Doppler indices throughout pregnancy

The introduction of a novel ratio combining mean arterial pressure and uterine artery Doppler indices provides a physiologically grounded marker for assessing maternal cardiovascular adaptation to pregnancy. Its association with embryonic volume, fetal growth, and birthweight suggests potential utility in second trimester risk stratification. Uterine artery Doppler measurements, particularly between 20 and 24 weeks of gestation, have been shown to improve the prediction of small-for-gestational-age (SGA) infants [11,14]. Several studies have also highlighted the additional value of incorporating mean arterial pressure in this context [15,16]. Integrating the ratio of mean arterial pressure and uterine artery Doppler indices into routine prenatal assessments could further improve the identification of pregnancies at risk for placental insufficiency. In fact, current UK guidelines recommend second-trimester uterine artery pulsatility index (UtA-PI) screening for women at increased risk of fetal growth restriction [17]. Given its potential clinical value, uterine artery Doppler indices should be systematically measured during second- and third-trimester ultrasound evaluations.

An integrated and risk-based follow-up approach after adverse pregnancy outcomes

Adverse pregnancy outcomes, particularly preeclampsia, are now widely recognized as early indicators of future cardiovascular disease risk. This recognition has led to their inclusion in guidelines from the Dutch Society of Obstetrics & Gynecology (NVOG), the European Society of Hypertension (ESH), the European Society of Cardiology (ESC), and the American Heart Association (AHA) [18-21]. These guidelines consistently recommend postpartum follow-up starting between 3 and 6 months after delivery. However, they differ in who should provide this care: some emphasize the role of obstetricians, while others advocate for shared or multidisciplinary care. The Dutch CVRM guideline suggests that the care setting (primary or secondary) should be determined by a woman's individual risk [19]. Despite these recommendations, none of the guidelines provide a formal risk stratification model for long-term cardiovascular disease specifically tailored to women with a history of preeclampsia. To improve care, we must move beyond general advice and implement structured, evidence-based follow-up strategies. This is where our find-

ings come into play. Our results demonstrate that rising systolic blood pressure during pregnancy, the use of four or more antihypertensive agents, and initiation of therapy before 24 weeks of gestation are associated with the development of chronic hypertension later in life. These clinical characteristics highlight the potential for stratifying women into low- and high-risk groups. When combined with additional risk factors identified in the literature review (Chapter 7), these variables could form the basis of a postpartum risk stratification model. Although such a model requires formal validation, we recommend that clinicians begin systematically documenting these risk factors in routine practice. This would not only support future research but also promote greater clinical awareness and facilitate early identification of women at increased risk. Integrating such risk factors into postpartum care also calls for a standardized and structured approach to cardiovascular health assessment, an element currently lacking in European and Dutch guidelines. Among the reviewed guidelines, only the AHA explicitly recommends structured tools such as the Life's Essential 8 (LE8) score [21, 22]. This score includes eight components: diet, physical activity, nicotine exposure, sleep, body mass index, blood lipids, blood glucose, and blood pressure. Already widely applied outside of pregnancy, LE8 has recently been shown to be associated not only with long-term cardiovascular outcomes following adverse pregnancy events but also with the recurrence of pregnancy complications in subsequent pregnancies [23, 24]. This dual relevance also underscores the importance of integrating cardiovascular health assessment into preconception care, aiming to improve outcomes in future pregnancies following complications.

Societal Impact and Organizational Implications

While the concept of hypertensive pregnancy disorders as indicators of future maternal cardiovascular health is increasingly recognized among clinicians and researchers, it remains underappreciated in broader society. Raising public awareness about the long-term health implications of pregnancy for both mother and child is essential. Importantly, many risk factors contributing to cardiovascular disease, such as hypertension, BMI, and lifestyle factors, are modifiable, offering a unique opportunity for prevention through timely intervention and education. Pregnancy therefore provides a critical window to engage women in preventive care, promote healthy lifestyle habits, and initiate cardiovascular surveillance for those at increased risk. Embedding pregnancy into the broader framework of preventive medicine requires a shift in both societal perception and healthcare practice. The evidence based mHealth lifestyle coaching program www.SmarterPregnancy.co.uk and initiatives like the Healthy Pregnancy Week in the Netherlands represent promising steps toward this goal [25]. For example, participating in the Smarter Pregnancy program for 26 weeks, starting in the first trimester of pregnancy, significantly reduces blood pressure [26]. As a next step, these efforts to improve lifestyle behavior should be expanded to adopt a life-course perspective: connecting pregnancy, postpartum care, and preconception counselling [27].

Organizationally, implementing such a perspective, particularly in research aimed at understanding and developing preventive strategies, requires better integration across disciplines and care settings. It is important to embed implementation research early on, so that practical barriers and facilitators are identified from the start. This requires collaborating closely with all involved parties and care providers, including midwifery practices, general practitioners, gynecologists, internal medicine specialists, to ensure that proposed workflows align with real-world clinical practice. In such a multidisciplinary context, the question of who should take the lead will depend on the woman's individual risk profile, underscoring the need for flexible and risk-stratified coordination using accessible and standardized measures and models. However, achieving such coordination is hindered by the fragmentation of data across cohorts and healthcare facilities, often due to differences in informed consent procedures and registration systems [28]. One practical solution is to ask women at study inclusion whether they are already participating in another cohort, and request consent to access that data. This approach could facilitate data-driven research and reduce duplication. Additionally, the use of standardized measurements and documentation within healthcare settings would significantly enhance the feasibility of AI-driven prediction models and personalized follow-up strategies. Finally, easier and more secure data sharing across these disciplines is essential, as it would support continuity of care, reduce administrative burden, and strengthen the foundation for integrated, life-course–oriented preventive strategies.

Conclusion

The overarching aim of this thesis was to improve our understanding of maternal hypertensive and placenta-related pregnancy disorders by investigating their underlying mechanisms, with the ultimate goal of enabling earlier identification of women at risk. In pursuit of this aim, we identified several physiological and molecular factors that contribute to pregnancy adaptation and development of chronic hypertension. By elucidating the roles of the corpus luteum and glycosylation mechanisms, this thesis deepens our understanding of how endocrine and immunological pathways influence maternal vascular adaptation to pregnancy. Furthermore, pregnancy features, including uterine artery resistance and blood pressure trajectories, appear to reflect some degree of cardiovascular and placental adaptation, revealing patterns of resilience or vulnerability that influence both pregnancy outcomes and the long-term risk of developing chronic hypertension.

REFERENCES

1. Rousian M, Schoenmakers S, Eggink AJ, Gootjes DV, Koning AHJ, Koster MPH, et al. Cohort Profile Update: the Rotterdam Periconceptional Cohort and embryonic and fetal measurements using 3D ultrasound and virtual reality techniques. Int J Epidemiol. 2021 Nov 10;50(5):1426-7.
2. Steegers-Theunissen RP, Verheijden-Paulissen JJ, van Uitert EM, Wildhagen MF, Exalto N, Koning AH, et al. Cohort Profile: The Rotterdam Periconceptional Cohort (Predict Study). Int J Epidemiol. 2016 Apr;45(2):374-81.
3. van der Bijl MF, Al-Hassany L, Wijtzes AI, Verdonk K, Duvekot JJ, Roeters van Lennep J. FUPEC study, a prospective open-cohort on severe pre-eclampsia and cardiovascular risk factors based in the Netherlands. BMJ Open. 2024 Nov 12;14(11):e093423.
4. van de Geijn FE, Wuhrer M, Selman MH, Willemsen SP, de Man YA, Deelder AM, et al. Immunoglobulin G galactosylation and sialylation are associated with pregnancy-induced improvement of rheumatoid arthritis and the postpartum flare: results from a large prospective cohort study. Arthritis Res Ther. 2009;11(6):R193.
5. Bondt A, Nicolardi S, Jansen BC, Kuijper TM, Hazes JMW, van der Burgt YEM, et al. IgA N- and O-glycosylation profiling reveals no association with the pregnancy-related improvement in rheumatoid arthritis. Arthritis Res Ther. 2017 Jul 5;19(1):160.
6. Štambuk T, Klasić M, Zoldoš V, Lauc G. N-glycans as functional effectors of genetic and epigenetic disease risk. Molecular Aspects of Medicine. 2021 2021/6//;79:100891-.
7. Birukov A, Plavsa B, Eichelmann F, Kuxhaus O, Hoshi RA, Rudman N, et al. Immunoglobulin G N-Glycosylation Signatures in Incident Type 2 Diabetes and Cardiovascular Disease. Diabetes Care. 2022 Nov 1;45(11):2729-36.
8. Hoshi RA, Plavsa B, Liu Y, Trbojevic-Akmacic I, Glynn RJ, Ridker PM, et al. N-Glycosylation Profiles of Immunoglobulin G and Future Cardiovascular Events. Circ Res. 2024 Mar;134(5):e3-e14.
9. Conrad KP, von Versen-Hoynck F, Baker VL. Pathologic maternal and neonatal outcomes associated with programmed embryo transfer. J Assist Reprod Genet. 2024 Apr;41(4):821-42.
10. Baksh S, Casper A, Christianson MS, Devine K, Doody KJ, Ehrhardt S, et al. Natural vs. programmed cycles for frozen embryo transfer: study protocol for an investigator-initiated, randomized, controlled, multicenter clinical trial. Trials. 2021 Sep 27;22(1):660.
11. Poon LC, Lesmes C, Gallo DM, Akolekar R, Nicolaides KH. Prediction of small-for-gestational-age neonates: screening by biophysical and biochemical markers at 19-24 weeks. Ultrasound Obstet Gynecol. 2015 Oct;46(4):437-45.
12. Velauthar L, Plana MN, Kalidindi M, Zamora J, Thilaganathan B, Illanes SE, et al. First-trimester uterine artery Doppler and adverse pregnancy outcome: a meta-analysis involving 55,974 women. Ultrasound Obstet Gynecol. 2014 May;43(5):500-7.
13. Melchiorre K, Leslie K, Prefumo F, Bhide A, Thilaganathan B. First-trimester uterine artery Doppler indices in the prediction of small-for-gestational age pregnancy and intrauterine growth restriction. Ultrasound Obstet Gynecol. 2009 May;33(5):524-9.
14. Familiari A, Bhide A, Morlando M, Scala C, Khalil A, Thilaganathan B. Mid-pregnancy fetal biometry, uterine artery Doppler indices and maternal demographic characteristics: role in prediction of small-for-gestational-age birth. Acta Obstet Gynecol Scand. 2016 Feb;95(2):238-44.
15. Lesmes C, Gallo DM, Saiid Y, Poon LC, Nicolaides KH. Prediction of small-for-

gestational-age neonates: screening by uterine artery Doppler and mean arterial pressure at 19-24 weeks. Ultrasound Obstet Gynecol. 2015 Sep;46(3):332-40.
16. Papastefanou I, Nowacka U, Syngelaki A, Mansukhani T, Karamanis G, Wright D, Nicolaides KH. Competing risks model for prediction of small-for-gestational-age neonates from biophysical markers at 19 to 24 weeks' gestation. Am J Obstet Gynecol. 2021 Nov;225(5):530 e1- e19.
17. Morris RK, Johnstone E, Lees C, Morton V, Smith G, Royal College of O, Gynaecologists. Investigation and Care of a Small-for-Gestational-Age Fetus and a Growth Restricted Fetus (Green-top Guideline No. 31). BJOG. 2024 Aug;131(9):e31-e80.
18. De Backer J, Haugaa KH, Hasselberg NE, de Hosson M, Brida M, Castelletti S, et al. 2025 ESC Guidelines for the management of cardiovascular disease and pregnancy. Eur Heart J. 2025 Aug 29.
19. (NVOG) NVvOeG. CVRM na een reproductieve aandoening. 2023 [cited 2025 August 26]; Available from: https://richtlijnendatabase.nl/richtlijn/cvrm_na_een_reproductieve_aandoening/cvrm_na_reproductieve_aandoening_-_startpagina.html.
20. Thomopoulos C, Hitij JB, De Backer T, Gkaliagkousi E, Kreutz R, Lopez-Sublet M, et al. Management of hypertensive disorders in pregnancy: a Position Statement of the European Society of Hypertension Working Group 'Hypertension in Women'. J Hypertens. 2024 Jul 1;42(7):1109-32.
21. Brown HL, Warner JJ, Gianos E, Gulati M, Hill AJ, Hollier LM, et al. Promoting Risk Identification and Reduction of Cardiovascular Disease in Women Through Collaboration With Obstetricians and Gynecologists: A Presidential Advisory From the American Heart Association and the American College of Obstetricians and Gynecologists. Circulation. 2018 Jun 12;137(24):e843-e52.
22. Lloyd-Jones DM, Allen NB, Anderson CAM, Black T, Brewer LC, Foraker RE, et al. Life's Essential 8: Updating and Enhancing the American Heart Association's Construct of Cardiovascular Health: A Presidential Advisory From the American Heart Association. Circulation. 2022 Aug 2;146(5):e18-e43.
23. Chen H, Li R, Liu S, Zhao S, Guo T, Tian S, et al. Life's Essential 8 and cardiovascular disease in women with a history of adverse pregnancy outcomes. Eur J Prev Cardiol. 2025 Jan 31.
24. Dodds LV, Feaster DJ, Kiefe CI, Gunderson EP, Bello NA, Rundek T, et al. Lower Prepregnancy Cardiovascular Health is Associated With Hypertensive Disorders of Pregnancy: The CARDIA Study. Hypertension. 2025 Oct;82(10):1696-704.
25. van Dijk MR, Koster MPH, Oostingh EC, Willemsen SP, Steegers EAP, Steegers-Theunissen RPM. A Mobile App Lifestyle Intervention to Improve Healthy Nutrition in Women Before and During Early Pregnancy: Single-Center Randomized Controlled Trial. J Med Internet Res. 2020 May 15;22(5):e15773.
26. Hojeij B, Schoenmakers S, Van Mullem I, Willemsen SP, Dinnyes A, Sinclair KD, et al. Smarter Pregnancy Coaching and Maternal Blood Pressure. Am J Prev Med. 2025 Mar;68(3):455-64.
27. van Zundert SKM, van Rossem L, Schoenmakers S, Steegers-Theunissen RPM. Lifestyle care for transformation of medical care using an early life course approach. Reprod Biomed Online. 2022 Nov;45(5):847-50.
28. Mulder ST, Omidvari AH, Rueten-Budde AJ, Huang PH, Kim KH, Bais B, et al. Dynamic Digital Twin: Diagnosis, Treatment, Prediction, and Prevention of Disease During the Life Course. J Med Internet Res. 2022 Sep 14;24(9):e35675.

10

Summary (English)
Samenvatting (Dutch)

SUMMARY (ENGLISH)

Understanding maternal hypertensive and placenta-related disorders of pregnancy requires an integrated approach that examines both underlying mechanisms and clinical characteristics, with a focus on predicting and managing associated complications. This thesis addresses these aspects in two parts: **Part I** investigates pathophysiological mechanisms contributing to hypertensive and placenta-related pregnancy complications, and **Part II** focuses on the identification of risk factors and predictors for maternal and neonatal outcomes, including development of chronic hypertension.

Part I – Immune modulation, hormonal signaling and vascular adaptation in pregnancy

In **Chapter 2**, we investigate immunological adaptation during pregnancy by analyzing changes in maternal glycosylation patterns of immunoglobulins IgG and IgA. These glycosylation traits change significantly throughout gestation and differ by parity and mode of conception. First pregnancies and those conceived after IVF/ICSI show more anti-inflammatory glycosylation profiles, marked by higher levels of galactosylation and sialylation.

Chapter 3 extends this work by comparing glycosylation profiles between pregnancies with and without placenta-related complications. Pregnancies complicated by disorders such as preeclampsia or fetal growth restriction exhibit a more pro-inflammatory glycosylation profile, including lower sialylation and galactosylation. Specific glycopeptides—particularly IgA Asn327/Asn340 (LAGY)—emerge as potential biomarkers of placental dysfunction, offering a novel insight into the role of maternal immunity in pregnancy pathology.

In **Chapter 4**, we focus on hormonal factors by examining the association between the number of corpora lutea and cardiovascular adaptation during pregnancy. Pregnancies conceived without a corpus luteum, i.e. those following programmed frozen embryo transfer, show higher mean arterial pressure and lower uterine artery resistance indices. These findings suggest suboptimal maternal cardiovascular adaptation in the absence of corpus luteum-derived hormones such as relaxin and prorenin.

In **Chapter 5**, we investigate the association between the number of corpora lutea and maternal pregnancy and neonatal outcomes. Pregnancies without a corpus luteum show a significant increase in gestational diabetes and a non-significant increase in hypertensive disorders. Conversely, pregnancies with multiple corpora lutea show a lower risk of hypertensive complications but also a tendency towards a lower birthweight, especially in male neonates. Together, these results highlight the importance of the periconception-

al hormonal environment in the (patho)physiology of maternal pregnancy and neonatal outcomes.

Part II – Clinical markers associated with adverse pregnancy outcomes and maternal cardiovascular health

Building on the mechanistic insights from Part I, **Part II** examines predictors of embryonic and fetal growth, and risk factors for chronic hypertension in women with a history of preeclampsia.

In **Chapter 6**, we introduce a novel hemodynamic marker: the ratio of mean arterial pressure to uterine artery pulsatility or resistance index. This ratio is positively associated with embryonic volume, fetal growth at the 20-week anomaly scan, and birthweight. We suggest that this ratio reflects the efficiency of maternal cardiovascular adaptation and provides a simple tool for early risk stratification of placental function and fetal development.

Chapter 7 presents a systematic review of the literature on long-term hypertension risk following preeclampsia. We identify 32 factors associated with chronic hypertension occurring at least five years postpartum. Risk factors include a higher pre- or postpartum BMI and blood pressure, increased left ventricular mass or septal thickness, early-onset or preterm preeclampsia, gestational diabetes, decidual vasculopathy, and recurrence of preeclampsia. No consistent associations are found for lifestyle factors such as smoking or exercise, nor for several biochemical markers.

Finally, in **Chapter 8**, we combine data from the prospective Rotterdam periconception cohort with data from the Follow-up After Preeclampsia Cohort (FUPEC study) to assess whether pregnancy blood pressure trajectories could predict chronic hypertension one to three years after delivery in women with a history of preeclampsia with severe features. Women who develop chronic hypertension exhibit a distinct pattern of rising systolic blood pressure during pregnancy. All women who initiate antihypertensive therapy before 24 weeks of gestation or require four or more different drugs develop hypertension in follow-up, suggesting that medication timing and intensity during pregnancy may serve as early indicators of long-term cardiovascular risk.

Together, the studies in this thesis demonstrate how immune modulation, hormonal signaling, and cardiovascular adaptation are involved in shaping pregnancy outcomes and maternal health trajectories. The findings provide novel insights into early mechanisms of placental dysfunction and underscore the potential of pregnancy and the periconceptional period as a stress test and intervention window for future health.

SAMENVATTING (DUTCH)

Het begrijpen van maternale hypertensieve aandoeningen en placenta-gerelateerde complicaties tijdens de zwangerschap vereist een geïntegreerde benadering waarin zowel de onderliggende mechanismen als de implicaties voor voorspelling en behandeling van complicaties worden onderzocht. Dit proefschrift behandelt deze aspecten in twee delen: **Deel I** richt zich op pathofysiologische mechanismen die bijdragen aan het ontstaan van hypertensieve en placenta-gerelateerde zwangerschapscomplicaties, terwijl **Deel II** zich richt op identificatie van risicofactoren en voorspellers van maternale en neonatale uitkomsten.

Deel I – Immuun modulatie, hormonale signalering en vasculaire adaptatie in de zwangerschap

In **Hoofdstuk 2** onderzoeken we de immunologische adaptatie tijdens de zwangerschap door veranderingen in maternale glycosyleringsprofielen van de immunoglobulines IgG en IgA te analyseren. Deze patronen veranderen significant gedurende de zwangerschap en verschillen op basis van pariteit en conceptiemethode. Eerste zwangerschappen en zwangerschappen na in-vitro fertilisatie (IVF) vertonen een meer anti-inflammatoir glycosyleringsprofiel, gekenmerkt door hogere niveaus van galactosylering en sialylering.

Hoofdstuk 3 bouwt hierop voort door glycosyleringsprofielen te vergelijken tussen zwangerschappen met en zonder placenta-gerelateerde complicaties. Zwangerschappen gecompliceerd door aandoeningen zoals pre-eclampsie of foetale groeirestrictie vertonen een meer pro-inflammatoir profiel, gekenmerkt door een afname in sialylering en galactosylering. Specifieke glycopeptiden, waaronder IgA Asn327/Asn340 (LAGY), komen naar voren als potentiële biomarkers voor placentadisfunctie. Ook bieden de profielen nieuwe inzichten in de rol van het maternale immuunsysteem bij zwangerschapscomplicaties.

In **Hoofdstuk 4** focussen we op hormonale factoren, waarbij het aantal corpora lutea werd onderzocht in relatie tot cardiovasculaire adaptatie tijdens de zwangerschap. Zwangerschappen zonder corpus luteum, zoals het geval is bij terugplaatsing van een cryo-embryo in een kunstmatige cyclus, gaan gepaard met een hogere gemiddelde arteriële bloeddruk en lagere weerstand in de arteria uterina. Deze bevindingen suggereren een suboptimale maternale cardiovasculaire adaptatie bij afwezigheid van hormonen afkomstig uit het corpus luteum, zoals relaxine en prorenine.

In **Hoofdstuk 5** beschrijven we de associatie tussen het aantal corpora lutea en zwangerschapsuitkomsten. Zwangerschappen zonder corpus luteum vertonen een significante toename in zwangerschapsdiabetes en een niet-significante toename in hypertensieve aandoeningen. Daarentegen gaat een hoger aantal corpora lutea gepaard met een ver-

laagd risico op hypertensieve aandoeningen, maar ook met een lager geboortegewicht bij mannelijke neonaten. Deze hoofdstukken onderstrepen het belang van de hormonale omgeving in de periconceptionele periode voor maternale gezondheid en zwangerschapsuitkomsten.

Deel II – Klinische markers voor negatieve zwangerschapsuitkomsten en maternale cardiovasculaire gezondheid

Voortbouwend op de inzichten uit Deel I, richt **Deel II** zich op associatie tussen bloeddruk, vaatweerstand en foetale groei, evenals de risicofactoren voor het ontwikkelen van hypertensie bij vrouwen die pre-eclampsie hebben doorgemaakt.

In **Hoofdstuk 6** introduceren we een nieuwe marker: de verhouding tussen de gemiddelde arteriële bloeddruk en de pulsatility index (PI) of resistance index (RI) van de arteria uterina. Deze ratio is positief geassocieerd met embryonaal volume, foetale groei bij de 20-weken echo, en geboortegewicht. We denken dat deze maat de efficiëntie van maternale circulatoire adaptatie weerspiegelt en dat deze mogelijk gebruikt kan worden voor het eerder voorspellen van foetale groeirestrictie, in combinatie met andere klinische markers of foetale biometrie.

Hoofdstuk 7 beschrijft een systematische literatuurstudie naar risicofactoren voor het ontwikkelen van hypertensie ten minste vijf jaar na een zwangerschap gecompliceerd door pre-eclampsie. Van de 32 onderzochte factoren zijn er 14 consequent geassocieerd met verhoogd risico, waaronder een hogere BMI of bloeddruk (preconceptioneel of postpartum), een verhoogde linker ventrikelmassa of septumdikte, ontstaan van pre-eclampsie voor 34 of 37 weken zwangerschap, zwangerschapsdiabetes, aanwezigheid van deciduale vasculopathie bij pathologisch onderzoek van de placenta, en het doormaken van pre-eclampsie in meerdere zwangerschappen. Factoren zoals roken, lichaamsbeweging en verschillende biochemische markers vertonen geen consistente associatie met ontwikkeling van hypertensie.

In **Hoofdstuk 8** combineren we data uit twee cohorten, namelijk het Rotterdam Periconception Cohort (de Predict studie) en het Follow-up After Preeclampsia Cohort (de FUPEC-studie), om bij vrouwen met pre-eclampsie met ernstige kenmerken te onderzoeken of bloeddrukpatronen tijdens de zwangerschap geassocieerd zijn met hypertensie één tot drie jaar postpartum. Vrouwen die bij follow-up een te hoge bloeddruk hebben of medicatie hiervoor gebruiken, vertonen een kenmerkend stijgend patroon in systolische bloeddruk tijdens de zwangerschap. Opvallend is dat alle vrouwen die vóór 24 weken zwangerschap met antihypertensiva starten of vier of meer middelen gebruiken op een moment tijdens de zwangerschap, hypertensie hebben bij follow-up. Dit suggereert dat timing en intensiteit van medicatiegebruik tijdens de zwangerschap belangrijke voorspellers kunnen zijn voor latere cardiovasculaire gezondheid.

Gezamenlijk tonen de studies in dit proefschrift aan hoe factoren binnen immuun modulatie, hormonale signalering en cardiovasculaire adaptatie elkaar beïnvloeden en hoe deze samenhangen met zwangerschapsuitkomsten en het ontwikkelen van hypertensie na de zwangerschap. De bevindingen bieden nieuwe inzichten in vroege mechanismen van placentadysfunctie en onderstrepen de waarde van zwangerschap en de periconceptionele periode als een moment van inzicht in de toekomstige gezondheid en als een kans voor interventie om deze gezondheid te verbeteren.

Addendum

Authors and affiliations
List of abbreviations
List of publications
PhD portfolio
About the author
Dankwoord

AUTHORS AND AFFILIATIONS

A. Daniels	Department of Obstetrics and Gynecology, Erasmus University Medical Center, Rotterdam, the Netherlands
A.H.J. Danser	Department of Internal Medicine, Erasmus University Medical Center, Rotterdam, the Netherlands
J.J. Duvekot	Department of Obstetrics and Gynecology, Erasmus University Medical Center, Rotterdam, the Netherlands
J.J. Koerts	Department of Obstetrics and Gynecology, Erasmus University Medical Center, Rotterdam, the Netherlands
J.E. Roeters van Lennep	Department of Internal Medicine, Erasmus University Medical Center, Rotterdam, the Netherlands
M. Rousian	Department of Obstetrics and Gynecology, Erasmus University Medical Center, Rotterdam, the Netherlands
R.P.M. Steegers-Theunissen	Department of Obstetrics and Gynecology, Erasmus University Medical Center, Rotterdam, the Netherlands
M. van Hoek	Department of Internal Medicine, Erasmus University Medical Center, Rotterdam, the Netherlands
K. Verdonk	Department of Internal Medicine, Erasmus University Medical Center, Rotterdam, the Netherlands
L.W. Voskamp	Department of Internal Medicine, and Department of Obstetrics and Gynecology, Erasmus University Medical Center, Rotterdam, the Netherlands
R.E. Wiegel	Department of Obstetrics and Gynecology, Erasmus University Medical Center, Rotterdam, the Netherlands
S.P. Willemsen	Department of Epidemiology, and Department of Obstetrics and Gynecology, Erasmus University Medical Center, Rotterdam, The Netherlands
M. Wuhrer	Center for Proteomics and Metabolomics, Leiden University Medical center, Leiden, the Netherlands

LIST OF ABBREVIATIONS

AC	Abdominal circumference
AC-FET	Artificial cycle frozen embryo transfer
ADCC	Antibody-dependent cellular cytotoxicity
AGA	Appropriate-for-gestational-age
AHA	American Heart Association
ALT	Alanine aminotransferase
ART	Assisted reproductive technology
AST	Aspartate aminotransferase
AUC	Area under the curve
BMI	Body mass index
BP	Blood pressure
BPD	Biparietal diameter
CDC	Complement dependent cytotoxicity
CI	Confidence interval
CL	Corpus luteum
CRL	Crown–rump length
DBP	Diastolic blood pressure
DDD	Defined daily dose
DR	Detection rate
eNOS	Endothelial nitric oxide synthase
EFW	Estimated fetal weight
ENI	Joining chain Asn71
eoPE	Early onset preeclampsia
ESC	European Society of Cardiology
ESH	European Society of Hypertension
EV	Embryonic volume
Fc	Fragment crystallizable region
FcγR	Fc gamma receptor
FDR	False discovery rate
FGR	Fetal growth restriction
FL	Femur length
FUPEC	Follow-Up after Preeclampsia Outpatient Clinic
GA	Gestational age
Gal	Galactose

GalNAc	N-acetylgalactosamine
GDM	Gestational diabetes mellitus
GGT	Gamma-glutamyl transferase
GlcNAc	N-acetylglucosamine
GnRH	Gonadotropin-releasing hormone
HC	Head circumference
HCG	Human chorionic gonadotropin
HDL	High-density lipoprotein
HELLP	Hemolysis, elevated liver enzymes, and low platelets
hMG	Human menopausal gonadotropin
HOMA-IR	Homeostatic model insulin resistance
HYT	T106/T109/S111/S113/T114/T117
ICSI	Intracytoplasmic sperm injection
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IQR	Interquartile range
IUI	Intra-uterine insemination
IVF	In vitro fertilization
IVST	Interventricular septum thickness
LAGC / LAGY	Asn327 / Asn340
LC-MS	Liquid chromatography-mass spectrometry
LDL	Low-density lipoprotein
LE8	Life's essential 8 score
LGA	Large-for-gestational-age
LH	Luteinizing hormone
LMP	Last menstrual period
LSL	Asn144 / Asn131
LVMi	Left ventricular mass index
MAP	Mean arterial pressure
MESA	Microepididymal Sperm Aspiration
MOOSE	Meta-analysis of observational studies in epidemiology
NC-FET	Natural cycle frozen embryo transfer
NK cells	Natural killer cells
NVOG	Nederlandse vereniging voor obstetrie & gynaecologie
PCOS	Polycystic ovarian syndrome
PE	Preeclampsia
PESA	Percutaneous epididymal sperm aspiration

PI	Pulsatility index
PlGF	Placental growth factor
POI	Premature ovarian insufficiency
PRISMA	Preferred reporting items for systematic reviews
PTB	Preterm birth
RAAS	Renin-angiotensin-aldosterone system
rFSH	Recombinant follicle stimulating hormone
RI	Resistance index
SBP	Systolic blood pressure
SD	Standard deviation
SES	Asn47
sFlt-1	Soluble fms-like tyrosine kinase-1
SGA	Small-for-gestational-age
TC	Total cholesterol
TESE	Testicular Sperm Extraction
TPL	Asn205
UtA	Uterine artery
VEGF	Vascular endothelial growth factor

LIST OF PUBLICATIONS

This thesis

LW Voskamp, M Rousian, A Daniels, M Wuhrer, AHJ Danser, RPM Steegers-Theunissen, K Verdonk. *Longitudinal Maternal IgG and IgA Glycosylation Profiles in Pregnancy Reveal Early Immune Alterations in Placenta-related Complications: The Rotterdam Periconception Cohort*. Hypertension. 2026.

Voskamp LW, Koerts JJ, Wiegel RE, Verdonk K, Danser AHJ, Steegers-Theunissen RPM, Rousian M. *Corpus Luteum Number and Maternal Hemodynamic Adaptation from Early Pregnancy Onwards: the Rotterdam Periconception Cohort (Predict Study)*. Hum Reprod. 2025 Nov 1;40(11):2078-2087.

Voskamp LW, Verdonk K, Roeters van Lennep JE, Duvekot JJ, Willemsen SP, Danser AHJ, Steegers-Theunissen RPM, Rousian M. *Blood pressure patterns during pregnancy and early postpartum in preeclampsia with severe features: predictors of chronic hypertension.* Pregnancy Hypertens. 2025 Aug 1;41:101240.

Voskamp LW, Rousian M, Koerts JJ, Steegers-Theunissen RPM, Danser AHJ, Verdonk K. *Risk factors for chronic hypertension 5 years after a pregnancy complicated by preeclampsia: a systematic review and meta-analysis.* J Hypertens. 2025 Jun 1;43(6):939-948.

Koerts JJ, **Voskamp LW**, Rousian M, Steegers-Theunissen RPM, Wiegel RE. *Impact of corpus luteum number on maternal pregnancy and birth outcomes: the Rotterdam Periconception Cohort*. Fertil Steril. 2024 Dec 6:S0015-0282(24)02431-2.

LW Voskamp, A Daniels, M. Rousian, M van Hoek, M Wuhrer, RPM Steegers-Theunissen, AHJ Danser, K Verdonk. *Longitudinal Profiling of IgG and IgA Glycosylation in Pregnancy Reveals Early Associations with BMI, Primigravidity and Conception Mode: The Rotterdam Periconception Cohort*. Submitted.

Voskamp LW, Verdonk K, Danser AHJ, Steegers-Theunissen RPM, Rousian M. *Maternal blood pressure to vascular resistance ratio is positively associated with embryonic and fetal growth: the Rotterdam Periconception Cohort*. Submitted.

Other

Wieles TAM, Bijma HH, Smith S, **Voskamp LW**, Willemsen S, Steegers-Theunissen RPM. The periconceptional vulnerability risk score is associated with reduced embryonic growth in naturally conceived pregnancies and in overweight women: The Rotterdam Periconception Cohort. Reprod Biomed Online. 2026 Jan;52(1):105085.

Van Zundert SKM, Van Rossem L, Mirzaian M, Willemsen SP, **Voskamp LW**, Bastiaansen WAP, Nikpayam D, Griffioen PH, Schilleman WF, Koning AHJ, Van Den Berg SAA, Rousian M, Van Schaik RHN, Steegers-Theunissen RPM. *The impact of maternal vulnerability on stress biomarkers and first-trimester growth: the Rotterdam Periconceptional Cohort (Predict Study).* Hum Reprod. 2024 Nov 1;39(11):2423-2433.

Bezstarosti H, Metsemakers WJ, van Lieshout EMM, **Voskamp LW**, Kortram K, McNally MA, et al. *Management of critical-sized bone defects in the treatment of fracture-related infection: a systematic review and pooled analysis.* Arch Orthop Trauma Surg. 2020 Aug 29.

Bezstarosti H, Van Lieshout EMM**, Voskamp LW**, Kortram K, Obremskey W, McNally MA, et al. *Insights into treatment and outcome of fracture-related infection: a systematic literature review*. Arch Orthop Trauma Surg. 2019 Jan 20;139(1):61–72.

PHD PORTFOLIO

PhD candidate	Lotte Voskamp
Departments	Obstetrics and Gynecology – Periconception Epidemiology
	Internal Medicine – Pharmacology and Vascular Medicine
PhD period	2022-2025
Promotors	Prof. dr. Régine P.M. Steegers-Theunissen
	Prof. dr. A.H. Jan Danser
Copromotors	Dr. Melek Rousian
	Dr. Koen Verdonk

Description	Year	EC
Courses		
Erasmus MC - BROK® (Basic course Rules and Organisation for Clinical researchers)	2022	1,5
Erasmus MC - Systematic literature retrieval in PubMed	2022	0,4
Erasmus MC - EndNote	2022	0,2
Erasmus MC - Systematic literature retrieval in Embase	2022	0,2
Erasmus MC - Reviews: project management, other databases and EndNote	2022	0,2
Erasmus MC - Basic Course on 'R'	2023	2,0
Erasmus MC - ESP01 Principles of Research in Medicine and Epidemiology	2023	0,7
PhD Introduction session	2023	0,2
Clinical Cardiovascular Epidemiology: Risk Factors. Diagnosis and Treatment	2023	0,5
Presenting Skills for PhD students	2023	0,8
Causal Mediation Analysis ESP69	2024	1,4
Scientific Integrity	2025	0,3
Teaching		
Lecturing		
SELF Leiden onderwijsavond	2023	0,2
VERGO onderwijsavond	2023-2024	0,30
Minor 'Innovations in primary care' LUMC: lecture on the periconception period	2023	0,15
Obstetric nurses in training: lecture on healthy pregnancy (Erasmus MC Academy)	2023	0,3
VERGO lunchlezing	2025	0,15

Description	Year	EC
Supervising		
Supervising Philip Starmans (medical student Erasmus MC)	2024	2,0
Supervising Rebecca Haasnoot (medical student Erasmus MC)	2024	2,0
Supervising Joni Koerts (research master student Erasmus MC)	2023-2024	4,0
Attended seminars, conferences and research meetings		
Weekly research meeting Department of Obstetrics and Gynecology	2022-2025	3,0
Weekly research meeting Pharmacology and Vascular Medicine	2022-2025	3,0
NEDWEP (Nederlandse Werkgroep Preeclampsie)	2023-2024	0,6
Annual Sophia Research Day	2023-2024	0,6
Annual Wladimiroff award meeting department Obstetrics and Gynecology	2023-2025	0,5
Erasmus MC PhD day	2023	0,3
Erasmus MC Clinical and Health Sciences day	2023	0,3
Erasmus MC PhD Symposium	2024	0,35
71st Annual Meeting Society of Reproductive Investigation (SRI) – Vancouver, Canada	2024	1
72nd Annual Meeting Society of Reproductive Investigation (SRI) – Charlotte NC, USA	2025	1
33rd Annual Meeting European Society of Hypertension (ESH) – Berlin, Germany	2024	1
34th Annual Meeting European Society of Hypertension (ESH) – Milan, Italy	2025	1
Presentations at (inter)national conferences		
Sophia Research Day - oral presentation	2024	0,50
NEDWEP bijeenkomst - oral presentation	2024	0,50
Annual Wladimiroff award meeting - oral presentation	2024	0,50
Annual Wladimiroff award meeting - oral presentation	2025	0,50
Erasmus MC PhD Symposium - poster and elevator pitch	2024	0,50
71st SRI Annual Meeting - 2 poster presentations	2024	1,00
72nd SRI Annual Meeting - 2 poster presentations	2025	1,00
33rd Annual Meeting ESH - oral and poster presentation	2024	1,00
34th Annual Meeting ESH - poster presentation	2025	0,50
Awards and Grants		
Award for Best New Investigator Poster presentation at the 71st SRI Annual Meeting	2024	
Accommodation grant 33rd Annual Meeting ESH	2024	
Total ECTS		36,15

ABOUT THE AUTHOR

Lotte Willemijn Voskamp was born on the 9th of March 1997 in Vlaardingen, the Netherlands. She completed her secondary education at the Stedelijk Gymnasium Schiedam in 2014, after which she began her medical training at the Erasmus University Medical Center in Rotterdam.

During her studies, Lotte developed a strong interest in obstetrics, gynecology, and internal medicine, with a particular focus on the cardiovascular complications that can arise during and after pregnancy. This interest was reflected in her master's thesis, in which she investigated the effect of omeprazole on angiogenic markers and clinical outcomes in women with preeclampsia. After graduating as a medical doctor in 2021, she worked as a resident in Obstetrics and Gynecology at Franciscus Gasthuis in Rotterdam, where she built her clinical foundation.

In 2022, she started her PhD at the Erasmus MC, within the Departments of Obstetrics and Gynecology and Internal Medicine. Her research focused on periconceptional mechanisms and maternal cardiovascular adaptation in relation to hypertensive pregnancy disorders and their long-term health consequences. Following her PhD, she worked as a resident in Obstetrics and Gynecology at the Erasmus MC, gaining experience in tertiary-level care for high-risk pregnancies. She then joined the Department of Cardiology as a postdoctoral researcher, where she investigates how pregnancy complications can inform long-term cardiovascular risk in women. She hopes to dedicate her career to improving cardiovascular health in women across the reproductive lifespan.

DANKWOORD

Dit proefschrift was niet, of pas veel later, tot stand gekomen zonder de directe of indirecte hulp van een heel aantal personen. Hoewel het dankwoord de enige plek in dit proefschrift is waar ik niets wetenschappelijk hoef te onderbouwen, vond ik dit tegelijkertijd misschien wel het moeilijkste stuk om te schrijven. In de wetenschap dat ik ongetwijfeld mensen vergeet die hebben bijgedragen, wil ik hieronder toch een aantal van jullie persoonlijk bedanken en een aantal herinneringen aan mijn promotietijd voor altijd vastleggen.

Allereerst mijn promotoren**, prof. Steegers-Theunissen** en **prof. Danser.** Toen ik van een klinische collega hoorde dat er mogelijk een positie voor een promotietraject over 'iets met pre-eclampsie' zou komen, heb ik snel een brief gestuurd om mijn interesse kenbaar te maken. Tijdens het sollicitatiegesprek bespraken we het voorlopige plan: pre-eclampsie, het RAAS-systeem, het samenvoegen van verschillende zwangerschapscohorten en iets met 'big data'. Slechts onderdelen daarvan zijn uiteindelijk in dit proefschrift terechtgekomen, ook door alle nieuwe ideeën die steeds weer naar boven kwamen. Bedankt voor jullie vertrouwen destijds en de begeleiding tijdens mijn promotietraject!

Geachte **prof. Steegers-Theunissen**, beste Régine. Allereerst wil ik je bedanken voor het opzetten en beheren van de Predict-studie, waaruit de meeste data van dit proefschrift afkomstig is. Ik kon altijd bij je binnenlopen met een vraag of om gewoon even iets te bediscussiëren. Ik bewonder je inzet voor een gezondere zwangerschap, en de samenwerkingen die je daarin opzet met personen vanuit allerlei vakgebieden. Ook mij wist je steevast te motiveren voor het uitwerken van nieuwe ideeën, en je begeleiding bij het schrijven van subsidieaanvragen heeft me veel geleerd.

Geachte **prof. Danser,** beste Jan. Doordat je op een andere verdieping zit, zagen we elkaar voornamelijk bij ingeplande meetings, waarbij we altijd welkom waren op jouw kamer. Ik waardeer en bewonder de snelheid en zorgvuldigheid waarmee je reageert op mijn vragen en manuscripten, gecombineerd met een pragmatische instelling die het tempo in het onderzoek hoog houdt, zoals jouw reactie op het rondsturen van de (zoveelste) uiteindelijke versie: "*Nu eerst maar eens zien wat de reviewers ervan denken*!".

Beste **dr. Rousian,** beste **Melek**, de eerste keer dat ik jou zag was als keuze-co in de kliniek, wat je vast zelf niet meer weet. Ik herinner me vooral je enthousiasme over een drieling. Des te blijer was ik toen je als gynaecoloog in mijn promotieteam kwam — toen wist ik nog niet dat het plannen (of het daadwerkelijk aansluiten bij) meetings lastig zou zijn en dat manuscripten vooral in het vliegtuig of rond middernacht werden gelezen. Gelukkig gaf je al snel aan dat je er helemaal geen probleem mee had dat ik je – eerst via de mail, en dan via WhatsApp of met een belletje – net zo lang stalkte tot ik mijn

antwoord had. Tijdens een van onze eerste gesprekken stelde je voor dat ik na mijn promotietraject nog wel een postdoc zou kunnen doen. Ik weet nog dat ik toen dacht: *'ik wil zo snel mogelijk weer terug naar de kliniek'*. Maar je had (zoals wel vaker) gelijk, en ik ben je ontzettend dankbaar dat je het laatste jaar van mijn PhD hebt meegedacht en meegewerkt aan hoe we dit konden realiseren!

Beste **dr. Verdonk,** beste Koen. Voor mij was jouw belangrijkste bijdrage in mijn team het inbrengen van originele en innovatieve ideeën. Je dacht vaak net een stapje verder en wanneer je zelf écht overtuigd was van een idee, had je het plan diezelfde middag nog uitgewerkt. Bedankt voor het aanmoedigen van die instelling in mij, en voor het opzetten van de samenwerkingen met Manfred en Mandy en met de FUPEC-studie! Dankzij jou was ik ook altijd op de hoogte van de laatste ontwikkelingen in het gebruik van AI-tools en de steeds groeiende mogelijkheden ervan. En tot slot jouw charmeoffensief voor de Interne Geneeskunde en Obstetric Medicine: ik kan je nog niet vertellen of het heeft gewerkt op klinisch gebied, maar ik denk dat inmiddels wel duidelijk is dat mijn onderzoekshart daar ligt. Ik kijk uit naar onze verdere samenwerking!

Graag wil ik de leden van de promotiecommissie bedanken voor de tijd en aandacht die zij hebben gestoken in het beoordelen van dit proefschrift. Geachte **prof. dr. Kavousi, prof. dr. Dolhain en dr. Ganzevoort,** hartelijk dank.

Ook wil ik graag de co-auteurs bedanken voor hun waardevolle inbreng in één of meerdere projecten. Beste **prof. Jeanine Roeters van Lennep** en **dr. Hans Duvekot**, bedankt voor het oprichten van het FUPEC-cohort. Als coassistent Interne Geneeskunde had ik al eens het geluk gehad om ingedeeld te worden bij jullie spreekuur. Hier legden jullie mij haarfijn het belang uit van de opvolging van vrouwen met ernstige pre-eclampsie, en zoals jullie kunnen lezen is dat mij altijd bijgebleven. Onze samenwerking en het beschikbaar stellen van de data hebben uiteindelijk geleid tot hoofdstuk 8. Beste **dr**. **Mandy van Hoek** en **prof. Manfred Wuhrer,** via jullie kwam het onderwerp glycosylering van immunoglobulines op mijn pad. Jullie expertise en ervaring in dit vakgebied heeft bijgedragen aan hoofdstuk 2 en 3 van dit proefschrift. Bedankt! Beste **dr. Sten Willemsen**, bedankt voor het beantwoorden van mijn statistiekvragen Ook al kan ChatGPT inmiddels een goed woordje R-code spreken, de aanpak met jou kunnen verifiëren blijft toch echt onmisbaar.

Zonder de Predict-studie had dit proefschrift niet bestaan. Allereerst dank aan alle deelnemers: zonder jullie was dit onderzoek simpelweg niet mogelijk geweest. Ook dank aan alle voorgaande promovendi, studenten en medewerkers die deze studie hebben opgezet en draaiende gehouden. Het resultaat: alweer een proefschrift met Predict-data.

Sophie, Eline, Nicole, Marjolein, Eleonora en Batoul: toen ik begon bij de Predict waren jullie de promovendi. Bedankt voor alle tips en tricks rondom de dataset en de studieflow, maar zeker ook voor alle hoe-overleef-ik-een-PhD-gesprekken, de koffie- en theepauzes en de maandelijkse etentjes. **Rianne en Marcella,** ook al hadden jullie je eigen studies en projecten, ik vond het erg fijn dat jullie de Predict groep kwamen versterken! **Anne, Lotte en Nikolai,** inmiddels hebben jullie het stokje overgenomen. Veel succes bij jullie promotietraject!

Lieve **Wietske,** inmiddels dr. Bastiaansen. Toen ik net het onderwerp 'big data' had gekregen, spraken wij elkaar voor het eerst. Ik was meteen onder de indruk van hoe snel jij ingewikkelde modellen in begrijpelijke taal kon uitleggen. Ik heb met veel plezier samengewerkt aan de projecten over het embryonaal volume, en ik ben blij dat je na je PhD als postdoc in de groep bleef. Bedankt voor je motiverende aanwezigheid, vrolijkheid, kritische blik en authenticiteit. **Bruno**, ongeveer halverwege mijn PhD-tijd kwam jij als postdoc bij de groep en al snel begonnen de eerste gesprekken over een project over medicatiegebruik. Dat zou eerst nog in dit proefschrift komen, maar uiteindelijk zijn we samen Shané gaan begeleiden. Bedankt voor deze fijne samenwerking!

Anna, June, Rebecca en Philip, jullie onderzoek en werk als masterstudenten hebben direct of indirect bijgedragen aan dit proefschrift, waarvoor dank!

Marije, wat een gezellige aanvulling ben je voor het team. Zeker met alle regelzaken aan het einde van mijn promotietraject was je enorm behulpzaam, dankjewel! **Joke**, bedankt voor het includeren van patiënten en je vrolijke aanwezigheid. **Ann**, bedankt voor het databeheer en de hulp bij aanvragen vanuit HiX. **Lorette**, voor de gezond-zwanger-spreekuren en de altijd gezellige koffie-dates tussendoor.

Lieve **Tamara,** als kamergenootje was je al snel mijn partner in crime binnen de Predict, en na onze onvergetelijke trip naar de SRI in Vancouver werd dat ook buiten het werk zo. Dat je vandaag als paranimf naast me staat, voelt dan ook niet meer dan logisch. Zonder jou was mijn promotietijd een stuk minder gezellig geweest, en (dat klinkt misschien tegenstrijdig) óók minder productief, want ik durf toch wel te stellen dat wij het beste in elkaar naar boven halen. Jouw doorzettingsvermogen en kracht zijn voor mij een groot voorbeeld. Bedankt voor je luisterend oor, levenswijsheden, en natuurlijk het fluffen van de melk.

Ook wil ik de collega's van de 14e verdieping bedanken voor de maandagochtendmeetings en de gezelligheid op de kerst- en promotiefeesten. **Ans,** als mede-PE-onderzoeker vond ik het, hoewel we uiteindelijk minder direct hebben samengewerkt dan gedacht (wat nog niet is, kan nog komen), altijd fijn om met je te kunnen sparren.

Tot slot gaat mijn dank en liefde uit naar de mensen buiten de EE-toren en de kliniek, zonder wie dit allemaal een stuk minder de moeite waard zou zijn geweest.

Lieve **Anouk, Paulien en Vera – de Kostailtjes.** Volgens mij hield ik toen ik jullie leerde kennen nog altijd vol dat ik absoluut géén dokter wilde worden. Dat is inmiddels goed mislukt. Bedankt voor jullie jarenlange vriendschap, ik blijf met veel plezier jullie altijd-bereikbare gynaecoloog in spé. **Vera,** in het bijzonder bedankt dat je mijn paranimf wil zijn!

Lieve **Christa, Katie, Larissa, Monique en Sophie** – de **Party & Co**. Wat ben ik onwijs blij dat ik jullie als cogroepje (en Monique als appendix) heb getroffen. Inmiddels zijn we ruim 8 jaar verder, en de pluche placenta die ik van jullie kreeg bij mijn afstuderen is nog steeds toepasselijk. Dankzij jullie leerde ik ook de mannelijke Party&Co-ude kant kennen: **Jeroen, Martijn, Thomas en Thomas**. Bedankt voor jullie mentale steun en afleiding tijdens alle skivakanties, weekendjes weg, bruiloften en kerstdiners! Lieve **Sophie,** ik vind het een eer dat je jouw creatieve energie hebt gestoken in de cover van dit proefschrift, het resultaat mag er zijn!

Lieve leden van de boekenclub, bedankt voor alles wat tussen de regels door kon worden gelezen. Sommige dingen blijven hier beter onbesproken.

Lieve **Alessia, Edwin, Noor en Yoeri**, ook al heb ik jullie pas tegen het einde van mijn promotietraject leren kennen, wil ik jullie graag hier noemen. Jullie open blik op het leven laat mij steeds zien wat er allemaal nog meer mogelijk is, en samen met jullie lukt het altijd nóg beter om te genieten, van goede muziek, gekke uitjes of gewoon van het moment. Bedankt voor jullie vriendschap, ik hoop op nog vele jaren!

Dan mijn broers, lieve **Mats en Jens**. Ik krijg nog vaak te horen dat jullie vroeger altijd schooltje met mij moesten spelen, waarbij ik als schooljuf (net als mama) voor de klas stond en jullie de opgaven uit mijn oude schriften liet maken. Misschien maar goed dat ik toen nog niet wist in welk vakgebied ik zou eindigen, want gynaecoloogje spelen lijkt me toch wat minder gepast. Volgens mij heb ik dat imago van grote zus die altijd beter weet inmiddels wat losgelaten — dat hoop ik dan tenminste, of jullie laten me dat in ieder geval minder merken. Ik ben blij dat jullie mijn grote kleine broertjes zijn.

Lieve **Opa en Oma**, jullie hebben mij van jongs af aan aangemoedigd nieuwsgierig te zijn naar de wereld om me heen: naar de bloemen en vlinders tijdens wandelingen op de Nederlandse heide en in de bergen, maar ook naar dingen ver daarbuiten — de telescoop die ik ooit van jullie kreeg is daar een mooi voorbeeld van. Ook jullie reislustigheid heeft z'n sporen nagelaten: ik heb een rijtje reisgidsjes van jullie in de kast staan, en jullie

waren altijd het meest enthousiast om te horen wat ik had gezien op mijn congrestripjes. Dat jullie altijd zo oprecht geïnteresseerd zijn geweest, betekent veel voor mij.

Lieve ouders, lieve **mam en pap**. Jullie hebben mij altijd de ruimte gegeven om mijn eigen weg te kiezen, en me daarin onvoorwaardelijk gesteund — van *"wil je even mee-eten"* tot hele verbouwingen. Dat ik daardoor altijd de vrijheid heb gevoeld om te studeren en doen wat ik écht leuk vind, is misschien wel het mooiste dat jullie me hebben meegegeven. Toen ik vertelde dat ik ging promoveren wisten jullie misschien niet precies wat dat inhield, maar 'je ergens heel goed in verdiepen' vonden jullie wel meteen iets voor mij. Natuurlijk vond ik het ook gezellig om af en toe m'n vader tegen te komen op het werk. Bij mijn afstuderen konden jullie er helaas niet bij zijn vanwege de coronamaatregelen. Volgens mij maken we dat vandaag meer dan goed.

