Determinants of perinatal outcomes in Norway: 1982-2020

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Dissertation presented for the degree of Philosophical Doctor (Ph.D.)

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2022

Series of dissertations submitted to the
Faculty of Medicine, University of Oslo

ISBN 978-82-348-0213-3

Cover: UiO.
Print production: Graphic Center, University of Oslo.

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ACKNOWLEDGMENTS

This thesis is based on data from several projects, with linkages between different national registries, all based in projects at the Norwegian Institute of Public Health. The ART study is funded by the Research Council of Norway's Centre of Excellence funding scheme, project number 262700. The HbA1c project is embedded within the Norwegian Environmental Biobank within the Norwegian Mother, Father and Child Cohort Study (MoBa), which is supported by the Norwegian Ministries of Health and Care Services and Education and Research. I am grateful to the participants in MoBa for their contributions.

I am thankful to all my supervisors; Siri E. Håberg, Maria C. Magnus, Tone K. Omsland and Per M. Magnus. They have been supportive both scientifically and on a personal level, cheering me along during the whole process. In particular, I wish to thank main supervisor Siri and co-supervisor Maria for being available at all times and letting me develop my own ideas, while giving useful feedback and providing assistance with technical details whenever necessary.

A warm thanks to Allen Wilcox, who has co-authored all the papers in this thesis. He has proven an invaluable source of knowledge and has been available for extensive discussions during the whole period, filled with a never-ending curiosity regarding the study of perinatal outcomes and their determinants. I would also like to thank everyone else involved in the studies; Hans Ivar Hanevik, Lars Christian Stene, Iris Erlund, and Helle M. Meltzer.

Further, I would like to thank all my other colleagues and fellow Ph.D.-students at the Centre for Fertility and Health and the Norwegian Institute of Public Health. They have been helpful and have provided a stimulating learning environment that I could never have dreamt of before I started working at the Centre.

Finally, thanks to my extended family for support, proofreading and baby-sitting, and particularly my husband Matias for cheering me on whenever the nerves were threatening to take control. Thanks to my son Albert for his love and laughter, who have experienced a slightly more absentminded mother than usual during the finishing stages of writing. Also, thanks to my coming son whose due date set the deadline for the submittal of this thesis, and who hopefully has not experienced an adverse intrauterine environment due to my increased stress-levels this fall.

Bærum, December 2022

SUMMARY

Background: Perinatal outcomes are associated with immediate and later health outcomes in both mother and child. The determinants of perinatal outcomes differ between and within countries and have changed over time. A lot is known about these determinants, but the mechanisms of what determines perinatal outcomes such as birthweight and gestational age at birth, are far from completely understood. More knowledge on the determinants of perinatal outcomes in a contemporary setting is needed to understand the processes determining fetal growth and timing of delivery, and in general to improve perinatal health.

Aims: The overall aim of this thesis was to study contemporary determinants of perinatal outcomes in a developed country, namely Norway, in the period between 1982 and 2020, with particular focus on the outcomes gestational length and birthweight. I have used three approaches to explore potential determinants of perinatal outcomes. In paper I, the aim was to examine potential causes of the rise and fall in mean birthweight that occurred in Norway between 1991 and 2007. In papers II and III, more contemporary determinants on a broader range of perinatal outcomes were studied. For paper II, I examined whether maternal glycated haemoglobin levels (HbA1c) in week 18 were associated with perinatal outcomes. In paper III, I explored whether a person's own mode of conception impacted their later reproductive and perinatal outcomes as adults.

Methods: All three studies included in this thesis are observational cohort studies, with study populations in papers I and III selected from registered births in the Medical Birth Registry of Norway (MBRN). The study population in paper I consisted of all registered livebirths in Norway between 1982 and 2016, while the study population in paper III included men and women born in Norway between 1984 and 2002. The study population in paper II consisted of a subsample of pregnancies in the Norwegian Mother, Father and Child Cohort Study (MoBa), with available measurements of HbA1c levels during pregnancy. Information on the perinatal outcomes and possible determinants of these were collected from MBRN for all three papers. Additionally, questionnaire data and biological samples were used in paper II. All three studies used a complete-case analysis approach. In paper I, we used multivariate linear regression models as well as stratification by different covariates to try to disentangle factors potentially impacting the birthweight trend. We used multivariate linear, logistic and log-binomial regression models to assess the associations between mid-pregnancy HbA1c levels in the normal range and perinatal outcomes in paper II, and to assess whether these

outcomes were different in women and men conceived naturally or by assisted reproductive technologies (ART) in paper III. Additionally, we assessed the likelihood of having a registered pregnancy by the end of follow-up using a Cox proportional hazards regression model in paper III.

Results: Mean birthweights in Norway increased about 50 grams between 1991 and 1997, before a similar decrease from 2001 to 2007. The trend was only apparent among term births to Scandinavian-born women and was not accompanied by corresponding changes in neonatal mortality or other adverse outcomes. The trend was not explained by offspring sex, parity, onset of delivery, marital status, seasonality, maternal age, maternal year of birth, gestational length, or changes in occurrence of congenital anomalies.

We found a positive linear association between maternal HbA1c levels measured in gestational week 18 and fetal growth parameters standardized for gestational age. The association was strongest for birthweight and risk of delivering a large-for-gestational age infant, and weaker for head circumference and length. Additionally, increasing HbA1c levels within the upper normal range was associated with a shorter pregnancy duration, and increased risks of preeclampsia and preterm delivery.

There was a decreased likelihood of having a registered pregnancy between 1999 and 2020 among women and men conceived by ART compared to their non-ART-conceived peers. This association was attenuated after adjustments for their own mother's age at birth and country of birth and geographical location, suggesting that social selection mechanisms into pregnancy may play a role. Except for decreased odds of having a boy among women conceived by ART, no apparent difference in perinatal outcomes was seen between pregnancies to ART-conceived and naturally conceived parents.

Conclusions: Known predictors of birthweight could not explain the temporal trend among live term births to Scandinavian-born women in Norway with an increase in the 1990's followed by a decrease in the 2000's. The results suggest that there are still undiscovered predictors of birthweight that are not associated with adverse neonatal health. Maternal HbA1c levels in gestational week 18 were associated with fetal growth parameters, gestational length, and risk of preeclampsia, and suggests that HbA1c levels reflect glycaemic control also in pregnancy. Perinatal outcomes were similar in women and men who were themselves conceived by ART, although they had fewer pregnancies by the end of follow-up compared to their peers. Longer follow-up time to complete the reproductive periods are needed for more in-depth assessment.

SAMMENDRAG

Bakgrunn: Svangerskapsutfall er assosiert med umiddelbare helseutfall og helse senere i livet hos både mor og barn. Determinanter for disse utfallene varierer både innad og mellom land, og er til en viss grad gjenstand for forandring over tid. Vi vet en del om disse determinantene, men komplette mekanismer for en rekke av utfallene, slik som fødselsvekt og svangerskapslengde, er ukjent. Økt kunnskap om determinantene for svangerskapsutfall i en moderne setting er nødvendig for å forstå prosessene omkring fostervekst og svangerskapsvarighet og dermed kunne forbedre disse utfallene.

Mål: Det overordnete målet i denne avhandlingen var å studere determinanter for svangerskapsutfall, spesielt fødselsvekt og svangerskapslengde, i et utviklet land, nemlig Norge, i perioden 1982-2020. Jeg har brukt tre ulike fremgangsmåter for å studere ulike determinanter for en rekke svangerskapsutfall. I artikkel 1 var målet å forsøke å finne årsakene til oppgangen og nedgangen i fødselsvekt i Norge mellom 1991 og 2007. I artikkel II og III vår målet å studere mer moderne determinanter for en rekke svangerskapsutfall. I artikkel II undersøkte jeg om glykosylert hemoglobin (HbA1c) i uke 18 i svangerskapet var assosiert med disse svangerskapsutfallene. I artikkel III var målet å undersøke om menn og kvinner unnfanget med assistert befruktning (ART) har endrete svangerskaps- og reproduktive utfall når de blir voksne sammenlignet med de som er unnfanget «naturlig».

Metode: Alle studiene inkludert i denne avhandlingen er observasjonelle kohortstudier. Studiepopulasjonene i artikkel I og III er basert på registrerte fødsler i Medisinsk Fødselsregister (MFR). I artikkel I består studiepopulasjonen av alle levendefødte i Norge mellom 1982 og 2016, mens studiepopulasjonen i artikkel III inkluderte kvinner og menn født i Norge mellom 1984 og 2002. Studiepopulasjonen i artikkel II omfattet et utvalg svangerskap i den norske mor, far og barn-undersøkelsen (MoBa) med tilgjengelige HbA1c-målinger fra midt i svangerskapet. Informasjon om svangerskapene ble hentet fra MFR i alle studiene. Vi brukte tilgjengelig informasjon fra MFR om ulike determinanter, i tillegg til spørreskjemadata i artikkel II. Alle studiene brukte en komplett-kasus analyse. I artikkel I brukte vi en multivariabel lineær regresjonsmodell i tillegg til å stratifisere på ulike variabler for a undersøke ulike faktorer sin eventuelle påvirkning på fødselsvekttrenden. Vi brukte multivariable lineære, binomiale og logistiske regresjonsmodeller for å undersøke sammenhengen mellom HbA1c-nivået halvveis i svangerskapet og svangerskapsutfall i artikkel II, og til å undersøke om svangerskapsutfallene var ulike mellom personer unnfanget

med og uten hjelp av ART. I tillegg brukte vi overlevelsesanalyser for å undersøke om det var ulik sannsynlighet for å være registrert med et svangerskap i MFR i artikkel III.

Resultater: Gjennomsnittlig fødselsvekt i Norge økte med cirka 50 gram mellom 1991 og 1997, før den falt tilsvarende mellom 2001 og 2007. Denne trenden var kun tilstede hos nyfødte født til termin av mødre født i Skandinavia. Det var ikke tilsvarende trender i neonatal dødelighet eller andre uheldige svangerskapsutfall. Trenden kunne ikke forklares gitt kjønn, paritet, induksjoner/keisersnitt, sivil status, årstid, mors alder eller fødselsår, svangerskapsvarighet eller endringer i forekomsten av medfødte misdannelser.

Vi fant en positiv, lineær sammenheng mellom mors HbA1c-nivå og størrelsesmessige mål av den nyfødte standardisert for svangerskapsvarighet, kjønn og paritet. Assosiasjonen var sterkest for fødselsvekt og risikoen for å føde et barn som var stort for gestasjonsalder, og mindre sterk for hodeomkrets og lengde. I tillegg fant vi at økende nivå av HbA1c innenfor normalområdet var assosiert med kortere svangerskap og økt risiko for for tidlig fødsel og svangerskapsforgiftning.

Det var lavere sannsynlighet for å ha et registrert svangerskap mellom 1999 og 2020 blant dem som selv var unnfanget ved hjelp av ART. Denne assosiasjonen var svakere etter justering for morens alder og bostedsfylke da de selv ble født, samt morens fødeland, noe som kan bety at sosial seleksjon inn i svangerskap kan spille en rolle for sammenhengen. Det var lavere sannsynlighet for å få en gutt blant kvinner unnfanget med ART, men for øvrig var det ingen forskjeller i svangerskapsutfallene hos mødre og fedre basert på deres egen unnfangelsesmetode.

Konklusjoner: Kjente determinanter for fødselsvekt kunne ikke forklare den norske trenden med en økning sent i nittiårene etterfulgt av en nedgang på starten av totusentallet blant spedbarn født til termin blant kvinner født i Skandinavia. Dette kan bety at det fortsatt er uoppdagete determinanter for fødselsvekt som ikke er knyttet til økt sykelighet hos barnet.

Mors HbA1c-nivå i 19. svangerskapsuke var assosiert med størrelsen på den nyfødte, svangerskapsvarigheten og risikoen for svangerskapsforgiftning. Dette antyder at også HbA1c kan brukes som et mål på glukosekontroll i svangerskapet.

Svangerskapsutfallene vi studerte var like mellom kvinner og menn som var unnfanget med og uten bruk av assistert befruktning, selv om de som var unnfanget med ART hadde lavere sannsynlighet for å ha et registrert svangerskap under oppfølgingstiden. Dog er lengre oppfølgingstid nødvendig for en fullstendig vurdering av fertilitet og risiko for ulike svangerskapsutfall.

LIST OF PUBLICATIONS

Paper I

Stumped by the Hump: The Curious Rise and Fall of Norwegian Birthweights, 1991-2007.

Carlsen EØ, Magnus MC, Omsland TK, Magnus PM, Håberg SE, Wilcox AJ. Epidemiology. 2020 Jul;31(4):587-594. PMID: 32427635. DOI: 10.1097/EDE.0000000000001211

Paper II

Glycated haemoglobin (HbA1c) in mid-pregnancy and perinatal outcomes. Carlsen EØ, Harmon Q, Magnus MC, Meltzer HM, Erlund I, Stene LC, Håberg SE, Wilcox AJ. Int J Epidemiol. 2022 Jun 13;51(3):759-768. PMID: 34993542. DOI: 10.1093/ije/dyab270.

Paper III

Reproductive outcomes in women and men conceived by assisted reproductive technologies.

Carlsen EØ, Wilcox AJ, Magnus MC, Hanevik HI, Håberg SE.

Submitted, under review.

TERMS AND ABBREVATIONS

aOR: adjusted odds ratio

ART: assisted reproductive technologies

BMI: body-mass-index

CI: confidence interval

DAG: directed acyclic graph

DM: diabetes mellitus

GA: gestational age

GDM: gestational diabetes mellitus

GWG: gestational weight gain

HbA_{1c}: glycated haemoglobin

HDP: hypertensive disorder of pregnancy

HELLP: hemolysis, elevated liver enzymes and low platelets

HR: hazard ratio

ICSI: intracytoplasmic sperm injection

IVF: in-vitro fertilization

IQR: inter-quartile range

LGA: large-for-gestational-age

LMP: last menstrual period

MBRN: Medical Birth Registry of Norway

MoBa: Norwegian Mother, Father and Child Cohort Study

NEB: Norwegian Environmental Biobank

NICU: neonatal intensive care unit

NIPH: Norwegian Institute of Public Health

OGTT: oral glucose tolerance test

OR: odds ratio

RCT: randomized controlled trial

RR: relative risk

SES: socioeconomic status

SGA: small-for-gestational-age

US: ultrasonography

WHO: World Health Organization

1 INTRODUCTION

Perinatal outcomes are widely studied and an important component of newborn and maternal health. Measures such as birthweight and gestational length are available for nearly all pregnancies around the world, and are important predictors of perinatal mortality and morbidity, which are explicitly stated as goals for improvement by the World Health Organization (WHO) [1]. The predictors of perinatal outcomes are many; some with large individual effects, others with small; some are well-known, others not yet discovered. This thesis is about determinants of perinatal outcomes in Norway, a developed and high-resource country. Different study designs were exploited to be able to assess a broad range of perinatal outcomes and specific possible determinants of these.

1.1 Perinatal outcomes

Perinatal outcomes include medical conditions, measures and interventions experienced by the pregnant woman or the newborn around the time of delivery. There are a multitude of perinatal outcomes that are of both clinical and scientific value to study. Some are related to the "maternal side" of the pregnancy and complications she experiences before or during labour, while other are related to the "fetal side" with intrauterine complications or complications experienced in relation to delivery or postnatally. In this thesis, I have a special emphasis on gestational length (papers II-III) and birthweight (papers I-III). These two measures are important surrogates for the intrauterine environment and are both important predictors of adverse immediate and later health outcomes [2-4]. The studies also include a broader range of outcomes, such as preeclampsia and other hypertensive disorders of pregnancy (papers II-III), placental weight (papers II-III), length and head circumference of the newborn (paper II), low Apgar score in the newborn (papers I and III), congenital malformations (papers II and III), and neonatal mortality (paper I).

1.2 Gestational age

Gestational length is the estimated duration of a pregnancy. Before birth, it is used to estimate how much time is left of a pregnancy, and to evaluate whether the fetus would be viable in case of a preterm delivery. Postnatally, gestational age (GA) at birth is used to determine whether the infant was born preterm, term or postterm, and thus assess the risk of various postnatal complications. The duration of a gestation is measured in days since the last menstrual period (LMP), and thus starts approximately 14 days before conception in a menstrual cycle lasting 28 days. The pregnancy duration was 280 days according to Nägele's

rule, where one adds 9 months and 7 days to the day of the LMP. Exactly when conception occurred remains unknown for most pregnancies, so the exact duration of a pregnancy is usually an approximation. Today, the expected duration of a pregnancy is 283 days from the LMP [5,6], corresponding to 40 completed weeks of gestation plus 3 days. A pregnancy is divided into trimesters, and referring to these is useful when discussing pregnancy duration in broader terms. A common definition is that the first trimester starts at the LMP date and ends on week 13 and 6 days, or day 97, and the second trimester ends on week 27 plus 6 days, or day 195. The three trimesters mark different periods of fetal development, with major organ formation occurring mainly before gestational week 9 [7]. The second trimester continues the development and maturation of the organ systems, and fetal growth accelerates towards the end. The third trimester marks the growth spurt and maturing of fetal lungs and brain [8].

In developed countries, including Norway and the other Nordic countries, we no longer base the estimated due date on LMP alone, as this is prone to uncertainties in recall of the exact date, variations in time between LMP and conception, or differences in time between conception and implantation [9]. Rather, the use of ultrasonography (US) is the gold standard to obtain a more precise dating of gestational age, although this method of estimation is not without flaws either. In Norway, US is offered routinely to all pregnant women around week 18 [6], and a combination of the biparietal diameter, femur length and abdominal circumference of the fetus is used to estimate the age of the fetus at that time, and thus the estimated time left before the pregnancy reaches the expected date of delivery, here defined as 283 days. However, only about 5% of pregnancies end on the estimated due date [10]. Although a combination of several factors is thought to play a role in the timing of delivery, such as the size of the fetus, medical conditions, and hormones, it is still unknown exactly what triggers its onset [11].

When studying gestational age at birth, it is common to include live births and births of a dead fetus old enough to be viable outside the womb, namely stillbirths. In Norway, the cut-off is now usually set at 22 completed weeks or a birthweight of 500 grams or more [12].

The definition of term births are those occurring between 37 and 42 completed weeks of pregnancy [13]. A preterm birth is usually defined as a birth that occurs before the completion of 37 gestational weeks [13], further divided into “very preterm”, often defined as birth before 32 completed weeks, and “extremely preterm”, defined as a birth before 28 weeks. Postterm pregnancies are usually defined as those proceeding beyond 42 completed weeks [14]. The number of deliveries that start spontaneously has declined over the years in the Nordic

countries, and now more than 30% of Norwegian deliveries are induced medically or by caesarean section [15]. This is to a large extent due to medical interventions to prevent stillbirths and other complications associated with postterm births [16,17], and thus the number of pregnancies allowed to reach postterm has declined markedly in Norway [18]. An increasing proportion of pregnancies conceived after the use of assisted reproductive technologies (ART), increased maternal age and other medical conditions in pregnancy leading to preterm births or medical inductions before term, as well as a general increase in interventions, have contributed to a downward shift in the distributions of gestational length over time [19].

1.2.1 Importance of gestational age

Given the different stages of fetal development by gestational weeks, it is not surprising that GA at birth is associated with immediate health outcomes. Furthermore, the infant's health may be affected by the pathology triggering an early delivery, such as placental pathology. However, health in childhood and later in life is also associated with GA at birth.

For most immediate health outcomes in the neonate, including perinatal mortality (defined as a stillbirth or death of a liveborn infant within 7 days after birth) and neonatal mortality (defined as death of a liveborn infant within 28 days after birth), the risks of adverse outcomes are highest at the youngest gestational ages [2,20-22], decrease steadily with increasing GA, and then shows an increase again with postterm births [16,23,24]. The same is seen for later health outcomes, with an increased risk for adverse neurodevelopmental outcomes, hospitalizations, and mortality amongst others among those born at earlier GAs, and possibly an increased risk again with postterm births [2,22,25-28]. However, the complete picture of long-term health consequences of the gestational age at birth, and in particular for those born extremely preterm, is unclear, as an increasing number of very preterm babies with increased risk of sequala are surviving, while on the other hand improved care may reduce the risk of long term health consequences compared to earlier years [22].

1.3 Birthweight

Birthweight is a widely attainable perinatal outcome to examine, as newborns are routinely weighed at birth in most countries. It can be quite easily compared between countries and regions and over time, and is a heavily studied subject. It is the direct result of fetal growth prior to birth, and is measured more exactly than gestational length. The weight of a newborn will by definition be somehow dependent on its gestational age, as the duration of the pregnancy will set some limitations for how much a fetus has time to grow. However, some

babies are born heavier compared to the mean weight of the peers at the same gestational age, and some are born at a lower weight. There must therefore be some mechanisms affecting fetal growth *in utero* that does not directly depend on timing of birth or the GA of the growing fetus, or may act in opposite directions on birthweight and gestational length. Consequently, the study of birthweight by itself can be an important measure of intrauterine growth conditions not exclusively related to pregnancy duration.

As when studying GA, we include stillbirths and live births, but not miscarriages. "Low birthweight" has commonly been defined as a birthweight below 2500 grams, and "very low birthweight" as a birthweight below 1500 grams. Conversely, "high birthweight", or macrosomia, is commonly used for those born with a birthweight of either 4000 or 4500 grams or more.

1.3.1 Importance of birthweight

In general, lower birthweights than 2500 grams are associated with adverse infant outcomes [20], and among very preterm babies, birthweight is an important predictor of survival [21]. Perinatal and neonatal mortality and morbidity show an inverse J-shaped relationship with birthweight [29-31], and a similar pattern for the risk of adverse neurodevelopmental outcomes [32]. Furthermore, birthweight shows an inverse association with risk of adult mortality and morbidity by all causes and circulatory disease, but more of a positive association with cancer mortality [33]. The exact values of the mean birthweight in a population may change without these associations changing. Even if birthweight, or fetal growth *in utero*, is not a cause of immediate and later health outcomes, it is an important predictor of those outcomes. Identifying determinants of birthweight may help pinpoint weaknesses in antenatal care that could improve perinatal and later health outcomes in individuals otherwise exposed to these determinants.

1.4 Other perinatal outcomes

There is a multitude of other perinatal outcomes that are of both clinical and scientific interest. Some are associated with birthweight or gestational age, either by being affected through the same mechanisms, by causing a change in the other or increased risk of an adverse outcome, or simply by some underlying unknown common cause. Below, I introduce the other perinatal outcomes studied in this thesis.

1.4.1 Fetal anthropometrics and placental weight

It is common to measure newborn length and head circumference at the same time as weight. These are partly determined by the duration of the pregnancy. However, the proportion between weight and length can vary among infants and the ratio increases with increasing gestational length [34]. Furthermore, fetal head size relative to total length decreases from week 20 until birth [8], and preterm newborns may have a relatively larger head size for their weight or length than term newborns. Additionally, some abnormalities in fetal development may in rare cases lead to a pathologically large head, macrocephaly, or small head, microcephaly. The growth and development of the brain, and consequently the head, is the most important for the growing fetus to maintain even in a state of suboptimal growth conditions. This has led to the clinical division into "symmetrical growth restriction" where all anthropometric parameters are reduced, and are thought to be induced by an early insult, compared to an "asymmetrical growth restriction" where only the birthweight is restricted but head circumference and length are normal, and are thought to be induced later in pregnancy [35].

The growing placenta is the platform through which maternal nutrients and oxygen supply are provided to the fetus and acts as a buffer between the maternal and fetal blood circulation [7]. The placenta grows as the pregnancy progresses, and a dysfunctional placenta may be a limiting factor for both fetal growth and pregnancy duration [36]. Both the placental weight relative to the GA and the ratio between the birthweight and placental weight may be an indicator of the fetal growth conditions and a predictor of newborn health [37-39].

1.4.2 Preeclampsia and other hypertensive disorders of pregnancy

Another frequently studied pregnancy outcome is preeclampsia, a hypertensive disorder of pregnancy (HDP). It is a syndrome consisting of hypertension and either end-organ dysfunction or proteinuria, manifesting in the second half of pregnancy, and is associated with maternal and infant morbidity and mortality [40-42]. Offspring of preeclamptic pregnancies may have higher risk of all-cause mortality extending beyond childhood [40,43].

Hypertension discovered before pregnancy or during the first half of pregnancy is considered to be chronic hypertension, and these women are at increased risk of developing an HDP (then called "superimposed" preeclampsia) [44]. Hypertension manifesting only after 20 gestational weeks without any additional symptoms is considered to be gestational hypertension [44], and up to 25% of these women will develop preeclampsia by the end of pregnancy. Preeclampsia is diagnosed by a combination of criteria: hypertension (systolic

blood pressure of ≥140 mmHg and/or diastolic blood pressure of ≥90 mmHg) in a previously normotensive patient at 20 weeks of gestation or more, accompanied by either proteinuria or signs of end-organ dysfunction [44]. Pregnancies complicated by preeclampsia are routinely induced by gestational week 37 in Norway [44]. If complications arise before 37 completed weeks, birth may be induced earlier as this is the only certain treatment. Between weeks 34-36^{+6}, induction is practiced quite liberally [44]. Given these interventions, both gestational length and birthweight tend to be shorter/lower than in unaffected pregnancies. Earlier onset of preeclampsia during pregnancy is usually more severe with poorer outcomes [45], and increases the risk of recurrence in subsequent pregnancies [46,47]. However, the exact pathogenesis of preeclampsia and the other HDPs are not completely understood. Likely, both maternal and fetal genetic and immunological factors combine to induce an abnormal development of the placental vasculature in the early part of the pregnancy [48]. Later GA at onset of preeclampsia in pregnancy has been suggested to be more related to maternal lifestyle and metabolic regulation, though women with cardiometabolic risk factors are at increased risk of developing preeclampsia with both early and later onset [49].

A complication of preeclampsia is the progression to eclampsia, which is defined as a grand mal epileptic seizure in a patient with preeclampsia (including within 7 days postpartum), with no other explanatory factors that could account for the seizure [44]. HELLP (hemolysis, elevated liver enzymes and low platelets) is a severe syndrome that mostly occurs in patients with preeclampsia. In total, 5-10% of pregnancies in Norway are complicated by an HDP and they are more common among first-time mothers [15,50]. HDPs are a major cause of maternal deaths in both low- and high-income countries, accounting for 10-30% [51-54]. Due to increased risk of cardiovascular disease in women who have had one or more pregnancies complicated by an HDP [55-57], postpartum follow-up is recommended according to Norwegian guidelines [44].

1.4.3 Apgar score, neonatal mortality, and congenital malformations

Right after birth, the neonate is evaluated using an internationally recognized system to assess its condition, namely the Apgar score. This is scored at 1 and 5 minutes after birth, and each item; heart rate, respiratory effort, muscle tone, reflex irritability and skin colour, is scored with a value of 0, 1 or 2, and added together [58]. 90% of neonates have Apgar scores between 7 and 10, and this is an indicator that no further interventions are required, although the individual predictive power of infant morbidity is not accurate [59]. However, an Apgar score below 7 is at a population level associated with an increased risk of both neonatal and child mortality and morbidity and in general require further evaluation and possibly interventions

such as resuscitation [60-62]. Both low and high birthweight and shorter and postterm gestations are associated with lower Apgar scores in the neonate [61,62].

Neonatal mortality is often defined as death of a live-born infant within the first 28 days of life. Both perinatal and neonatal mortality risk show inverse J-shaped associations with GA and birthweight [63]. Due to low absolute numbers of perinatal and neonatal deaths in the Nordic countries, with decreasing rates over time [64], exposures that may lead to an increase in the risk of perinatal and neonatal death may be hard to identify. However, the rate of neonatal deaths is used to measure the level of neonatal and maternal care on a country-wise level.

An abnormal development of an organ system leads to a congenital malformation, which is any structural anomaly present at birth or in an earlier pregnancy loss. Among livebirths, around 4% present with a congenital malformation, while the prevalence is higher in miscarriages and stillbirths [15]. Congenital malformations present in liveborn neonates can be classified as minor or major. Minor malformations are mainly of cosmetic significance, while major malformations may have medical implications and require correction. Some neonates present with a pattern of anomalies that correspond to a certain syndrome. Congenital malformations are often seen with genetic syndromes, which are associated with increased perinatal mortality and morbidity, continuing into adult life [7,65,66]. The prevalence of congenital malformations has been relatively stable over time in Norway [15], but given that it represents an important failure in the development of the fetus, any risk factors should be identified to better guide women and men before and during pregnancy. Fetuses with a congenital malformation or syndrome are in general more likely to be born early and with lower birthweights [67,68], although some syndromes lead to postterm pregnancies or excess intrauterine growth [69].

1.5 Contemporary determinants of perinatal outcomes

The determinants, including risk factors, of perinatal outcomes are many and diverse, and differ by setting. My aim was to increase knowledge on determinants for perinatal outcomes using contemporary data from Norway, which is an example of a developed country with one of the best antenatal care settings and lowest infant and maternal mortality levels in the world [70,71]. I will therefore discuss known determinants of perinatal outcomes mainly in a contemporary setting in developed countries. I have chosen to include findings on associations from Nordic countries, and prioritized these over other or conflicting associations from less comparable countries (from a Norwegian standpoint). Gestational age and

birthweight will be given main attention, but the other perinatal outcomes included in this thesis will also be touched upon.

I have chosen to divide the modern predictors of perinatal outcomes into categories based on mechanism of biological or indirect impact, namely infant factors, maternal factors, paternal factors, and external/environmental factors, with the use of assisted reproductive technologies (ART) as a separate category, although some of these may overlap. In the following subchapters each of the categories will be discussed in detail with regards to the various perinatal outcomes included in this thesis.

1.5.1 Infant influence on perinatal outcomes

A non-systematic summary of infant factors and their associations with perinatal outcomes is provided in Table 1.

Transgenerational recurrence of perinatal outcomes may to a large extent be explained by genetic factors inherited from the parents to the fetus, although shared environmental factors, including intrauterine conditions, lifestyle and behaviours may play an important role. Interestingly, increased birthweight in the father have been associated with shorter gestational age in the offspring, suggesting that fetal genetic factors inherited from the father affecting growth velocity *in utero* may trigger delivery [11]. As we did not have access to genetic data in our studies, inherited genetic components will only be discussed to a small extent, but are important to keep in mind when interpreting the results.

Table 1. Infant factors as predictors of perinatal outcomes.

Infant factors	Impact on perinatal outcomes
Sex	Boys are bigger, have shorter gestations with US and longer with LMP [72]. Boys are at increased risk of perinatal death and conditions requiring resuscitation [20,21,73].
Multiple gestation (twins, triplets)	Increased risk of adverse outcomes, including perinatal death [20,21], shorter gestation [74], lower birthweights, and increased risk of HDP [75,76].
Genotype	Strong correlation between maternal birthweight and offspring birthweight [77,78]. May contribute to the risk of preeclampsia [79].
Medical conditions	Congenital malformations or impaired growth may lead to either spontaneous earlier or later delivery [80], or to iatrogenic causes of altered timing and mode of delivery [81].

1.5.2 Maternal influence on perinatal outcomes

A non-systematic summary of maternal factors and their associations with perinatal outcomes are provided in Table 2. Publication bias of positive findings may influence the presented summaries below, and as most are based on observational studies, any causal interpretation of

the associations should be done with caution. Genetic factors are not listed as a separate category, as genetics may be part of all the associations observed and may act through multiple mechanisms. For instance, maternal genetics related to her own body-mass-index (BMI) may both directly affect fetal size through inherited genes, as well as through her own lifestyle choices and/or intrauterine conditions associated with BMI. I have not noted the direction of effect between ethnicity or migrant status on the various pregnancy outcomes, but in general ethnicity/country of origin other than the host-country has been associated with an increased risk of adverse pregnancy outcomes [82], with some exceptions indicating a "healthy migrant effect". Admittance to a neonatal intensive care unit (NICU) is more likely in newborns with lower Apgar score, and as these two outcomes are associated, I have chosen to include NICU admission in the summary tables. As both admittance to the NICU and the Apgar score are surrogates for the newborn health condition, I have not assessed the transgenerational recurrence risk of these outcomes, but would expect that a recurrence risk of low birthweight, preterm birth and HDPs would be reflected in an increased risk of NICU admittance or a low Apgar score in the second generation.

Table 2. Maternal factors as determinants of perinatal outcomes.

	PERINATAL OUTCOME				
Maternal factor	**Gestational age at birth**	**Birthweight**	**Preeclampsia**	**Low Apgar score or NICU admission**[1]	**Congenital malformations**
Maternal age	Negative association [83,84]	Negative association [83]	Positive association [45,75,76,83]	Positive association [83]	J-shaped or positive association [85,86]
Primiparity	Negative association [87,88]	Negative association [88,89]	Increased risk [75,76,90]	Increased risk neonatal mortality [91]	Increased risk for some organ systems [92]
Inter pregnancy interval	Inverse U-shaped or negative association [93-95]	Inverse U-shaped or negative association [93-95]	Positive association [90,94]	U-shaped association [95]	U-shaped association [96]
Female-factor infertility	Negative (adenomyosis, endometriosis, unspecified) or no association (PCOS) [97-100]	Negative (adenomyosis, unspecified) or uncertain association (PCOS, endometriosis) [97-100]	Increased risk (PCOS, endometriosis, adenomyosis, unspecified) [97-101]	Increased risk (NICU, PCOS) [97]	Increased (unspecified) or no association (PCOS, endometriosis) [97,99,102]
Ethnicity / migrant	Associated [82,103-107]	Associated [104,105,107]	Associated [45,108,109]	Associated [104,107]	Little or no association [107]

	PERINATAL OUTCOME				
Maternal factor	**Gestational age at birth**	**Birthweight**	**Preeclampsia**	**Low Apgar score or NICU admission**[1]	**Congenital malformations**
Diabetes mellitus[2]	Negative association [110-112]	Associated [111,112]	Increased risk [75,76,112]	Increased risk [111,112]	Increased risk [112,113]
Other chronic medical conditions[3]	Negative (asthma, coeliac disease) [114-116] positive (other atopic) [115] association	Negative association (asthma, coeliac disease) [114,116]	Increased risk (antiphospholipid syndrome, hypertension, asthma) [45,75,76,114]	Increased risk (NICU and asthma) [117]	Increased risk (asthma) [117]
Height	Positive association [78,87,118]	Positive association [87,118,119]	Negative or no association [49,119-121]	Negative association neonatal mortality low/middle-income countries [122]	Negative or no association [123,124]
BMI	Negative or inverse J-shaped association [125,126]	Positive association [125,127-129]	Positive association [49,75,76,120,125,128]	Little or no association [128]	J-shaped or positive association [130,131]
Own perinatal outcomes[4]	Positive association [11,26,77,132]	Positive association [77,133]	Increased risk [134]	Not assessed	Increased risk [135,136]
Educational level	Positive association [107,137-139]	Positive association [107,137,139]	Negative association [49,109]	Negative association [107,140]	Negative or no association [107,139]
Smoking	Negative association [103,125]	Negative association [125]	Decreased risk [49,125]	Increased risk (NICU) [141]	Increased risk [142-144]
Physical activity (PA), diet & supplements (DS)	Little/no association PA [145-147], associated DS [148]	Little or no association PA, [145-147], associated DS [149]	Associated PA [150-153] and DS [148,154]	Little or no association PA [145,147]	No association PA [155], associated DS [156,157]
Marital status	Associated [26,103,158,159]	Associated [158,159]	Associated [45,160,161]	Associated [162]	Little or no association [163]

PCOS: polycystic ovary syndrome; PA: physical activity; DS: diet and supplements

[1] I have here added admission to NICU together with risk of low Apgar score, as these two outcomes are related, and many studies have not had available information on Apgar scores. Where I have not found large studies on either of these outcomes, I have included studies on perinatal or neonatal mortality.

[2] Diabetes mellitus includes pre-existing DM as well as GDM.

[3] Other chronic medical conditions include maternal asthma, maternal phospholipid syndrome, maternal hypertension.

[4] Own perinatal outcomes mainly referring to the same outcome in her own pregnancy unless otherwise specified.

1.5.3 Paternal influence on perinatal outcomes

A non-systematic summary of paternal factors and their associations with perinatal outcomes is provided in Table 3. Like in Table 2, genetic factors are not listed as a separate category, as genetics may be part of all of the associations observed, and may act through multiple mechanisms. Of note, paternal gestational age shows a linear association with offspring GA, but high paternal birthweight has been associated with shorter GA in the offspring, suggesting that fetal growth may trigger delivery [11]. Although not included in this table, it is noteworthy that older paternal age is associated with an increased risk of stillbirth [164], and paternal smoking with increased risk of congenital malformations for some organ systems [124,144,164]. Paternal factors may influence perinatal outcomes either directly through biological mechanisms via the fetus, or via factors related to the health behaviour of both parents.

Table 3. Paternal factors as determinants of perinatal outcomes.

	PERINATAL OUTCOME				
Paternal factor	**Gestational age at birth**	**Birthweight**	**Preeclampsia**	**Low Apgar score or NICU admission**[1]	**Congenital malformations**
Paternal age	Negative/inverse J-shaped or no association [84,164,165]	J-shaped or no association [164,165]	J-shaped or no association [165]	J-shaped or no association [165,166]	J-shaped or positive for some organ systems [164,167]
Male-factor infertility	No association [168,169]	No or negative association [168-170]	No association [168]	No association ICSI and perinatal mortality [171]	No association [168,169]
Ethnicity / migrant	Probably associated [82,104,172,173]	Associated [104,172,173]	Associated [173]	Probably associated [104,172,173]	Associated with some organ systems [124]
BMI and height	No association [118,174]	Small non-linear association BMI [164,174,175], positive height [118,164,175]	J-shaped association BMI, not with height [121,174]	No association BMI and live births rates in ART pregnancies [176]	No association with height for some organ systems [124]
Own perinatal outcomes[2]	Positive association [11,132]	Positive association [133]	Increased risk [134]	Not assessed	Increased risk [136,177]
Educational level (EL) and household income (HI)	Positive association EL [103,137,138]	Positive association EL [137]	Decreased risk HI [178,179]	Negative association perinatal mortality HI, neonatal mortality EL [180,181]	Negative association some organ systems EL [124]

EL: educational level; HI: household income; ICSI: intracytoplasmic sperm injection

[1] I have here added admission to NICU together with risk of low Apgar score, as these two outcomes are related, and many studies have not had available information on Apgar scores. Where I have not found large studies on either of these outcomes, I have included studies on perinatal or neonatal mortality.

[2] Own perinatal outcomes mainly referring to the same outcome in the partner's pregnancy unless otherwise specified.

1.5.4 External and environmental influence on perinatal outcomes

As the main focus of the determinants of perinatal outcomes in this thesis is not related to external or environmental factors aside from the use of assisted reproductive technologies, which is discussed in the next subchapter, I will only briefly discuss some major groups of exposures and their impact on perinatal outcomes.

Maternal infections during pregnancy may be relatively harmless, or lead to increased risk of both maternal and fetal morbidity and mortality. Some infections early in pregnancy may lead to an increased risk of congenital malformations or fetal loss, such as with Zika virus [182,183], cytomegalovirus [184] and rubella [185]. Due to an altered immunological state and increased cardiovascular load, pregnant women face an increased risk of hospitalizations and mortality with infections such as influenza [186] and malaria [187], which may also increase the risk of adverse perinatal outcomes such as stillbirth [188]. Less severe maternal infections, such as bacterial vaginosis and genital chlamydia, may lead to an increased risk of preterm delivery [189,190].

Exposure to some environmental toxicants during pregnancy has been linked to adverse perinatal outcomes including restriction of fetal growth [191], but these possible associations are still uncertain [192] and need to be explored further. Intake of certain medications during pregnancy has been associated with increased risk of adverse outcomes in the newborn [193-195], but the type of adverse outcome and magnitude of increased risk differ by type of medication.

The degree of medical interventions during labour and delivery varies by country and by delivery institutions [196,197]. The rate of medical interventions may impact on perinatal outcomes, and even within Norway the risk of delivery complications and perinatal mortality differ among delivery institutions [198].

1.5.5 Assisted reproductive technologies (ART)

1.5.5.1 What is ART

ART is here defined according to the International Committee for Monitoring Assisted Reproductive Technologies (ICMART) guidelines as "*All interventions that include the in vitro handling of both human oocytes and sperm or of embryos for the purpose of reproduction*" [199]. This includes for instance in vitro fertilization (IVF) with and without intracytoplasmic sperm injection (ICSI), but not intrauterine inseminations. This means that any pregnancies conceived by ART will have had an egg retrieval performed in the woman, a

semen sample from a male partner or a sperm donor, and conception and at least some developmental steps for the early embryo outside the uterus. The use of oocyte donation was not allowed in Norway until January 2021, which is after the study periods of the projects in this thesis, and will therefore not be discussed here. The use of ART to achieve pregnancy is increasing worldwide as it is in Norway, mostly due to a delay in childbearing, but possibly also increasing levels of infertility [200]. The first birth in Norway from a pregnancy conceived by ART was in 1984, and most pregnancies in the first 15-20 years were accomplished using the transfer of fresh embryos in the same menstrual cycle as the oocyte retrieval. Historically, ART pregnancies were often multiple gestations due to a practice of the transfer of more than one embryo for insertion, and consequently an increased risk of implantation of two or more embryos. This practice has changed to a general recommendation of insertion of only one embryo due to similar live birth rates and a decreased risk of adverse pregnancy outcomes [201].

The option of cryopreserving an embryo became an increasingly popular technique after the production of more than one blastocyst after a cycle of hormonal stimulation and oocyte retrieval was successful. In 2021, about 46% of ART livebirths in Norway were conceived by the transfer of fresh, and 40% with the transfer of frozen, embryos, with the rest of unknown method (my own estimates from MBRN).

ICSI includes direct injection of a sperm into the oocyte and is thus a more invasive procedure than IVF without ICSI. This method became more common in Norway around year 2000, and in 2021 about 35% of ART births are a result of IVF with ICSI (my own estimates from MBRN). This may be combined with either fresh or frozen transfer. The success rates with ART differ according to type of procedure used and time of follow-up, but the general rates are between 20-50% after a couple of years [202,203], with live birth rates from one fresh embryo transfer around 35% [204].

1.5.5.2 ART as a determinant of perinatal outcomes

ART-conceived pregnancies are at increased risk of a number of adverse outcomes. However, whether these are related to the underlying cause of use of ART or the actual procedures imposed on the mother and embryo, is to some extent unknown [205], though studies indicate that the procedures play at least some part in the increased risks [206]. Transfer of multiple embryos explained some of the increased risks in ART-conceived pregnancies, but not all [206-208]. In general, ART-conceived pregnancies face increased risks of preterm birth, perinatal and neonatal mortality, giving birth to a small-for-gestational-age (SGA) or low birthweight

newborn, hypertensive disorders of pregnancy (HDP) and placental abnormalities, gestational diabetes mellitus (GDM) and medical interventions [206-210]. However, these risks are distributed differently by ART method used [206,211,212]. Perinatal outcomes associated with the major categories of fresh embryo transfer, frozen embryo transfer and IVF with ICSI, are listed in Table 4. Overall, the perinatal outcomes of pregnancies conceived by ART have improved over the years, and frozen embryo transfer and possibly ICSI provide the outcomes most closely resembling naturally conceived pregnancies [206,213].

Table 4. Overview of associations between different ART methods and perinatal outcomes in pregnancies with single embryo transfer.

ART method, single embryo transfer	Impact on perinatal outcomes
Fresh embryo transfer	Increased risk of preterm birth, SGA and low birthweight, and HDPs compared to non-ART [206,209].
Frozen embryo transfer	Increased risk of LGA, high birthweight and HDPs, and lower risk of preterm birth, compared to fresh embryo transfer [209,211,212].
IVF with ICSI	Increased risk of birth defects.[210] Maybe lower risk of preterm birth when compared to conventional IVF [206].

1.6 Existing knowledge of determinants of the outcomes studied in this thesis

For comparison of my studies to existing literature, I have mainly examined studies with determinants of perinatal outcomes from countries that are somewhat similar to the Norwegian population, including studies from (northern and western) European countries, as well as the United States, Canada, Australia and New Zealand. I will discuss each of the topics covered in this thesis separately below, providing a non-structuralized review of existing literature, in English or Scandinavian languages, on the topic of each paper at that point in time.

1.6.1 Time trends in birthweight

Studying trends in birth outcomes over time and possibly disentangling underlying driving factors may further help understand the aetiology of those birth outcomes. Changes in birthweights in whole populations over time is such an example, where one may study mean birthweights, or some aspects of the distribution, such as the risk of having “low birthweight”, “high birthweight”, or the proportion of SGA and large-for-gestational-age (LGA) newborns

compared to a specific reference population. One challenge in studying these time trends, is that simultaneous processes occurring over the same period may work in different directions, thus diluting or even completely masking potential shifts in fetal growth patterns.

In general, as nutritional status and energy intake increased in the latter half of the 20th century, many Western countries saw an increase in mean birthweights, including Norway, summarized in Table 5. However, some countries experienced opposite trends, or the increase in mean birthweights was accompanied by an increase in low birthweight newborns. Many possible explanations for this increase in birthweights were suggested, and some studies found indications of maternal BMI, smoking behaviour and induction practices to play an important role in some countries [129,214], while the increase was not fully explained by these and other factors in other countries [215,216]. Even more puzzling was the emergence of a different pattern in some countries, such as Norway and Sweden, where birthweights began to decrease again in the early 2000's after a period of increase (Table 5).

The increase and then decrease in birthweights seen in Norway was suggested to be induced by a change in the intake of sugar levels, specifically in the form of sugar-sweetened beverages [217]. However, a study in the Norwegian Mother, Father and Child Cohort Study (MoBa) found an inverse association between sugar-sweetened beverage intake and birthweight in women without diabetes [218], thus suggesting that the hump pattern was not explained by intake of sugar-sweetened beverages.

Table 5. Summary of birthweight trends in Western countries between 1980 and 2019 with suggested explanatory factors.

Study	Country and inclusion criteria, No. Newborns	Time period	Birthweight trend	Suggested explanatory factors
Ananth CV, et al. 2002. [219]	USA, GA 20-44, (N=48,637,680) and Canada (not Ontario/ Newfoundland) (N=3,167,702), live singletons	1985-1998	Increase in birthweight among term US births and all Canadian births. Decline in birthweight among preterm US births.	No formal analyses. Increased rate of SGA births particularly among blacks during the period.
Ananth CV, et al. 2003. [220]	USA (except 7 states), live singletons, GA 28-41, no malformations. No N given.	1989-1998	Term SGA births declined by 8.2% between 1989 and 1998 among whites and by 10.3% among blacks. Increase in % SGA among preterms.	Adjustment for maternal age, parity, education, marital status, prenatal care, prior preterm/SGA birth, hypertension, placental abruption and previa, bleeding, DM and smoking/alcohol use, removed most of the decline in term SGA births. Largest effect for smoking and alcohol.
Ananth CV, et al. 2004. [221]	USA, live singletons 37-44 GA. N=16,869,705	1975+'80+'85+'90+'95+2000	Term SGA births declined by 23% among black women, and 27% among white women over the whole period.	U-shaped relationship between maternal age and SGA, stronger than the period effect. Decrease in SGA by later maternal birth cohorts.
Arbuckle TE, et al. 1989. [222]	Canada (not Newfoundland), live births, GA 25-42 between 500-5000 gr. N=682,817	1972 and 1986	Increase in mean birthweight for singletons of 4.1% and 5.7% for twins.	No formal analyses. Decrease in % singleton preterm births, and a decrease in % singletons <2500 gr. Increase in preterm twin births and decrease in % twins <2500 gr.
Bell R. 2008. [223]	England, Northern part, live births and stillbirths ≥500 gr and GA ≥28. N=682,895 live births and n=3386 stillbirths.	1982-2000	The proportion of births with birthweight <2500 grams (10%) and ≥4000 grams (25%) increased. Not given trend in mean birthweight.	No formal analyses. Increase in proportion of macrosomic babies only among singletons. Increase in low birthweight only among live births, and more for multiples than singletons.
Bergmann RL, et al. 2003. [224]	Germany, Berlin, singletons, GA ≥37. N=185,322	1993-1999	Proportion with birthweight ≥4000 gr increased from 10.0-11.3%, no change in mean birthweight among all births, not shown among term.	After adjustment for maternal BMI, height, age, nationality, postterm births, GWG, DM, smoking, parity, "pregnancy risk factors", sex, the trend in proportion of high birthweights disappeared. Increasing proportion of women ≥165 cm, BMI≥26, GWG≥16 kg, age ≥30 years.
Blondel B, et al. 2002. [225]	Canada (not Newfoundland/Ontario), England, Wales, France, USA, live births. N=25,878,028	1981-'83 + '95-'97, FR '81+'95+'98	The % of singletons with birthweight <2500 increased slightly in all countries except Canada, and among twins for all countries.	Rates of twins and multiples increased in all countries during the period. Increase in multiple births explained why there was a lack of decrease in low birthweights over the study period.
Bonellie S, et al. 1997. [226]	Scotland, live singletons. N=765,592.	1980-1992	56 gr increase in mean birthweight.	Adjustment for maternal height, age and parity explained slightly less than 50% of the increase.
Bonellie S, et al. 2008. [227]	Scotland, singletons, no lethal malformations, >250 gr. N unknown.	1980-2003	Increase in mean birthweight of about 60 gr, mostly 1980-1995.	No formal analyses.

Study	Country and inclusion criteria, No. Newborns	Time period	Birthweight trend	Suggested explanatory factors
Boulet SL, et al. 2005. [228]	USA, live singletons, GA 37-44 >3000 grams. N=4,598,784.	1989-2000	Decrease in median birthweight and % >4000 grams between '89/'92 and '01/'02.	No formal analyses. Delivery by c-section among births ≥4000 grams increased during the time period.
Branum AM, et al. 2002. [229]	USA, live births. N=8,788,806 newborns to white women and n=1,714,401 newborns to black women.	1981+'90+'98	Increased % of births <2500 gr among white women'90-'98. For singletons the increase was seen <1500 gr, while for multiples both <1500 and 1500-2499 gr increased.	Increased % preterm births among whites. For blacks, the % preterm births among singletons decreased and preterm multiples increased. The % of multiples increased for all. The increase in multiple births explained some of the trend, but not all. Maternal age and parity did not explain trends of low birthweight or preterm births.
Brynhildsen J, et al. 2009. [230]	Sweden, 2 delivery wards in Southeast part. N=4330.	1978+'86+'92+'97+2001	Increase in mean birthweight '78-'97, then slight decrease until 2001.	The increase from '92 disappeared after adjustment for maternal BMI, age, parity, employment, study year, smoking and GA. A decrease in birthweight '92-'01 was seen among women with BMI<25, while it continued to increase among those with BMI≥25.
Daltveit AK, et al. 1999. [231]	Norway, all births GA ≥ 16. N=1,700,000	1967-1995	Mean birthweight increased 43 gr '67-'81, then declined 25 gr by '90, increased by 44 gr by '95. The % <2500 gr declined '67-'79, then increased until '95.	Not explained by maternal age and parity. The % multiple births increased '86-'95. The % births <2500 gr among term births decreased. The increase in birthweight was greatest among primipara <20 years. An increase in multiple births and those <1000 gr explained the increase in <2500 gr after '87.
Diouf I, et al. 2011.[232]	France, National Perinatal Surveys, live singletons, GA ≥37. N=49,321.	1972+'81+'95+'98+2003.	Mean birthweight increased '72-'81 before declining to '72-levels in '95. Decrease in % SGA and % LGA.	After adjustment for GA, maternal age, parity, country of origin, sex, induction and maternal smoking, birthweight increased '72-'95 (48 gr), decreased until '03 (-51 gr), similar trends in SGA and LGA.
Donahue SM, et al. 2010. [233]	USA (not California), singletons GA 37-41, women ≥18. N=36,827,828.	1990-2005	Decreased mean birthweight of -52 gr, decreased % LGA. Majority of decline '00-'05.	Not explained by GA, maternal age, SES, smoking, DM, hypertension, GWG, maternal BMI and height, mode of delivery, prenatal care and US, parity, sex, previous preterm birth or high birthweight.
Fairley L 2005. [234]	Scotland, live singletons N=1,282,172.	1980-2000	Increase in mean birthweight of 88 gr, decreased % with birthweight <2500 gr.	Not explained by parity, age, height, SES.
Ghosh RE, et al. 2017. [235]	England and Wales, singleton live births, 500-5999 gr. N=17,254,624.	1986-2012	Increase in mean birthweight of 59 gr. Decreased % <2500 gr and increased % ≥4000 gr.	Not explained by maternal age, marital status, area-level deprivation, area-level ethnicity and individual ethnicity. The increase in birthweight was largest among preterm births.
Glinianaia, SV, et al. 2008. [236]	England, Newcastle upon Tyne, PAMPER database, singleton births, N=97,809.	1961-1992	Increase in mean birthweight of ~70 gr. Mainly among term births.	No formal analyses. Maternal age decreased until '73 and then increased. Decrease in GA. Increase in c-sections.
Grundt JH, et al. 2012. [217]	Norway, Oppland county, live singletons, no malformations, GA 37-42. N=33,088.	1989-2010	Increase in birthweight '90-'01 of 53 grams, then down 50 grams by '09. Same trend % births >4500 gr.	Half of the increase (not decrease) explained by maternal age, GA, sex, parity, smoking, preeclampsia, DM, thromboembolism, prior preterm birth or c-section, anaemia, oligo- and polyhydramnios.

Study	Country and inclusion criteria, No. Newborns	Time period	Birthweight trend	Suggested explanatory factors
Joseph KS, et al. 1999. [237]	Canada (not Newfoundland/Ontario), all births. N=1,393,173.	1985-‘87 + ‘93-‘95	Proportion (live and stillbirths) with birthweight <2500 gr increased slightly.	No formal analyses. Main change among those <500 gr.
Kinnunen TI, et al. 2003. [238]	Finland, cross-sectional population surveys, Helsinki and Tampere, singletons, GA 22-44. N=4404.	1954-‘63 + ‘85-‘86 + 2000-‘01	GA-adjusted birthweight increased 174 gr ’63-’85 and 71 gr ’85-‘00. % birthweight >4000 gr increased, and % birthweight <2500 gr decreased.	No formal analyses. Increase in maternal age, height, BMI and GWG. Among all cohorts, higher GWG was associated with increased birthweight and % births >4000 gr. No difference in % <2500 gr by GWG in any of the cohorts.
Kramer MS, et al. 2002. [239]	Canada, 1 hospital, live singletons, no malformations, GA 22-43. N=61,437.	1978-1996	Term birthweight increased 57 grams. Decrease in % SGA and increased % LGA among term births.	Explained by postterm births, US dating, maternal height and BMI, GWG, GDM, smoking, age, parity, SES, pre-pregnancy hypertension, and pregnancy-induced hypertension.
Lahmann PH, et al. 2009. [215]	Australia, Queensland, live singletons ≥400 gr, GA ≥20. N=830,231.	1988-2005	Birthweight increased 32 gr, increase % ≥4000 gr. Increase % <2500 gr.	Not explained by sex, GA (decreased), maternal age and ethnicity. Increase only among non-indigenous women, greatest in term births.
Maher J, et al. 2004. [240]	England and Wales, live singletons, birthweight 500-6000 gr. N=1,203,375.	1983+2000	Proportion with birthweight ≥4000 gr increased, while % <2500 gr increased slightly.	No formal analyses. The increase in heavier newborns was more apparent among women ≥30. The % of low birthweight newborns born to women aged ≥35 increased.
Millar WJ, et al. 1991. [241]	Canada (not Newfoundland), live singletons. N=6,600,000.	1971-1989	Decrease in % with birthweight <2500 gr.	No formal analyses. Increase in % preterm births among low birthweights. Stable % <1500 gr. Increase in % term births.
Odlind V, et al. 2003. [242]	Sweden, all deliveries, no N reported.	1973-2000	Increase in mean birthweight and % with birthweight >4500 gr, decrease in % <2500 gr.	No formal analyses. Decrease in maternal smoking, increased maternal age. Among live singletons, % with GA<32 increased.
Oja H, et al. 1991. [243]	Finland, Northern part, live births, GA 25-45. N=20,632.	1966 + ’85-‘86	Increase in mean birthweight.	After conduction of nonparametric regression function models to the GA distributions, the GA-adjusted birthweights were similar between the two periods.
Orskou J, et al. 2001. [244]	Denmark, 1 hospital, singletons. N=43,561.	1990-1999	Birthweight increased 45 gr, more in term births. Increased % ≥4000 gr.	Not explained by GA (decreased) and inductions (increased).
Orskou J, et al. 2003. [245]	Denmark, 1 hospital, non-DM. N=24,093.	1990-1999	Increase in % with birthweight >4000 gr.	Explained by weight, height, age, parity, smoking, alcohol and caffeine intake, cohabitation, education and GA.
Power C 1994. [246]	Scotland (n=1,172,205), England and Wales (n=4,646,200), live births.	1975-‘92 S, ‘83-‘89 E&W.	Increase % birthweight ≥4000 in Scotland. Decrease % <2500 gr in Scotland and E&W.	No formal analyses.

Study	Country and inclusion criteria, No. Newborns	Time period	Birthweight trend	Suggested explanatory factors
Schack-Nielsen L, et al. 2006. [216]	Denmark, live singletons, birthweight ≥500 gr and GA ≥20. N=1,863,456.	1973-2003	Increase in birthweight of 160 gr. Increase in % >4000 gr.	Maternal age (increased), GA (decreased) and maternal smoking (decreased) between '91-'03 explained ~20% of the increase. Increased ponderal index of 0.8kg/m^3.
Schiessl B, et al. 2009. [247]	Germany, Bavaria, singletons, GA ≥37. N=695,707.	2000-2007	Mean birthweight decreased by 19 gr. % ≥4000 gr decreased.	Increase in GWG and GDM, smoking decreased. Stratified analysis on GWG and parity did not explain the trend.
Skjaerven R, et al. 2000. [248]	Norway, singletons, GA 16-44. N>1,800,000.	1967-1998	Term GA-adjusted birthweight increased (~100 grams for week 40) and decreased among preterm births.	Among preterm births, the increase in c-sections between '87 and '98 explained most of the decrease. No analyses on term births.
Spencer, NJ, et al. 1999. [249]	England, Sheffield Child Development Study, live singletons. N=58,547.	1985-1994	Increase in birthweight of 34 gr. % with birthweight <2500 gr was stable.	No formal analyses. Social inequality partly predictive of birthweight, but not for trend.
Surkan PJ, et al. 2004. [129]	Sweden, live singletons without malformations, GA ≥37. N=874,163	1992-2001	Increase in mean birthweight of 35 gr, and % of LGA births and births ≥4500 gr.	Trend of LGA explained by maternal BMI, smoking, parity, age, cohabiting status, country of birth, height, preeclampsia, GDM and GA. Maternal smoking and BMI contributed most.
Wen SW, et al. 2003. [214]	Canada, 1 hospital, live singletons, no malformations, GA 22-43. N=61,437.	1978-1996	Mean birthweight increased by 55 grams during the period, % SGA down and LGA up.	Trends explained by postterm births, dating by US, maternal height, BMI, GWG, GDM, smoking, age, parity, education, marital status, hypertension.
Yang Q, et al. 2006. [250]	USA, live singletons, maternal age 15-49. N=73,628,288.	1980-2000	% <2500 gr decreased '80-'90 and increased'90-'00 among whites, opposite among blacks.	Changes in age-parity specific rates of low birthweight and age-parity distributions contributed to the observed trends (explaining 10-90%).
Zhang X, et al. 2010. [251]	USA (not California), live singletons to white women, GA ≥37. N=23,549,360.	1992-2003	Mean birthweight decreased by 37 gr, macrosomia (>4500 gr) by 25%.	No formal analyses. GA decreased. Induction and c-section rates increased. Birthweight by GA increased. Ecological analyses suggested >50% of variance explained by induction practices.

1.6.2 The role of maternal blood sugar regulation and HbA1c during pregnancy in perinatal outcomes

Maternal diabetes mellitus (DM), including gestational diabetes (GDM), is a risk factor for a number of adverse pregnancy outcomes (Table 2). Furthermore, compromised maternal blood sugar regulation not meeting criteria for overt DM or GDM has been linked to adverse outcomes, while lifestyle interventions have proven to decrease the risks to some extent. The main screening tool for detection of compromised blood sugar control in pregnant women is per now an oral glucose tolerance test (OGTT) performed around gestational week 24 [252]. Studies in women without diabetes and OGTT-results below the levels of GDM have shown linear associations between fasting blood glucose levels and OGTT-results with increased risks of caesarean sections, inductions, LGA, macrosomia, neonatal hypoglycaemia, preeclampsia, and shoulder dystocia, but not with preterm delivery [253].

Among patients with DM, glycated haemoglobin (HbA1c) assays are the standard tool for monitoring glycaemic control, as this reflects the overall blood sugar levels the previous 90-120 days before measurement [254]. However, increased haemoglobin turnover and changes in blood volume happening during pregnancy complicate the interpretation of HbA1c levels in pregnancy, which is generally thought to be lower than in the non-pregnant state, in particular during the second trimester [255-258]. Furthermore, HbA1c may not capture the short-term fluctuations in blood sugar that could be important for diabetes management during pregnancy.

Currently, screening with HbA1c may be used early in pregnancy to diagnose overt DM (HbA1c value ≥48 mmol/mol [6.5% units]) [259,260]. HbA1c is not recommended as a screening tool in the second and third trimesters of pregnancy [258,261,262]. However, the “late” GA at which OGTT is currently performed leaves limited time for lifestyle interventions, and fails to capture the long-term levels of blood glucose in the pregnant women. Indeed, there are indications that altered fetal growth patterns observed in women with GDM are apparent before the timing of normal OGTT screening [263]. Furthermore, OGTT is a more invasive test than HbA1c, as it requires the pregnant women to meet for fasting blood tests before performing the OGTT, which is time-consuming and dreaded by many due to the nausea commonly experienced [264]. HbA1c levels in early pregnancy in the upper normal/prediabetic range have been associated with an increased risk of developing GDM in the same pregnancy, although the sensitivity of screening for GDM with HbA1c early in pregnancy has been

reportedly low, between 10-20% [264-267]. Furthermore, the association between HbA1c levels measured at different timepoints in pregnancy and a number of pregnancy outcomes in studies including women with GDM have been conflicting, to a large extent without statistically significant associations after adjustment for possible confounders [265,266,268-271]. This could possibly be due to treatment of women with GDM leading to prevention of adverse outcomes among those with elevated HbA1c levels. Indeed, one study from New Zealand that examined pregnancy outcomes in women with HbA1c levels of 41-49 mmol/mol (5.9-6.6%) treated for GDM at an earlier or later timepoint according to whether the levels were measured around GA week 10 (inter-quartile range [IQR] 6-13) or 29 (IQR 26-32), found that the risk of preeclampsia was significantly reduced among those who began treatment for their GDM earlier compared to later [269]. Those with a later diagnosis of GDM and HbA1c levels within the "pre-diabetic range" were at higher risk of adverse pregnancy outcomes than those with HbA1c levels within the normal range [269]. Similarly, another study among women with GDM found that early HbA1c levels were less correlated with adverse pregnancy outcomes than later HbA1c levels, probably due to the earlier intervention to treat GDM among those with early detection [268].

The associations with HbA1c levels and perinatal outcomes during pregnancy in women without DM and GDM have been less well described than fasting glucose levels and OGTT-levels and are summarized in Table 6. Some of the studies found that HbA1c levels measured at different timepoints in pregnancy was associated with perinatal outcomes, although the study from China did not report when in the second trimester the HbA1c levels were measured [264,272-274]. A study from Greenland did not find a significant association between birthweight Z-score and HbA1c levels measured in the first trimester [275]. The generalizability to a Norwegian population may be limited for a number of reasons: Greenlanders are in general not of Caucasian European origin, the reported levels of maternal smoking during pregnancy was high, the mean birthweight was higher than in Norway, and about 50% of the women were overweight or obese before pregnancy. One Dutch study had very low numbers [255]. Another Dutch study examining trimester-specific effects of HbA1c levels and birthweight, found an indication that those without a decrease in HbA1c levels from the first to the second trimester gave birth to bigger babies, although the numbers were low [276]. There is thus a knowledge gap regarding HbA1c levels in early mid-pregnancy and perinatal outcomes, as well as in a Nordic setting.

Table 6. Summary of existing knowledge on HbA1c levels in pregnancy among women without DM and the association with perinatal outcomes.

Study	Country/region and time period	No. of study participants, inclusion criteria	Week of pregnancy HbA1c measured	Modelling of HbA1c	Association with perinatal outcomes
Bi J, et al. 2020. [272]	China, 1 hospital, births September 2014 - March 2018.	N=5658 pregnant women. Singletons, live birth, no birth defects, birthweight 500-5000 gr, GA ≤ 42 weeks, no GDM or DM, HbA1c <6.5%/48 mmol/mol.	Second trimester.	Linear exposure, in %-point units.	**Gestational age**: Increased risk of preterm birth, per %-point unit HbA1c: OR 1.85 (95% CI 1.27, 2.69) and adjusted OR (aOR) 1.62 (1.10, 2.37) adjusted for maternal age, infant sex, parity and BMI. Strongest among women with BMI ≤24. **Birthweight**: Increased risk of birthweight >4000gr, per 1%-point unit: OR 2.11 (95CI 1.37, 3.24) and aOR 1.58 (1.02, 2.44) adjusted for maternal age, infant sex, GA, parity and BMI. LGA: OR 1.73 (1.23, 2.43) and aOR 1.38 (0.98, 1.96). **Other outcomes**: N/A.
Hughes RC, et al. 2014. [264]	New Zealand, Christchurch, 2008-2010.	N=8475 pregnant women. Singletons, no treatment for GDM, HbA1c <6.5%/48 mmol/mol.	First trimester, median GA 47 days (IQR 38-62 days), =6+5 weeks.	Binary exposure, <5.7% versus 5.7-6.4% (n=200)	**Gestational age**: Preterm delivery increased risk among high, RR 1.66 (1.01, 2.74). **Birthweight**: No difference in mean birthweight. >4000 gr, RR 1.12 (0.78, 1.61), SGA RR 0.71 (0.46, 1.10), LGA 1.66 (1.11, 2.48). SGA and LGA adjusted for ethnicity, maternal height and weight, parity, GA, sex. **Other outcomes**: Induction RR 1.44 (1.01, 2.06), c-section RR 1.10 (0.82, 1.47), major congenital anomalies RR 2.67 (1.28, 5.53), preeclampsia RR 2.42 (1.34, 4.38), perinatal death RR 3.96 (1.54, 10.2), shoulder dystocia 2.47 (1.05, 5.85).
Karcaaltincaba D, et al. 2010. [273]	Turkey, Ankara, 1 Hospital. Year not stated.	N=102 pregnant women. "Abnormal" screening test for GDM [1], normal OGTT, singletons, no malformations, non-smokers, term, no chronic medical conditions.	Second trimester, GA week 26 (median 26.2, minimum 24.2 and maximum 28.3)	Linear exposure, in %-point units.	**Gestational age**: N/A **Birthweight**: Pearson correlation coefficient between HbA1c and birthweight of 0.373, p<0.001. ROC curve analysis after logistic regression analyses determined an HbA1c of 4.99 on prediction of LGA with 93.8% sensitivity and 61.6% specificity, and for birthweight >4000 gr, cut-off at 4.99 with 92.9% sensitivity and 60.2% specificity. **Other outcomes**: N/A
Lowe LP, et al. 2012. [274]	15 field centres. USA, Canada, Barbados, Northern Ireland, England, Thailand, Israel, Australia, Hong Kong, Singapore, Netherlands, 2000-2006.	N=21,064 pregnant women. Singletons, ≥ 18 years, no ART, no DM, no HIV, no Hepatitis B or C.[2]	GA 24-32, "as close to week 28 as possible".	7 categories of HbA1c levels, and continuous.	**Gestational age**: Preterm delivery, aOR 1.17 (1.10, 1.24), per %-point unit increase in HbA1c adjusting for field centre, age, BMI, height, parity, smoking, alcohol use, hospitalization prior to delivery, any family history of DM, GA at OGTT, cord glucose, mean arterial pressure. **Birthweight**: LGA, aOR 1.15 (1.09, 1.21) per %-point unit increase in HbA1c adjusting for the above. **Other outcomes**: Primary c-section aOR 1.09 (1.04, 1.13), neonatal hypoglycaemia aOR 1.13 (1.02, 1.25), preeclampsia aOR 1.27 (1.19, 1.37) per %-point unit increase in HbA1c adjusting for the above plus family history of hypertension (preeclampsia only), and except hospitalization prior to delivery and mean arterial pressure (preeclampsia only).

Study	Country/region and time period	No. of study participants, inclusion criteria	Week of pregnancy HbA1c measured	Modelling of HbA1c	Association with perinatal outcomes
Radder JK, et al. 2005. [255]	Netherlands, Leiden University Medical Centre, unknown year.	N=62 pregnant women. "Healthy women".	Group 1 measured at GA week 14.5 (all GA <18) and Group 2 at GA week 34.6 (all GA >30).	Linear exposure, in %-point units.	**Gestational age**: N/A **Birthweight**: Linear regression analyses: Group 1: HbA1c and birthweight, r=0.142, p=0.45. Group 2: HbA1c and birthweight r=0.266, p=0.14. Both adjusted for GA. **Other outcomes**: N/A
Rasmussen KV, et al. 2020. [275]	Greenland, Nuuk, 1 hospital, September 2015 - September 2016.	N=378 pregnant women (n=2 with GDM). Singletons, GA ≥28, no DM, no PCOS, no use of oral steroids.	First trimester, all GA <20 weeks, median 63 days (7+0 weeks), IQR 47-84 days.	Linear exposure, in mmol/mol units.	**Gestational age**: N/A **Birthweight**: No increase in birthweight Z-score: unadjusted change per %-point unit HbA1c 0.12 (-0.18, 0.23) and adjusted 0.6 (-0.25, 0.37), adjusted for maternal age, residence, smoking during pregnancy, ethnicity, parity. Among obese women, adjusted change was 0.66 (-0.03, 1.34). **Other outcomes**: N/A
Versantvoort AR, et al. 2013. [276]	Netherlands, Leiden University Medical Centre, November 2002 - October 2004.	N=103 pregnant women. Singletons, BMI <30, none of the following: miscarriage, induced abortion, severe malformation, DM, family history DM, hypertension, lipid disorders, renal disease, corticosteroids, recurrent abortions, history of preeclampsia/SGA/LGA/preterm/stillbirth.	Three measures: GA week 10-14 + 24-26 + 34-36.	HbA1c level as linear exposure as well as difference in HbA1c levels between the trimesters for each woman.	**Gestational age**: N/A **Birthweight**: No correlation between HbA1c at any timepoint and birthweight percentile. Correlation between difference in first and second trimester HbA1c and birthweight (decrease related to lower birthweight). **Other outcomes**: N/A

C-section: caesarean section; N/A: not assessed in the reported paper; PCOS: polycystic ovary syndrome

[1] Abnormal screening test defined as: 1-hour plasma glucose >140 mg/dl after a 50 gr glucose test [273].

[2] Women meeting criteria for GDM today were included, but were not treated as the criteria for GDM were dissimilar from today. Only those meeting criteria for "overt" DM were unblinded: The participants were excluded *"... if the 2-hour plasma glucose level was >200 mg per deciliter [11.1 mmol per liter]) or, for ethical and safety reasons, if the fasting plasma glucose level exceeded 105 mg per deciliter (5.8 mmol per liter), the random plasma glucose level was 160 mg per deciliter (8.9 mmol per liter) or more, or any plasma glucose level was less than 45 mg per deciliter (2.5 mmol per liter)."* [274]

1.6.3 Perinatal outcomes and reproductive health in persons conceived by assisted reproductive technologies

Perinatal outcomes, including birthweight, hypertensive disorders of pregnancy [134] and gestational age [26] are in general subject to some degree of transgenerational recurrence. Furthermore, parents using ART would usually have fulfilled clinical criteria for infertility to qualify for ART treatment, which by itself is associated with increased risk of adverse outcomes [100,206,277]. These "infertility genes" would to some extent be inherited by their offspring, which in turn may impact on perinatal outcomes in their own pregnancies when reaching adulthood. However, the extent of possible heritability of reproductive health and perinatal outcomes in offspring conceived by ART is little studied.

To what extent ART influences health in children are still unclear. There are some indications that ART-conceived children and adolescents may face a slightly elevated risk of adverse neurocognitive development [278]. Studies suggest that there might be an increased risk of elevated blood pressure and other markers of poor cardiometabolic health among those conceived by ART, but the study numbers were in general low and follow-up times short, and there have been conflicting results [279]. The observed smaller size at birth in ART-conceived newborns (specifically those born after fresh embryo transfer) seems to be compensated by faster growth during the first years of life, resulting in similar height and weight around entrance into adulthood [280]. Whether this added growth spurt in ART-conceived children and adolescents is reassuring or a reason for concern regarding implications for later cardiometabolic health is somewhat debated [281]. Furthermore, studies suggest that birthweight and infant growth velocity affect pubertal timing [282], which in turn may impact fertility [283]. However, studies on pubertal development among adolescents have not shown differences in those conceived by ART or to subfertile parents compared to their peers [284].

When starting work on the ART analysis, I conducted a systematic search via the Library at the Norwegian Institute of Public Health (NIPH), and was not able to identify any studies on perinatal or reproductive outcomes in people conceived by ART. There are some studies on reproductive hormonal status and other measures related to fertility such as semen quality, which are summarized in Table 7.

Of note, there are fewer studies on female than male reproductive measures in those conceived by ART. The results from the study on women are in general reassuring [285]. For men, the results are conflicting, with some indications of impaired semen quality and altered

sex hormone levels, while other studies do not find significant differences [286-289]. However, the numbers of study participants were in general low, and impacts of possible differences in these measures on actual later reproductive performance are unknown.

A study on fertility history in relatives of fertile couples compared to infertile couples who used ICSI to conceive, indicated that infertility may run in families, in particular in the male lineages [290]. Studies in Norway found that women and men who were born preterm had lower reproductive rates than those born at term [2,26], and a Danish study found lower fecundability among women born at lower gestational ages [291].

However, whether adults conceived by ART face increased risks of adverse pregnancy outcomes when they become pregnant, similar to pregnancies conceived by ART, is unknown. Furthermore, it is difficult to predict in what directions altered pregnancy outcomes could go compared to naturally conceived peers, as many factors may come into play: the possible transgenerational transmission of perinatal outcomes, the genetic inheritance related to infertility, the exposure to ART procedures at conception and *in utero* from maternal hormonal stimulation, the socioeconomic selection into ART-use in the earlier ART-conceived cohorts, and possibly other general health issues seen among ART-conceived offspring.

Table 7. Summary of existing knowledge on reproductive measures among ART-conceived men and women.

Study	Country, time	No. of participants	Case and control definition	Findings
Belva F, et al. 2016. [286]	Belgium, Brussel. 2013-2016	N=111; 54 ICSI-conceived men and 57 spontaneously conceived men, ages 18-22.	ICSI-conceived (fresh embryo transfer) versus naturally conceived **men**. ICSI performed mainly due to male-factor infertility. Only Caucasian, singleton men.	**Sperm quality:** ICSI-conceived had lower median sperm concentration, total sperm count, and total motile sperm count after adjustment for age, BMI, genital malformations, time from ejaculation to analysis, abstinence period. More likely to have sperm concentrations <15 million/ml (aOR 2.7, 1.1-6.7) and total counts <39 million (aOR 4.3, 1.7-11.3), more likely to have normal morphology in <4% (aOR2.3, 0.9-5.4). No difference in median percentage progressive and total motility, and median semen volume.
Belva F, et al. 2017. [287]	Belgium, Brussel. 2013-2016	N=111; 54 ICSI-conceived men and 57 spontaneously conceived men, ages 18-22.	ICSI-conceived (fresh embryo transfer) versus naturally conceived **men**. ICSI performed mainly due to male-factor infertility. Caucasian, singleton men.	**Sex hormones**: Similar levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone and Inhibin B after adjustment for age, BMI and season. ICSI-conceived more likely to have Inhibin B-levels below 10th percentile (aOR 4.0; 95% CI 0.9-18.4) and FSH levels above 90th percentile (aOR 3.3, 95% CI 0.9-11.9).
Belva F, et al. 2017. [285]	Belgium, Brussel. 2013-2016	N=152: 71 ICSI-conceived women and 81 spontaneously conceived women, ages 18-22.	ICSI-conceived (fresh embryo transfer) versus naturally conceived **women**. ICSI performed mainly due to male-factor infertility. Caucasian, singleton women.	**Sex hormones**: Similar levels of anti-Müllerian hormone, FSH, LH, dehydroepiandrosterone (DHEAS) and antral follicle count among ICSI-conceived and naturally conceived women also after adjustment for BMI.
Catford SR, et al. 2022. [288]	Australia, IVF-centres in Victoria and the Western Australian Raine Study. Born 1989-1992.	N=476, 120 ART-conceived and 356 non-ART conceived men. 18-25 years.	**Men** conceived with IVF with ICSI compared to non-ART conceived men.	**Sperm quality:** ART-conceived had lower total and progressive sperm motility with higher mean normal morphology, after adjustment for age, abstinence period, plurality, alcohol consumption, education level, parental age at birth, educational level and maternal smoking and alcohol consumption. Similar prevalence of oligozoospermia, similar semen volume, sperm concentration, total count, total motile count, proportion of other abnormal semen parameters, and testicular volume. **Sex hormones**: ART-conceived had lower mean LH levels and higher mean testosterone levels than non-ART conceived, but similar concentrations of FSH.
Jensen TK, et al. 2006. [289]	Denmark, Copenhagen and Aalborg. 2001-2005	N=1925 men, 47 "fertility treatment"-conceived, 1878 naturally. Age ~18.	**Men** where the mothers answered that they had received "fertility treatment" (25/47 hormonal treatment), compared to no treatment.	**Sperm quality:** Those conceived by fertility-treated had lower total sperm concentration, total sperm count, fewer motile sperm and morphologically normal sperm, and smaller testicles. Statistically significant differences after adjustments. **Sex hormones**: No statistically significant differences.

2 AIM AND SPECIFIC OBJECTIVES OF THE THESIS

The aim of this thesis was to investigate trends and determinants of perinatal outcomes, in particular gestational length and birthweight, in a developed country, namely Norway. I used different study designs to be able to assess a broad range of perinatal outcomes and explore selected potential determinants of these. My aim was to increase the knowledge about determinants of perinatal outcomes by studying factors that are not fully understood, including contemporary determinants and measures for which there still is a knowledge gap.

The specific objectives were:

1. To explore potential causes of the rise and fall in mean birthweight in Norway between 1991 and 2007 (paper I).
2. To investigate whether maternal glycated haemoglobin (HbA1c) levels within the normal range in individuals without diabetes mellitus measured during mid-pregnancy was associated with specific perinatal outcomes, in particular birthweight and gestational length (paper II).
3. To investigate whether men and women conceived by ART differ in their own reproductive outcomes, including gestational age and birthweight of their offspring, compared to their naturally conceived peers (paper III).

3 MATERIALS AND METHODS

3.1 Participants and design

All residents in Norway have a personal identification number of 11 digits, which allows individual level linkage between different registries. Two of the studies in this thesis defined the study population based on the Medical Birth Registry of Norway and are population-based registry studies (Table 8). The last study was based on a subsample of the Norwegian Mother, Father and Child Cohort Study (MoBa), and was a prospective cohort study following women from recruitment around gestational week 18 and until delivery.

Table 8. Summary of data sources, study population and study design of the research projects included in this thesis.

Paper	Participants	Study period	Design
Paper I	All liveborn newborns in Norway between 1982 and 2016 registered in MBRN. Participants: 1,859,312.	January 1982 to December 2016.	Population-based national registry study
Paper II	Pregnancies recruited at gestational week 18 in the Norwegian Environmental Biobank project within MoBa, with available HbA1c measures and no diagnosis of diabetes mellitus. Participants: 2937.	January 2003 to December 2009.	Prospective cohort-study
Paper III	Women and men born in Norway between 1984 and 2002. Participants: 1,092,151.	January 1999 to December 2020.	Prospective population-based registry study

3.2 Data sources

This thesis is based on epidemiological studies using existing health registries and a large pregnancy cohort. The conducted studies used information from two registries, namely the Medical Birth Registry of Norway and the National Population Registry. Paper II additionally used information from a Norwegian pregnancy cohort, MoBa, and its sub-project the Norwegian Environmental Biobank. Below, I list the data sources used in the three papers in this thesis, and what information was obtained from each of them:

1. The Medical Birth Registry of Norway (MBRN) was established in 1967 and contains information on pregnancies with a gestational length of 12 weeks or more. It is mandatory for the midwife or obstetrician to complete a birth record for all deliveries from gestational week 12 and later, including information on maternal, pregnancy and infant factors. In our studies, we used recorded information on the newborn, namely date of birth, sex, birthweight, birth

length, head circumference, gestational age at birth, congenital malformations, Apgar score at 5 minutes, death within the first 28 days after birth and placental weight. We obtained information about the delivery, including the onset (spontaneous versus medically or surgically induced), and whether it ended in a caesarean section. About the pregnancy and the mother, we obtained information on maternal age at delivery, use of ART to conceive, parity (nulliparous, multiparous), marital status (married, cohabiting, nonmarried/single, divorced/separated/widowed, other/unknown), maternal country of birth (Scandinavian [including Norway, Sweden and Denmark] or other), county of residence at time of birth, preeclampsia, eclampsia, HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count) or hypertension prior to or during pregnancy, diabetes mellitus (type 1, type 2, gestational or undefined), smoking status during pregnancy (recorded from 1999 onwards), pre-pregnancy BMI (available from 2006 onwards), folic acid supplement before and during pregnancy (recorded from 1999).

2. The Population Registry of Norway contains information on all residents in Norway with place on residence and vital status. We used information on date of death or emigration in the survival analysis in paper III.

3. The Norwegian Environmental Biobank (NEB) project is a substudy within the Norwegian Mother, Father and Child Cohort Study (MoBa) [292-294]. MoBa recruited pregnant women and their partners from 1999 to 2008 around gestational week 18, at the routine ultrasound scan offered to all pregnant women. The participation rate was 41%, leading to a cohort of 95,200 women (with some women contributing more than one pregnancy) and 114,500 offspring. Questionnaires were issued to the mothers around gestational weeks 15 and 30 as well as at regular intervals after birth. For this project, we used data available as of November 2015, version 9 of the quality assured data files.

The NEB was established as a substudy within MoBa, with permission to draw biological material on 2999 subjects within the MoBa biobank [295]. The study participants were randomly selected based on availability of biological samples, singleton pregnancies only, the mother had answered all questionnaires until age 3 of the child, and no recorded diagnosis in the children of autism, suspected autism or language delay.

As a part of the NEB project, maternal HbA1c levels were measured in blood samples drawn in gestational week 18, with recorded measures in 2979 of the 2999 pregnancies. Of these, 2937 were to women without any registration of diabetes mellitus in the MBRN, comprising the study population in paper II. From the MoBa questionnaires, which contains maternally

reported information on the woman and the pregnancy, we obtained information on maternal height and pre-pregnancy weight (used to calculate BMI), smoking during pregnancy, educational level (less than high school, high school, college ≤4 years, college >4 years), weight gain during pregnancy, and native language of the mother and her parents (Norwegian or other).

3.3 Gestational age as a research outcome

In the papers included in this thesis, we have assessed gestational age both as a continuous, mean outcome (papers II and III), and by dividing into preterm births (before 37 completed gestational weeks) versus non-preterm births (papers II and III).

In paper I, gestational age was used as a predictor of birthweights, and divided into preterm births (gestational days 154-258) and term births (comprising gestational days 259-315). We used LMP-based dating, as we looked at a time period that included a shift in dating from LMP to US (around 1999), to ensure that the same dating method was used on all pregnancies.

In papers II and III, we used ultrasound-based dating of GA if that value was between 154-315 days and used LMP-based dating if US was missing and LMP was within the stated range. Additionally, in paper III, we used date of embryo insertion for ART pregnancies to estimate GA if US measures were missing, using the same time restriction on GA as for LMP and US.

3.4 Birthweight as a research outcome

As discussed previously, birthweight is measured routinely at birth, and usually reported in grams [296]. The most straightforward way of presenting changes in birthweight in relation to an exposure, would therefore be as a mean for a given group or level of exposure, with change in mean according to a change in the exposure. For example, time trends often use the mean birthweight as the outcome of interest, showing changes in trends over time. In papers I and III, we have assessed changes in mean birthweight according to the examined exposures.

However, to assess changes or differences in the non-predominant distribution, one can use cut-offs of low and high birthweight, which may be useful to detect pregnancies and infants at statistically higher risk of complications. In paper III, we used these hard cut-offs to assess whether the risk of giving birth to a newborn with low birthweight (<2500 grams) or high birthweight (≥4500 grams) differed according to parental mode of conception.

Given that birthweight depends heavily on the duration of the pregnancy, birthweight can be converted to a Z-score standardized for gestational age, sex and other factors, in our case parity, to allow for investigations of exposures regardless of that exposure's possible impact or association with gestational age. Similarly, cut-offs into SGA comprise the distribution of newborns falling below the 10th percentile of birthweight while LGA comprise the newborns above the 90th percentile of birthweight. We saw a strong association between HbA1c levels and GA in paper II, and wanted to assess possible added or reversed effects of HbA1c levels on infant growth parameters from that on GA. We therefore chose to assess birthweight Z-score both as a continuous outcome and risk of SGA and LGA in paper II by maternal HbA1c levels.

3.5 Other perinatal and fertility outcomes as research outcomes

In the MBRN, the registration of medical conditions has changed over time according to current clinical guidelines. Since 2016, preeclampsia has been defined by the combination of elevated blood pressure (≥140 mmHg systolic blood pressure and/or ≥90 mmHg diastolic blood pressure measured at least twice with 4-6 hours between) and the presence of proteinuria (≥0.3 gr/24 hours or total protein/creatinine ratio of >0.3 or ≥1+ on a urine dipstick) [296].

Other hypertensive disorders of pregnancy studied included HELLP, eclampsia and gestational hypertension. HELLP is reported to MBRN according to clinical criteria of hemolysis, elevated liver enzymes and low platelets [44]. Eclampsia is defined as generalized seizures during pregnancy, delivery, or the first 7 days after birth in cases of underlying preeclampsia, while gestational hypertension is defined as elevated blood pressure after week 20 (see preeclampsia definition of elevated blood pressure) [296].

In papers I and II, we included eclampsia and HELLP in the preeclampsia definition. In paper III, preeclampsia was assessed by itself, and hypertensive disorders of pregnancy (HDP) was additionally assessed as a combined outcome of preeclampsia, HELLP, eclampsia or gestational hypertension. In a sensitivity analysis of the impact of preeclampsia on birthweight time trend in paper I, we restricted the time period to 1999 onwards due to changes in the reporting of preeclampsia to the MBRN around 1998-1999 [297]. In paper II, the whole period is after this change, and in paper III, the pregnancy outcomes in the second generation are all after 1999.

Apgar scores are scored on every newborn right after birth and at 5 minutes according to clinical guidelines described in the Introduction, and coded in whole numbers on the birth certificate [296]. However, as it is not normally distributed, we assessed it as a binary outcome, with a score <7 at 5 minutes defined as "low", and 7 or more as high/normal. This outcome was assessed in papers I and III.

On the birth certificate, congenital malformations are reported as "yes" or "no" according to whether any sign of a congenital malformation is present [296]. A malformation is only recorded in the MBRN if the diagnosis was set before hospital discharge after birth. If "yes", then a doctor is required to fill out diagnostic codes and describe the defect with words. For the three papers included in this thesis, the composite outcome of either "yes" or "no" to any congenital malformation was used. This might lead to failure to identify associations limited to specific birth defects, but gives more statistical power as more cases are combined together. This outcome was assessed in papers II and III, and used as an adjustment covariate in paper I.

Both head circumference and length of the newborn are measured shortly after birth, and reported to the MBRN in whole cm [296]. Although routinely measured, it is to be expected that measurements are less reproducible and more dependent on the individual measuring these anthropometrics than birthweight. Nevertheless, both anthropometric measures are commonly normally distributed in large populations. Both head circumference and length of the newborn were converted into Z-scores by GA, infant sex and parity for analyses as described for birthweight, and were assessed in paper II with maternal HbA1c levels as the exposure.

Placental weight is reported in whole 10 grams on the birth certificate to the MBRN [296]. The distribution is usually normally distributed, as for birthweight. This outcome was assessed in paper III as a continuous outcome, comparing differences in means according to parental mode of conception.

In paper III, we additionally included offspring sex, use of ART and age at first pregnancy as outcomes studied. Sex is reported on the birth certificate to the MBRN. Use of ART is mandatorily reported by fertility clinics to the MBRN, and what type of ART method in use is indicated (IVF with and without ICSI, fresh or frozen embryo transfer). Intrauterine inseminations are included on these reports, but were not defined as ART in the parent or offspring generation in paper III, according to international guidelines [199]. Age at first pregnancy might be an indicator of fertility, although it can be difficult to separate biological and social determinants. Sex, use of ART and age at first pregnancy are, strictly speaking, not perinatal outcomes, but could rather be defined as measures of fertility. Differences in these

outcomes between two subgroups or populations could thus indicate an underlying difference in fertility potential or degree of infertility, which in turn may impact the risk of perinatal outcomes. I do therefore not include the results of these associations as main outcomes in this thesis, but rather discuss the implications of the main results in light of the observed association between the exposure and these fertility measures.

Neonatal mortality is recorded in the MBRN according to time of death of a liveborn infant and grouped as: 0-24 hours after birth, 2-7 days after birth, and 8-28 days after birth. In paper I, we defined neonatal mortality as death of a liveborn infant within the first 28 days of life.

3.6 HbA1c measurement

In the NEB, venous blood samples drawn from the pregnant woman around gestational week 18 (mean 18.4 for the study sample included in paper II) were sent to the MoBa biobank for storage at -20 ºC until analysis was performed, ranging from 5-12 years. The blood samples were sent to the Biochemistry Laboratory, Forensic Toxicology Unit, Finnish Institute for Health and Welfare, Helsinki, Finland, which is accredited by the Finnish Accreditation Service (FINAS, Helsinki) and fulfils the requirements of the standard SFS-EN ISO/IEC 17025:2005, which covers the HbA1c assay. The laboratory took part in the HbA1c external quality assessment scheme organized by Lab quality (Helsinki, Finland). Using an Architect c8000 analyser (Abbott Laboratories, Abbott Park, Illinois, USA), HbA1c was measured in two batches (December 2014-January 2015 and July-October 2015). The precision levels between the series are expressed as the coefficient of variation (CV) [mean ± standard deviation (SD)] and were 2.0%±0.3 and 1.8%±0.2 in the first and second batches, respectively. For the entire study, the between-batch CV was 1.9%±0.3. Systematic error (BIAS%±SD) was evaluated using samples from the proficiency testing. Values were assigned by the European Reference Laboratory for Glycohemoglobin. In the first period, this was 3.0%±1.4, and in the second period, 4.1%±3.1. To evaluate the immunoturbidimetric method, samples from 100 subjects of four different nationalities were analysed by both this and an enzymatic method by Abbott Laboratories at the Biochemistry Laboratory, and compared. The regression equation indicated very good agreement between the two methods, with $y = 0{,}988x - 1{,}026$, $R^2 = 0{,}983$ [298]. The distribution of HbA1c levels in the complete study population was Gaussian, with a mean of 32.7 mmol/mol (5.1% in %-units), and an SD of 2.9 mmol/mol. The range among pregnancies to women without diabetes was 22-47 mmol/mol.

3.7 Statistical methods

The studies included in this thesis are all observational studies. We used common statistical methods applied in epidemiological research, based on current best practice [299]. Covariates in the multivariate regression models were mainly based on an *a priori* selection of possible confounders of the studied associations, using an approach based on drawing of directed acyclic graphs (DAGs). In this thesis, I have used the statistical software Stata (versions 15.0 and 16.0 SE) for data analysis, visualization, and modelling methods (Statacorp, College Station, TX).

3.7.1 Statistical methods in paper I *"Stumped by the Hump: The Curious Rise and Fall of Norwegian Birthweights, 1991-2007"*

Paper I is mainly a descriptive study showing mean birthweight in all live births per year between 1982 and 2016 after exclusion of those with a missing birthweight, birthweight <500 gr or a GA recorded as <154 or >315 days. We compared the pattern of mean birthweights by year using different stratifications including term and preterm births, maternal parity, season of birth, mother's country of birth (Scandinavian versus other), county of residence, offspring sex, onset of delivery (spontaneous, medically induced, caesarean section), maternal diagnosis of diabetes, and gestational age in whole weeks (Figure 1).

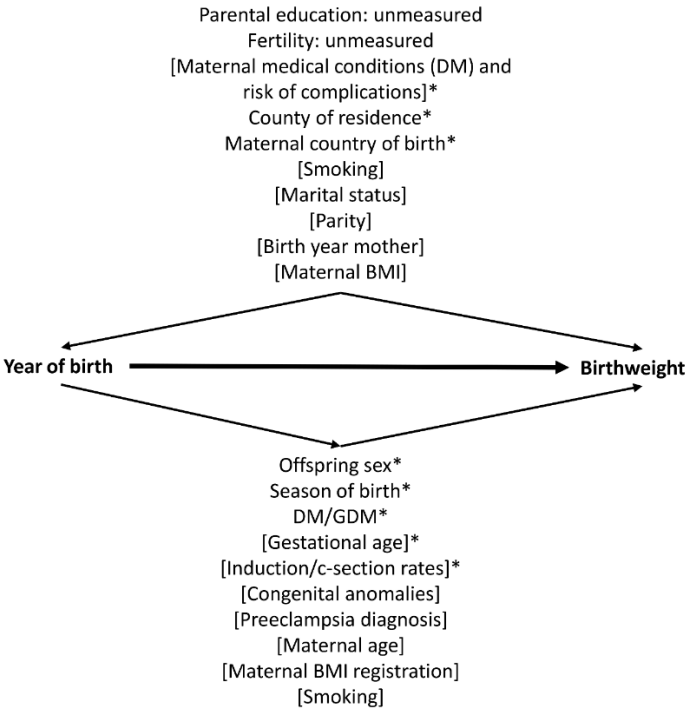

Figure 1: Directed Acyclic Graph (DAG) of the covariate selection in paper I. Factors in brackets were conditioned on in multivariate regression analyses, while factors with a * were studied by conducting stratified analyses. Smoking, maternal BMI, preeclampsia, DM and GDM could be seen as both confounders (true, when not differing by registration practices) and mediators (due to differences in registration in the MBRN by year).

We also applied linear regression analysis to try and disentangle the various determinants' roles on the increase and decrease in birthweight. If the observed mean change in birthweight by year of birth was possible to "adjust away", this would indicate that the major change in mean birthweight could be mediated through the included covariate(s) or by selection into pregnancy a given year by these underlying factors. However, spurious associations due to collider stratification bias could not be excluded. We obtained crude and adjusted change in mean birthweight within term born infants to Scandinavian-born women by year of birth, adjusting for selected covariates in the different models, using the year 1982 as the reference year. Adjustment variables in the fully adjusted model included maternal age (continuous and squared to allow for linear and non-linear associations), parity (primipara versus multipara), gestational age in days (continuous and squared to allow for linear and non-linear associations), onset of delivery (spontaneous, medical induction, and caesarean section), congenital malformation in the newborn (yes versus no) and marital status (married, cohabiting, nonmarried/single, divorced/separated/widowed, and other/unknown). We did a complete-case analysis, only including individuals with available information on all covariates in the analyses.

In separate sensitivity analyses we additionally adjusted for maternal year of birth in 10-year intervals (categorically), for preeclampsia in births between 1999 and 2016 (as there was a change in reporting guidelines of preeclampsia in 1999), for smoking in births between 1999 and 2016, and for maternal pre-pregnancy BMI (continuous) between 2007 and 2016. For the latter analyses, we used 2007 as the reference year, and examined whether the inclusion of the extra covariates impacted the total change in mean birthweight levels.

We also assessed time trends in the outcomes preterm birth, low Apgar score and neonatal mortality by dividing the proportion with the outcome among all infants with available information on that outcome annually between 1982 and 2016.

Finally, w plotted mean birthweights among all liveborns in Sweden and Finland from the same time periods (1982-2016 for Sweden and 1987-2016 for Finland), which was available from online summary statistics.

3.7.2 Statistical methods in paper II *"Glycated haemoglobin (HbA1c) in mid-pregnancy and perinatal outcomes"*

For the analyses in paper II, we excluded women with any diagnosis of diabetes mellitus in the MBRN. We did a complete-case analysis, only including pregnancies with available

information on the covariates in question. Gestational age estimation was based on routine ultrasound for n=2876 and LMP for n=51, where US measures were missing. 10 pregnancies were excluded due to missing on both. We assessed the associations between HbA1c levels and multiple possible predictors of HbA1c that could be confounders of associations with perinatal outcomes (parity, smoking, maternal education, native language [proxy for ethnicity], infant sex, weight gain before week 18, maternal height, use of ART) using linear regressions with HbA1c as the outcome, and presented results in univariate analyses, and adjusted both separately and combined for maternal age (continuous) and maternal pre-pregnancy BMI (continuous). As only maternal age, BMI and smoking remained associated with HbA1c levels in these exploratory analyses, and the possible confounders of associations between HbA1c levels and perinatal outcomes is uncertain, we chose to include these three variables in all multivariate analyses of perinatal outcomes. When we investigated gestational age as an outcome, we additionally adjusted for parity to improve precision of the estimates. Binary outcomes (preterm birth, preeclampsia, congenital malformations) were analysed by log-binomial regression and multinomial regression (LGA and SGA), while continuous outcomes (birthweight, head circumference, length of the newborn and gestational age at birth) were analysed by linear regression models.

As both mean birthweight, head circumference and length of the newborn relative to GA may vary over time and by country [8], we used singleton births in MBRN of the same time period (1999-2017) as reference population for the standardization of fetal anthropometrics in this paper, grouping by GA in completed weeks, sex and parity. The Z-scores were calculated by subtracting the mean value in the reference population from the individuals' values and dividing by the standard deviation of the reference population.

We explored the associations between HbA1c levels and the outcomes using a four-knot restricted cubic spline model, and identified which outcomes showed linear or non-linear associations (Table 9). We thereafter explored whether a restricted cubic spline model improved the model fit compared to either a linear regression model or a one-knot linear spline model using likelihood ratio testing. There was no indication of an improved fit for any of the outcomes. Therefore, we presented results from a linear regression model by each 5 unit increase in HbA1c levels. For non-linear associations, we present the results of the regression coefficients for the one-knot spline model by each unit increase in HbA1c levels up to 34 mmol/mol (lower 3 quartiles) and from 35 mmol/mol (upper quartile). For graphical displays of the associations in the published paper, we used the 4-knot restricted cubic spline

model for all outcomes.

For gestational age at birth, we additionally performed sensitivity analyses excluding pregnancies with preeclampsia, with preterm delivery, with non-spontaneous start of delivery, and stratified by parity.

In the analyses of fetal anthropometrics, we chose to standardize for gestational age. Adjusting for this mediating variable could in theory lead to collider stratification bias. However, given the strong association between HbA1c levels and pregnancy duration, failure to account for GA would raise the problem of interpreting fetal growth at higher levels of HbA1c.

Table 9. Statistical modelling of perinatal outcomes studied in paper II.

Outcome	Main exposure	Statistical method	Effect size measure	Covariates included in multivariate regression model
Birthweight Z-score	HbA1c, continuous, per 5 units increase	Multivariate linear regression	Change in birthweight Z-score	Maternal age (whole years, continuous), maternal pre-pregnancy BMI (continuous), smoking in pregnancy (yes or no)
LGA	HbA1c, continuous, per 5 units increase	Multivariate multinomial regression	Change in risk ratio of LGA	Maternal age, maternal pre-pregnancy BMI, smoking in pregnancy
SGA	HbA1c, continuous ≤34 and ≥35 mmol/mol separately using a one-knot linear spline for HbA1c levels	Multivariate multinomial regression	Change in risk ratio of SGA	Maternal age, maternal pre-pregnancy BMI, smoking in pregnancy
Length Z-score	HbA1c, continuous, per 5 units increase	Multivariate linear regression	Change in length Z-score	Maternal age, maternal pre-pregnancy BMI, smoking in pregnancy
Head circumference Z-score	HbA1c, continuous, per 5 units increase	Multivariate linear regression	Change in head circumference Z-score	Maternal age, maternal pre-pregnancy BMI, smoking in pregnancy
Gestational age	HbA1c, continuous, ≤34 and ≥35 mmol/mol separately using a one-knot linear spline	Multivariate linear regression	Change in whole gestational days	Maternal age, maternal pre-pregnancy BMI, smoking in pregnancy, parity (0 or ≥1)
Preterm birth	HbA1c, continuous, ≤34 and ≥35 mmol/mol separately using a one-knot linear spline	Multivariate logistic regression	Change in relative risk (RR) of preterm birth	Maternal age, maternal pre-pregnancy BMI, smoking in pregnancy
Preeclampsia	HbA1c, continuous, ≤34 and ≥35 mmol/mol separately using a one-knot linear spline	Multivariate logistic regression	Change in RR of preeclampsia	Maternal age, maternal pre-pregnancy BMI, smoking in pregnancy
Congenital malformations	HbA1c, continuous, ≤34 and ≥35 mmol/mol separately using a one-knot linear spline	Multivariate logistic regression	Change in RR of congenital malformation	Maternal age, maternal pre-pregnancy BMI, smoking in pregnancy

3.7.3 Statistical methods in paper III *"Reproductive outcomes in women and men conceived by assisted reproductive technologies"*

We identified liveborn males and females in the MBRN between 1984 and 2002 and dichotomized them to either belonging to the "naturally conceived" group or the "ART-conceived" group. The use of fresh transfer of embryos after conventional IVF was the most common method used among the ART-conceived liveborns (~65% of all ART-conceived pregnancies). We thereafter linked these individuals to the MBRN between 1999 and 2020 if they had a registered pregnancy being either the mother or the father.

We conducted analyses in all first registered births of at least 22 weeks gestation separately for men and women. We used linear regression to compare mean birthweight, gestational age and placental weight, and logistic regression to obtain the odds ratios of low birthweight, high birthweight, preterm birth, preeclampsia, any hypertensive disorder of pregnancy, congenital malformation in the newborn, low Apgar score, as well as the use of ART and offspring sex according to parental mode of conception. Possible confounders of the association between one's own mode of conception and later reproductive outcomes as an adult, can only be measured before one's own birth. As these transgenerational confounders can be hard to define accurately, and we did not have information on genetic makeup, socio-economic status etcetera on the parents, we used a different approach when choosing which covariates to adjust for in multivariate analyses (Figure 2).

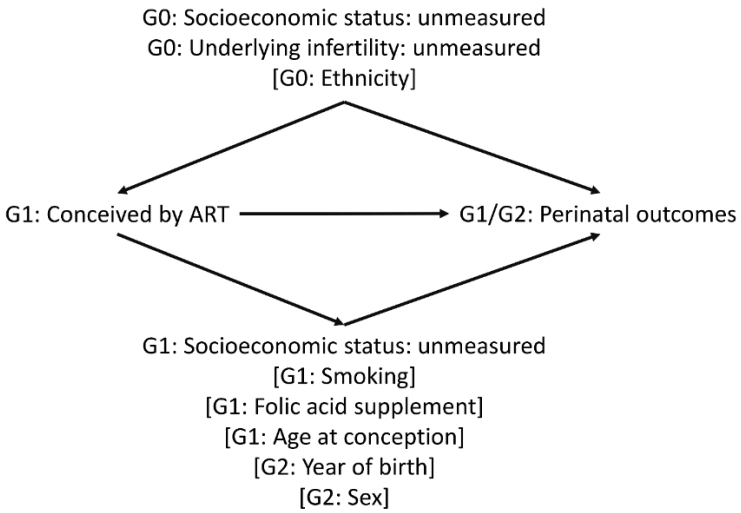

Figure 2: Directed Acyclic Graph (DAG) of the covariate selection in paper III in the analysis of perinatal outcomes. G0: the generation using ART or not to conceive. G1: the generation conceived by ART or not, in whom we later assessed the perinatal outcomes among those with a registered pregnancy as mother or father. G2: the offspring generation in which the birthweights, congenital malformations, Apgar scores and so on were assessed. Factors in brackets were conditioned on in multivariate regression analyses. Year of birth in G2 included due to temporal trends of perinatal outcomes. Smoking, folic acid supplement and age at conception in G1 could all be seen as possible surrogates of the socioeconomic statuses in both G0 and G1.

Namely, we chose those that could differ with mode of conception due to cohort effects induced by a skewed distribution of yearly number of births due to availability of ART. In multivariate analyses, we therefore adjusted for offspring sex (which we found differed between the two groups), their own age at conception as well as the partner's age at conception for analyses in men (categorically, <25 years, 25-29 years, and ≥30 years), year of pregnancy (categorically, <2011, 2011-2015, 2016-2018, 2019-2020), smoking at beginning of pregnancy (yes or no consent versus no), folic acid supplement (yes versus no), and their own mother's country of birth (Norwegian versus other, which could be seen as a "true" confounder if correctly reflecting ethnicity). We subsequently conducted similar analyses on all registered births, and in adjusted analyses we additionally adjusted for parity (0 versus ≥1).

To assess whether the likelihood of having a registered pregnancy differed according to one's own mode of conception, we used survival analysis stratified by sex. Starting at age 14 with those residing in Norway and not having a previously registered pregnancy in MBRN at that age, we used a Cox proportional hazards model with age as underlying time axis and obtained hazard ratios (HRs) with conception of a pregnancy in MBRN as the outcome. Individuals were censored if moving out of the country, registered as dead, or on December 31, 2020, whichever came first. In this analysis, all pregnancies in MBRN were included, and non-livebirths without a valid GA and missing birthweight or birthweight <500 grams were assigned a GA of 14 weeks (likely a miscarriage), and non-livebirths with missing GA and a birthweight ≥500 grams were assigned a GA of 22 weeks (likely a stillbirth).

In this analysis, we could only adjust for variables available in the women and men's own birth records. We speculated that year of birth (due to via availability of ART, as an approximation of timing of conception), ethnicity, county of residence at the time of their own birth and the age of their own mother could impact the likelihood of having a pregnancy either through social (including timing) or biological mechanisms, and could also have impacted on their own mode of conception (Figure 3). Therefore, in the minimally adjusted model, we adjusted for year of birth categorically (1984-1989, 1990-1993, 1994-1997, 1998-2002), and in the fully adjusted model we additionally adjusted for their own mother's country of birth (Norway versus other), their own county of birth (11 counties) and their mother's age at time of their own birth (continuous).

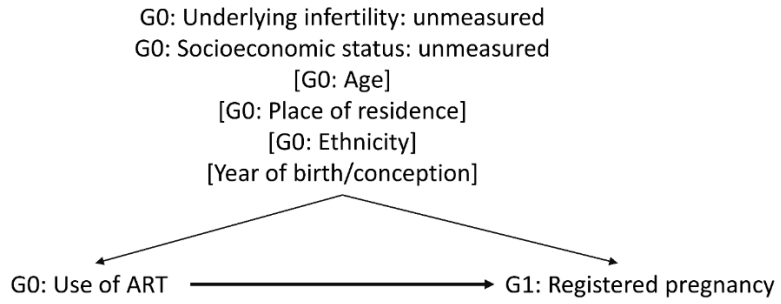

Figure 3: Directed Acyclic Graph (DAG) of the covariate selection in paper III in the Cox regression proportional hazards analysis of likelihood of having a registered pregnancy. G0: the generation using ART or not to conceive. G1: the generation conceived by ART or not, in whom we later assessed the likelihood of having a registered pregnancy as either the mother or father. Year of birth included as both an indicator of availability of ART and impacting the likelihood of having time to have a pregnancy in the G1 generation before the end of follow-up. Year of birth of G1 was conditioned on in the "minimally" adjusted model, while the three other variables in brackets were also conditioned on in the "fully" adjusted model.

3.8 Approvals

The studies reported in paper I and III were conducted in a project approved by the Regional Committee for Medical and Health Ethics of South/East Norway (No. 2014/404), which waived the need of consent from participants in these registry-based studies. The study reported in paper II was conducted partly in the aforementioned project as well as in a project approved by the Regional Committees for Medical and Health Research Ethics South/East Norway (2014/434). Data collection in MoBa was licensed by the Norwegian Data Protection Agency and approved by the Regional Committees for Medical and Health Research Ethics. The MoBa cohort is now regulated by the Norwegian Health Registry Act.

3.9 Ethical considerations in epidemiological research

Epidemiological health research is a broad scientific field that exploits a variety of research methods and aims to shed light on different questions related to health. In general, the aims are to study trends and occurrence of health or disease distributions in different populations, to identify causes of disease, or to undertake experiments to assess effectiveness of treatments or preventive efforts on diseases or other health conditions [299]. Consequently, most of the research is conducted on data material collected on human subjects, either through already established population registries, or through recruitment into specific trials or cohorts. As such, the potential harm for study participants, which should be minimized in all human research [300], is therefore in general limited, at least with regards to physical harm. However, cohort studies may involve more invasive procedures such as drawing of blood samples, which would require consent to participation according to the Helsinki Declaration [301].

All the research data used in the studies included in this thesis have been stored at secure servers at NIPH. The linkage keys used to link the registries with each other is stored at separate institutions. In general, all the data in these studies are anonymized, and remain non-identifiable to the researcher. However, when many variables are available on each individual, such as day of birth, county of residence, country of birth, date of birth of family members, health conditions, and more, it is possible to accidentally identify an individual. Consequently, it is important that the researcher protects the integrity of the research participants.

The researcher should strive to disseminate important research findings to both the policy makers and the populations included in the studies [302]. Below, I discuss further the ethical challenges associated with research on the different types of data sources used in this thesis, namely register-based and cohort-based.

3.9.1 Ethical considerations in register-based research

The studies in papers I and III are based on a register of all births in Norway, the Medical Birth Registry. Consequently, the Regional Ethics Committees can waive the need for consent from the participants. If these types of studies were to require consent from participants, it would likely lead to selection bias into the study, which makes interpretation and generalizability of the findings more difficult. As it is unethical to include pregnant women in most trials of medications, side-effects, and vaccines, the use of registries in epidemiological research is vital to obtain knowledge on possible consequences of these interventions [302]. Similarly, ART cannot be randomly assigned to couples aiming to achieve a pregnancy, so registry-based research may provide the best method to identify any adverse effects of this exposure on both the parents and child. As few countries outside the Nordic countries have the same opportunity to link information on "all" inhabitants across various health registries, the knowledge gained from research on the Nordic populations may be invaluable in a global setting. Although it is theoretically possible to identify individuals accidentally in a register-based study, in particular where the outcomes studied are very rare, the benefits of being able to do such research has been considered to outweigh the risks. Furthermore, each scientist using the data needs to be aware of the possible pitfalls and take necessary precautions. In paper III, which contains the smallest group of study participants (women and men conceived by ART who themselves parented a pregnancy registered in MBRN), we have taken care to not show any cells with numbers between 1-4, to avoid possible identification of individuals.

In tables included in paper I, no such precautions in presentation of the data were taken as the groups are very large.

3.9.2 Ethical considerations in cohort-based research

The study in paper II is based on a pregnancy cohort, namely MoBa. The data sources consist of a linkage between already established health registries, including MBRN which was used in this study, and data collected from the participating subjects. This includes questionnaires issued to the pregnant woman before and after birth, as well as blood samples collected from the woman around gestational week 18 and from cord blood at birth [294]. This additional collection of personal information requires written informed consent, both regarding the collection of biological samples, but also to the questionnaire data and the linkage to already established health registries [300]. Furthermore, the parents recruited to MoBa consent on behalf of their (unborn) children to the use of questionnaire information, registry linkages and biological samples, with the knowledge that the children will be informed of their participation at age 15 and on requirement of their own consent to remain in the project after they turn 18 years [303]. The study participants can withdraw at any time, which is in line with the Helsinki declaration and the International Ethical Guidelines for Epidemiological Studies by the Council for International Organizations of Medical Sciences [301,304]. Given that participants had consented to blood sample withdrawal and later analyses of these samples and no additional questionnaires or samples were required to conduct the analyses in the NEB, no additional consent was required before conducting the research. However, the consent given at the recruitment into the cohort is quite broad, which by some means may indicate that it is less valid [300]. Nevertheless, participants were informed at the time of consent that each new research project will require an approval from a Regional Ethics Committee before for instance blood samples can be analysed [303]. The results of the analyses are not disseminated directly to any study participant, but results from published studies are included in newsletters from MoBa going out to the participants in the cohort on a regular basis [305].

4 SUMMARY OF RESULTS

4.1 Paper I "*Stumped by the Hump: The Curious Rise and Fall of Norwegian Birthweights, 1991-2007*"

There were 1,859,312 live births in Norway with available information on birthweight and gestational age, and among these, mean birthweight increased by approximately 50 grams from 1991 to 1997, plateaued, and then declined by the same amount between 2002 and 2007. The same pattern was seen for Sweden, but not Finland. Similar patterns were not seen for neonatal mortality, preterm delivery or low Apgar score. The birthweight trend was not seen in preterm births (Figure 4) and was less clear among infants born to women who were not born in Scandinavia. It was less clear among infants where the birth started with a caesarean section, or where the birthweight was below the 10th percentile.

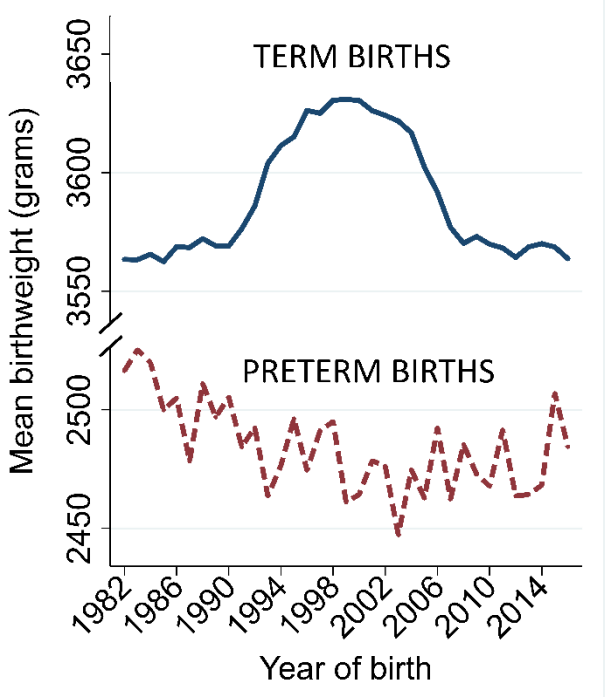

Figure 4. Mean birthweights among term births and preterm births in Norway between 1982 and 2016. Reprinted with permission from Carlsen EØ, Magnus MC, Omsland TK, et al. Stumped by the Hump: The Curious Rise and Fall of Norwegian Birthweights, 1991-2007. Epidemiology. 2020 Jul;31(4):587-594. Copyright © 2020 Wolters Kluwer Health, Inc. All rights reserved.

The trend was similar in term births in primiparous and multiparous women, by season of birth, offspring sex, in deliveries starting spontaneously or by medical induction, across all 19 counties in Norway, in women without a registration of DM, across the range of term gestational ages, and among infants with a birthweight above the 10th percentile. The pattern remained the same in adjusted analyses, although the birthweights did not decrease as much in the post-hump period as in the unadjusted analyses. The time trend was robust in all sensitivity analyses. We were thus not able to "explain" the hump shape of term birthweights.

4.2 Paper II "*Glycated haemoglobin (HbA1c) in mid-pregnancy and perinatal outcomes*"

Infant anthropometrics were linearly associated with maternal HbA1c levels in week 18 of pregnancy (Table 10), with a statistically significant association at the 5%-level for birthweight Z-score. Additionally, the risk of giving birth to an LGA infant rose with increasing HbA1c levels. The association with SGA was less clear, but indicated a U-shaped pattern.

Table 10. Association between maternal HbA1c levels and perinatal outcomes from paper II.

Outcome	**HbA1c level mmol/mol continuous,** estimate per 5 units (95% CI)	**HbA1c level ≤34 mmol/mol,** estimate per unit (95% CI)	**HbA1c level ≥35 mmol/mol,** estimate per unit (95% CI)	**Included observations for each outcome,** no. (% of study sample)
Birthweight (Z-score)	0.10 (0.03, 0.16) p=0.003	NA	NA	2874 (97.9)
Length (Z-score)	0.05 (-0.01, 0.11) p=0.09	NA	NA	2873 (97.8)
Head circumference (Z-score)	0.05 (-0.01, 0.12) p=0.10	NA	NA	2843 (96.8)
Large-for-gestational-age (risk ratio)	1.23 (1.01, 1.50) p=0.04	NA	NA	2874 (97.9)
Gestational age (days)	-0.81 (-1.47, -0.16) p=0.02	0.07 (-0.12, 0.26) p=0.49	-0.66 (-0.99, -0.33) p<0.001	2874 (97.9)
Small-for-gestational-age (risk ratio)	0.93 (0.72, 1.22) p=0.62	0.95 (0.88, 1.02) p=0.17	1.07 (0.95, 1.21) p=0.27	2874 (97.9)
Preterm birth (RR)	1.36 (0.95, 1.95) p=0.09	1.01 (0.90, 1.13) p=0.86	1.14 (1.00, 1.31) p=0.05	2874 (97.9)
Preeclampsia (RR)	1.07 (0.74, 1.54) p=0.73	0.92 (0.83, 1.02) p=0.12	1.20 (1.05, 1.37) p=0.007	2884 (98.2)
Any congenital malformation (RR)	0.93 (0.69, 1.24) p=0.61	0.94 (0.86, 1.01) p=0.10	1.10 (0.97, 1.25) p=0.13	2884 (98.2)

Results from multivariate regression analyses are presented. Modified from Carlsen EØ, Harmon Q, Magnus MC, et al. Glycated haemoglobin (HbA1c) in mid-pregnancy and perinatal outcomes. Int J Epidemiol. 2022 Jun 13;51(3):759-768. NA: not assessed.

The association between gestational age and HbA1c levels were inverse in the linear model with a decrease in GA with increasing HbA1c levels. However, a non-linear spline model with a single knot showed a better model fit. This showed an inverse association between HbA1c levels and GA only in the upper quartile of the HbA1c distribution (Table 10), with an adjusted decrease in GA of 0.66 days per increase in HbA1c mmol/mol unit. This association was robust in all sensitivity analyses.

Within the highest quartile of HbA1c levels, increasing levels increased the risk of both preterm birth and preeclampsia, while congenital malformations showed more of a U-shaped association (Table 10). Overall, we found indications that maternal HbA1c levels during week 18 of pregnancy were associated with a range of perinatal outcomes.

4.3 Paper III "*Reproductive outcomes in women and men conceived by assisted reproductive technologies*"

There were 162,991 naturally conceived women and 449 ART-conceived women with at least one live- or stillbirth by the end of follow-up. Birthweight, placental weight, HDPs, congenital malformations and gestational age were similar in the pregnancies conceived by these women. The odds of giving birth to a boy was lower among ART-conceived than naturally conceived women, also after adjustment (adjusted odds ratio [aOR] 0.79, 95% CI 0.66, 0.95) (Figure 5). There was an indication of an increased odds of giving birth to an infant with a low Apgar score, but confidence intervals were wide and included the null values. The results were similar when including subsequent pregnancies, with an increased aOR of a low 5-minute Apgar score among ART-conceived women (1.65, 95% CI 1.04, 2.61).

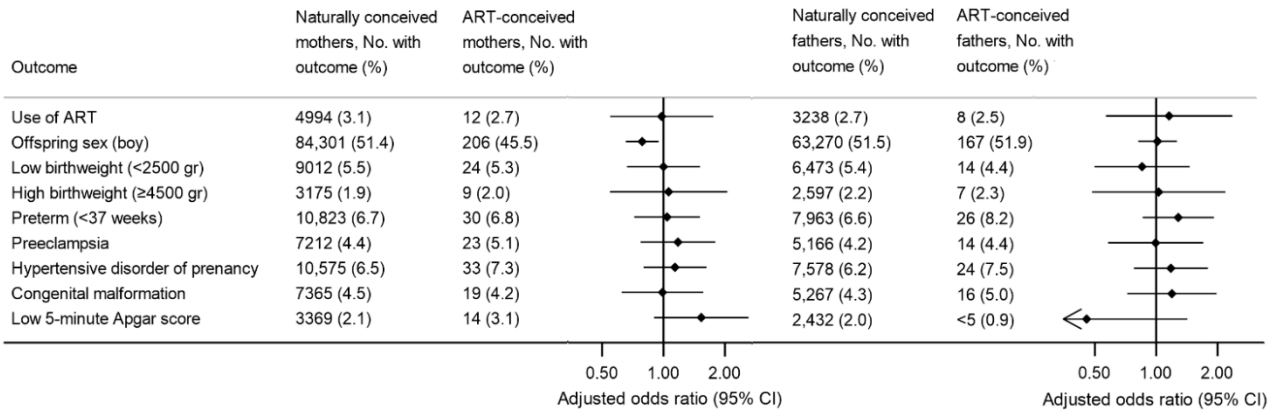

Figure 5. Association between parental mode of conception and a number of perinatal outcomes, where naturally conceived women and men are the reference and ART-conceived are the "exposed". Results from multivariate regression analyses are presented. Modified from Carlsen EØ, Wilcox AJ, Magnus MC, et al. Reproductive outcomes in women and men conceived by assisted reproductive technologies. Submitted manuscript.

121,580 naturally conceived men fathered at least one live- or stillbirth in the MBRN, compared to 318 ART-conceived men. Birthweight, placental weight, HDP risk, congenital malformations and gestational age did not differ in the pregnancies fathered by these men (Figure 5). There were lower odds of giving birth to an infant with a low 5-minute Apgar score among ART-conceived men, but the numbers were small and confidence intervals wide

and included the null values (aOR 0.46, 95% CI 0.15, 1.44). The results were similar when we included subsequent pregnancies.

The likelihood of having a registered pregnancy in MBRN during the follow-up period was lower among ART-conceived women (HR 0.69) and men (HR 0.77) than their naturally conceived peers. However, the differences were attenuated in the fully adjusted models (women: adjusted HR 0.87, 95% CI 0.79, 0.95; men: adjusted HR 0.91, 95% CI 0.82, 1.02).

Overall, we found no large differences in perinatal outcomes among women and men conceived by ART compared to their naturally conceived peers, but the likelihood of having a registered pregnancy in MBRN during the follow-up period was lower.

5 DISCUSSION

5.1 Methodological considerations

I will here discuss some methodological considerations not already discussed in the Methods chapter, namely study design and sources of bias in the papers included in this thesis.

5.1.1 Study design

The gold standard for identifying cause and effect, is often considered to be a randomized controlled trial (RCT). Then participants are assigned an intervention, and the outcomes are compared between those who received the intervention and those who did not receive the intervention. However, many exposures believed to impact health and disease are either not possible to assign at random to participants (such as educational attainment, ethnicity, and most exposures of interest in pregnancy studies), or it is not ethically acceptable to do so (such as smoking, high maternal age at childbirth). In these cases, an observational study design is required to study the exposure [306]. Furthermore, an interventional study can only be feasible if a hypothesis on a causal relationship is already established, or one would not know what kind of intervention or exposure to study. These hypotheses are often developed as a result of findings from observational studies [307]. Studies on pregnancy have in particular been exempted from interventional studies due to the feared double impact on the mother and the fetus [304]. A study of determinants of perinatal outcomes may comprise both the search for causal and noncausal factors, where both may predict perinatal outcomes to an equal extent in a given setting.

The studies in this thesis are all cohort studies [306]. The study population in paper I includes all births in Norway during the defined study period. In paper II, the study population includes a subgroup of a large pregnancy cohort, where participants were recruited at random from the whole pregnant population residing in Norway during the inclusion period, regardless of exposures or outcomes. The study population in paper III consists of a cohort of individuals born in Norway during the given study period and we assessed their own reproductive outcomes by the end of the follow-up period according to whether they were conceived by ART or not. However, given that the analyses in papers I and III are based solely on available information from health registries and paper II on a combination of health registries, questionnaire data and other material collected throughout the cohort's follow-up period, I will discuss the strengths and weaknesses of the study designs as either registry-based (papers I and III) or cohort-based (paper II) (Table 11).

Table 11. Strengths and limitations of the register-based and cohort-based study designs included in this thesis.

Strengths of register-based research design (papers I and III):	**Strengths of cohort-based research design (paper II):**
• Large numbers, in theory all births in Norway during the study periods included. • Generalizable to the complete Norwegian population (study sample = target population), probably also to countries with similar demographic characteristics. • Can assess the prevalence of a disease, exposure or other covariate in the population. • Rare outcomes may be more easily studied, as a larger study population is included. • Long follow-up time possible, less dependent on loss-to-follow-up, except for deaths, emigration (factors leading to not being registered in Norwegian registries).	• Designed questionnaires and obtainment of biological samples to the research questions intended to study. • Possible to sample participants based on desired covariates, exposures or outcomes for specific studies, such as for the sub-sample analysed from the NEB. • Possible to collect information on previous history of behaviour, medical conditions, exposures, family history of disease, that are not available in Norwegian registries.
Limitations of register-based research design (papers I and III):	**Limitations of cohort-based research design (paper II):**
• The data variables are predefined, not designed for research purposes. • Lack of detail in variables. • No information on history not recorded in Norwegian registers (for instance births or late miscarriages outside Norway). • No information on blood samples or other biological specimens taken during clinical care, as those are not available in the public health registries. • Less certain of timing of exposures in relation to outcomes, covariates etcetera.	• Lower numbers, limited by participation rates. • Costly to start up and manage. • Loss-to-follow-up, withdrawal from study. • May be less generalizable to the general population (study sample ≠ target population). • More prone to selection bias, depending on recruitment practices. • Cannot estimate the prevalence of a disease or exposure in the general population, only the study sample. • Low numbers may restrict the possibility to examine rare outcomes. This led to uncertainties regarding the associations between HbA1c and the binary perinatal outcomes.

Assessment of time trends of an outcome in a whole country, such as for birthweights in Norway between 1982 and 2016, is most ideally studied using the whole population, namely all births during the period. However, by using a registry-based approach to try to disentangle the underlying drivers of the trend, we might have missed important determinants of birthweight not recorded in the registries, including educational attainment, exercise, diet, and BMI as well as paternal factors.

The cohort study design of paper II allowed us to study an exposure not available in the Norwegian health registries, namely maternal HbA1c levels measured in early mid-pregnancy. Furthermore, we were able to control for possible confounders in more detail than

what are available in the registries. However, the generalisability to the whole Norwegian population may be questioned due to the selection of participants into both the MoBa cohort, and the even stricter selection into the NEB sub-sample. Also, due to lower sample size, the associations with binary outcomes under study, namely preeclampsia, preterm birth and congenital malformations, are probably less accurate than associations with continuous outcomes.

The use of registries to study the perinatal outcomes in women and men according to their own mode of conception allows for an early assessment of the oldest cohorts born after conception by ART in Norway. However, as for paper I, we might miss important explanatory factors that could impact fertility behaviour and perinatal outcomes, as these are not available in the registries. Nevertheless, a "tailor-made" prospective cohort study that included only a selected number of participants and followed them from birth based on their own mode of conception would require very long follow-up, and no guarantee that the study participants would end up having enough registered pregnancies as parents to conduct comparative analyses.

5.1.2 Sources of bias in observational studies

Sources of bias can be grouped into some main categories: confounding, selection bias, and information bias [299], and may differ according to study design (Table 12).

Table 12. Main sources of bias stratified by the register-based and cohort-based study designs included in this thesis.

Main sources of bias in the register-based research in this thesis (papers I and III):	**Main sources of bias in the cohort-based research in this thesis (paper II):**
• Selection bias: May arise by restricting the study population by year of follow-up, period, registration in available registries.	• Selection bias: Recruitment into the MoBa cohort, specifically recruitment into sub-cohort of NEB-study.
• Confounding: Residual and unmeasured confounding. Less knowledge on socio-economic factors and lifestyle factors, which are often confounding factors in studies on perinatal outcomes.	• Confounding: Unobserved confounding related to variables affecting HbA1c levels and perinatal outcomes that we did not have access to or did not account for.
• Information bias/measurement error: Missing data, wrong coding, lack of reporting to the MBRN.	• Information bias/measurement error: Self-report on smoking, height and weight. No information on country of birth/ethnicity except self-reported language. Measurement error of biologic samples.

Below, I will go more in depth on each of the main sources of error, how they may affect the findings in the included papers, and what we did to minimize possible effects of these errors in our analytic approach.

5.1.2.1 Confounding

A general concern in epidemiological studies of observational nature is confounding, which may arise through multiple processes. Confounding can be defined according to DAG terminology as an association that arises by an "open" biasing path between an exposure and an outcome, and is thus causally associated with both [308]. Of particular concern is the case where those who are exposed to a certain factor differ substantially in other areas that might impact the outcome, as opposed to those who are not exposed [306]. Such confounding can lead to spurious associations between the exposure and the outcome, as opposed to interventional study designs, where the interventions/treatments/exposures are assigned at random. Notably, confounding can lead to both overestimation and underestimation of the true association [308].

To get rid of these possible false estimates of association, we can adjust for variables that lead to confounding (Figure 6).

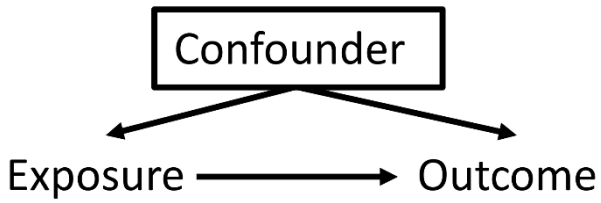

Figure 6. Graphical display of a simple directed acyclic graph (DAG).

In modern epidemiologic study design, it is common to include covariates as possible confounders in statistical analyses based on an *a priori* hypothesis of possible confounding, and not on level of statistical significance between the variables and the exposure and outcome, as was common earlier [308]. In paper I, it could be argued that there could not be any true confounders, as the exposure was year of delivery, and the outcome birthweight. However, one could also argue that some factors may impact both the decision or possibility to become pregnant and give birth in a given year, and also the birthweight in the offspring of that individual, thus fulfilling the criteria of a confounder. In our analyses, we therefore tried to account for possible factors that could impact year of delivery and birthweight, bus also adjusted for covariates that might *explain* the time trend, not as a confounder, but more of a mediator, lying between the exposure and outcome in the order of association (Figure 1). Some covariates could even be argued to be both possible confounders and mediators, such as

smoking, which could impact the year of birth by impacting fertility, but also be registered differently by year of birth. Covariates that would have been interesting to include but that are not recorded in the MBRN, include educational attainment, the mother's own birthweight, diet and level of physical activity.

Standardizing or adjusting for gestational age to assess the association between an exposure and the outcome by removing a mediating effect of GA can introduce collider stratification bias or over-adjustment [309-312], and should not be done without considering these possible pitfalls. Fetal size at the time of ultrasound measurement will impact the gestational age estimation [24], and indications of fetal growth restriction might lead to induction of delivery [81]. In Figure 7, this is illustrated where adjusting for GA (as a mediator) can introduce a spurious statistical association between the unmeasured confounder and the main exposure *through* GA, which were truly unrelated in the study population. This can lead to a change in the strength of the measured association between the exposure and birthweight, and in more extreme cases introduce reversal of statistical associations [309]. Our inclusion of GA in adjusted analyses could in theory therefore have led to biased estimates in papers I and II.

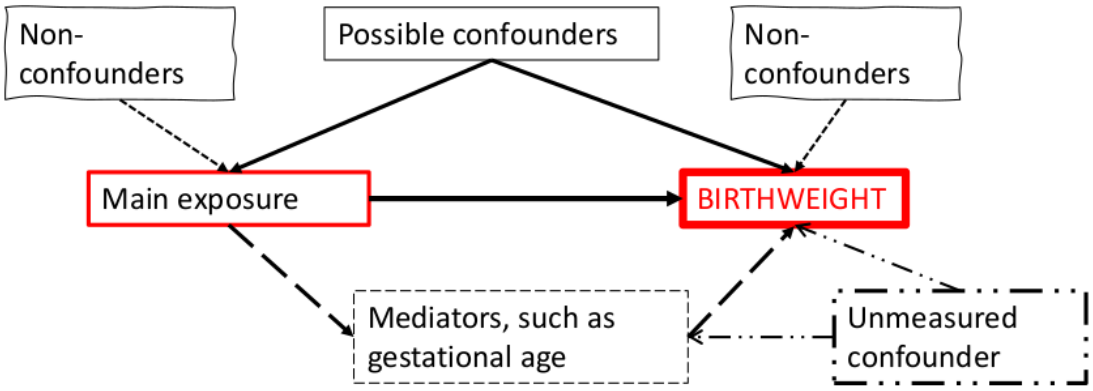

Figure 7. Illustration of a directed acyclic graph (DAG) examining birthweight as the outcome. The DAG does not differ whether you treat birthweight as a continuous or categorical variable. Variables denoted confounders should ideally be adjusted for, while non-confounders should not, except for the case where you assess continuous outcomes, and determinants of that outcome may be adjusted for to provide higher precision of the estimates (as when we included parity as an adjustment variable in the multivariate regression analysis of HbA1c levels and gestational age in paper II, but did not include it when assessing the risk of binary outcomes).

Similarly, in paper III, we had limited information on possible "true" confounders in the causal sense between mode of conception and their own adult reproductive outcomes, which would have been genetic factors, smoking in the pregnancy they were born to, and so on (Figure 2 and 3). The country of birth of their mother, if reflecting ethnicity, could be seen as a "true" confounder, and was included as a covariate. Additionally, we adjusted for factors that might confound the association due to selection into the two groups, based on cohort effects, as already discussed in chapter 3.7.3.

As already discussed in the chapter on study design, one of the strengths of a cohort-based design is the usually more extensive information available on possible confounders. We were therefore able to assess the possible inclusion of more potential confounders in paper II than in papers I and III. However, given the exploratory nature of the analysis of associations between HbA1c and perinatal outcomes, we chose to only include variables that we *a priori* considered might be possible confounders, that were additionally associated with HbA1c levels in our study sample. We also chose to include GA in the standardization of the anthropometric measures, not as a confounding factor but as a mediating or possibly reverse-acting factor, even though this in theory could have led to collider stratification bias.

5.1.2.2 Selection bias

I here define selection bias as confounding arising from collider bias introduced by conditioning on selection into the sample under study [308]. Although the use of registries on "all births" in Norway in papers I and III limits the risk of selection bias, there may still be mechanisms leading to biased findings.

In paper I, we had no information on "loss of follow-up" of ongoing pregnancies during the time period under study, which could lead to bias. If a certain exposure led to an increased rate of miscarriages or stillbirths among a group of pregnancies that would "normally" have gone on to be live births with "low" birthweights, this could have led to a failure to detect an exposure that impacted the mean birthweights. Similarly, selection into pregnancy and a live birth by a covariate we did not have information on or did not adjust for during the period between 1982 and 2016 may have impacted the mean birthweight trend, thus falsely rendering us without an explanation of the change in birthweight over time.

In paper III, there may have been selection bias if the health of the individuals born between 1984 and 2002 impacted their likelihood of having a registered pregnancy in MBRN by the end of follow-up, and this differed by their own mode of conception. Similarly, biological or social mechanisms for selection into pregnancy by the end of follow-up could have impacted the perinatal outcomes if these selection mechanisms differed by their own mode of conception. For instance, early miscarriages are not captured in MBRN, which could have led to selection into registration of a pregnancy if one of the groups were more likely to experience miscarriages. We did adjust for some factors that could be indicative of socio-economic status, such as the age of their own mother at time of birth, her country of birth, and their own county of birth, but we cannot exclude the possibility that selection into pregnancy

impacted our findings. Given that infertility has been associated with adverse perinatal outcomes [97-100], and we did not find a significantly different proportion of these outcomes according to parental mode of conception, this could suggest that only the more fertile women and men conceived by ART have had a registered pregnancy in MBRN by now.

Pregnancies resulting in multiple births were part of the study populations in papers I and III, but not in paper II, which was restricted to singletons. Unless a confounding factor of the association between the exposure under study and the risk of twinning were present in the studies, this should not impact our findings. Although twinning rates differ by maternal age and ethnicity [313,314], I do not expect the association between maternal HbA1c levels and perinatal outcomes to interact with maternal age. We did adjust for maternal age in the multivariate analyses in paper II, but we did not check for interactions between age, HbA1c levels and perinatal outcomes. Ethnicity is associated with perinatal outcomes [82,104,105,108] and possibly HbA1c levels [315]. We did not find an association between maternal native tongue (as a proxy for ethnicity) and HbA1c levels in our study population. However, the lack of specificity of maternal ethnicity could have limited our ability to estimate a true association, thus possibly leading to a bias either by lack of adjustment for confounding or selection bias. On the other hand, HbA1c levels, as a measure of glycaemic control, could be a mediator between ethnicity and perinatal outcomes, and in that case, ethnicity is not a confounder.

A limitation of the use of a pregnancy cohort requiring active consent and participation such as in paper II, is that the selection into the study may limit generalizability. The self-selection into MoBa led to an increased prevalence of older women with higher socio-economic status. However, although this pregnancy cohort cannot be used for prevalence estimates of outcomes and exposures, a study has suggested that exposure-outcome associations are less biased [316]. Regardless, the selection into the sub-study of NEB required completion of all questionnaire data until the child was 3 years of age as well as the exclusion of children with symptoms of autism or language delay. This subsampling led to a skewness in the time period of births from the whole MoBa cohort (more births included during the second half of the recruitment period), and fewer records with missing information on maternal BMI, educational level, household income, smoking and alcohol intake [295]. This may have impacted our findings if this selection also led to a change in the estimated associations between HbA1c levels and perinatal outcomes, which is unlikely but cannot be excluded.

5.1.2.3 Measurement error/information bias

The estimation of gestational length may lead to challenges with assessing differences across different subpopulations or exposures. As discussed in the Introduction, we cannot know the "true" gestational age of an infant or an ongoing pregnancy unless we have the date of ovulation or embryo implantation. As ultrasonography has become increasingly used in Western countries to determine the gestational length of a pregnancy clinically, this has become practice to use too when conducting research of GA as an outcome. However, if an exposure or a subpopulation associates with not only gestational length but also fetal size at ultrasound measurement, spurious associations between the exposure and GA at birth may occur. Similarly, when using US dating from the second trimester, girls and boys will already have deviated in size, such that the male to female ratio of postterm births will increase due to a false assignment of longer gestational ages to the males than females, which is not present when using LMP or earlier first-trimester US measurements [24,72]. In cases like these, LMP may be a better choice for estimating GA. Although less precise overall, the effect size will be more accurate if it is not related to the exposure of interest.

In all the papers, the GA may be related to the exposure; in paper I, with year of birth, although we did use LMP to account for the change in dating practice over the time period. However, induction practices and the clinical use of US from 1999 onwards may have impacted the mean yearly GA also as estimated by LMP during the study period. In paper II, increased HbA1c is associated with higher birthweight, which would indicate a larger fetus at ultrasonography as early ultrasound measurements and size at birth are correlated [317]. Therefore, the decrease in GA with increasing HbA1c may actually be even larger in effect size than what we found, as they are falsely assigned an older GA than "true", if the differences in fetal size were present already at the time of ultrasonography. In that case, we could have performed a sensitivity analysis using LMP, to see whether that differed in effect size from US dating. However, the dating precision using LMP may also be related to maternal BMI and age [318], which were associated with HbA1c in our study, but we did control for maternal BMI and age in our analyses.

Given that US-based estimation of GA depends on fetal size at the time of US, it could be argued that using US estimations on ART-conceived pregnancies may introduce a systematic bias when compared to non-ART-conceived pregnancies as the newborns in general are smaller with ART. Therefore, date of embryo insertion (corrected by 14 days plus the age of

the embryo at the time of the transfer) may be a more correct estimate [6]. However, this will necessarily also introduce some uncertainty as we don't know exactly how long an ART-conceived pregnancy is "supposed" to last compared to a non-ART-conceived pregnancy with regards to timing of implantation and age of the embryo, and the estimation method would differ by exposure group. In paper III, we show the gestational age in the parent generation, which may be slightly biased due to LMP-based estimates for the non-ART (in general) and ART insertion date-based estimates for the ART-conceived. However, we did no formal comparisons of the GA between these two groups. In their own perinatal outcomes, we do not expect the groups to differ in the estimates of GA based on US, as birthweights were similar between the two groups.

Variables used as confounders or possible predictors in papers I and III are based on variables reported to the MBRN. These may be missing, wrongly coded, or not capturing the desired information. For instance, we do not have information on maternal ethnicity, but use maternal country of birth as an approximation. The stratification of births based on maternal country of birth in paper I is therefore only a surrogate for ethnicity and may be a source of bias if ethnicity played an important role in the time trend. This cannot be excluded, as we found the time trend to only be present among infants born to Scandinavian-born women. Information on smoking, folic acid supplement and maternal BMI may also be subject to error in recording, wrong reporting by the mother or missing information in MBRN, though we did not expect this to differ between the exposure groups in paper III, and it was only included in sensitivity analyses in paper I from the years smoking and BMI were registered. Any implausible values were removed, and we did case-complete analyses.

Similarly, self-reported information from MoBa used in paper II may be prone to errors, and although it cannot be excluded, we do not expect this to differ according to maternal HbA1c levels. The exclusion of women from the study population in paper II based on a diagnosis of any DM could be prone to misclassification, as the screening for and coding of GDM has changed somewhat during the study period and historically women were less often screened than today [319]. However, if women would have fulfilled criteria had they been screened, but were not, this is of less concern, as we wanted to estimate the associations between HbA1c levels and perinatal outcomes given no clinical intervention to improve glycaemic control in the given pregnancy.

Assessing preeclampsia as a research outcome might be subject to information bias. A couple of studies have validated this diagnosis in the MBRN. They found that for the time period

prior to new diagnostic criteria and change in the birth certificate to the MBRN around 1998, the positive predictive value of the diagnosis was about 88% according to the criteria at the time, but they did not assess specificity or sensitivity [297]. For the subsequent period, the positive predictive value according to the new diagnostic criteria was reported to be around 81-84%, with a specificity in the MoBa cohort of 99% and a sensitivity of about 43%, with mainly false negatives seen among milder cases of preeclampsia [297,320]. I would expect any misclassification in papers II and III to be non-differential. I can therefore not exclude the possibility that a misclassification of the assigned pregnancies with preeclampsia in papers II and III could have attenuated (towards the null) any associations between the exposures (HbA1c in paper II and parental mode of conception in paper III) and preeclampsia.

Congenital malformations were only assessed as a composite outcome in papers II and III, and used as an adjustment covariate in paper I. This might have led to a failure to identify associations between HbA1c levels or parental mode of conception with specific birth defects. However, given the relatively small study samples in both these papers, we considered the best approach was to study the combined outcome. Furthermore, birth defects or syndromes might have been diagnosed after the infant was discharged from the hospital following delivery, leading to failure to identify neonates with this outcome in the analyses. This would be expected to be non-differentially misclassified, again increasing the likelihood of a bias toward the null.

The only biological sample measured "directly" and not obtained from questionnaire data or registries, were HbA1c levels from NEB. HbA1c is relatively stable in frozen samples stored at -80 ºC [321,322], but we have no direct evidence comparing samples stored at -20 to -80 ºC. Furthermore, HbA1c levels in relation to blood glucose may be altered in pregnancy due to physiological changes. Therefore, we recommend that our findings be interpreted with caution, not suggesting they be used to define new criteria for intervention, but rather to show that HbA1c levels and specific perinatal outcomes are associated on a continuous scale. Furthermore, we did not have information on factors that might interfere with HbA1c measurements, such as blood haemoglobin levels and hemoglobinopathies [256,323,324]. This could have biased our results due to unmeasured confounding. However, given that the genetic variants (HbS and HbC traits) known to affect the accuracy of the measurement method used in our study [324] are very uncommon in the Norwegian population [325], and anaemia in pregnancy is thought to be of minor importance as a confounder of pregnancy outcomes [326] we expect the associations to not be majorly affected.

5.2 Discussion of main findings

5.2.1 Temporal trends in birthweight (paper I)

In paper I, we explored whether various known predictors of birthweight were driving factors of the pattern of an increase and then decrease in mean birthweight in Norway occurring over a 17-year period. Given that this was a registry-based study, we could only explore those variables recorded in MBRN and available during the period of the hump pattern. The pattern was only present among Scandinavian-born women, which could indicate that the pattern may have been induced by culturally related behaviour. However, similar increases were seen in other countries [215,216,219-221,240,242,246], while a (subsequent) decrease has been reported less frequently [232,233,247].

The finding of the pattern only among term births could suggest that something affected the major distribution of birthweights, and not the extreme lower part of the distribution. Another explanatory hypothesis could be that there is an underlying shift in who ends up in the predominant and residual distributions, thus affecting the mean birthweight of the main distribution only. Some other countries also found the trend of increase in birthweights among term births only [236,239]. Secular trends in maternal age and parity distributions have partly explained changes in trends of proportion of infants with a birthweight <2500 grams [250,327], while the decline in birthweights seen in Japan between 1980 and 2004 was not explained by a number of predictors [328]. To be able to change the mean birthweight in a population, a factor would either have to be very prevalent, or have a very strong impact on birthweight. As discussed in paper I, such a factor with a prevalence of 5% would have to decrease birthweights by 1000 grams if we were to see a population decrease of 50 grams in a population with a mean birthweight of 3600 grams and standard deviation of 500 grams. A more realistic magnitude of effect for an exposure would be 200 grams (equivalent to the estimates for smoking), which would require a prevalence of 25%. The hump pattern was present among newborns born to women without a diagnosis of diabetes. As we found in paper II, a change in maternal HbA1c levels of 5 units corresponded to a change in around 40 grams in the birthweight of a term infant, which could imply that maternal glycaemic control during pregnancy could contribute to a change in mean birthweights, but probably not explain the whole hump pattern. Furthermore, we would expect an increase in the proportion of pregnant women with poor glycaemic control over time, as obesity and GDM rates have increased in Norway over the last decades [15,329].

Our study was limited by the lack of information on maternal BMI before 2006 and smoking before 1999. Maternal smoking rates did not impact the decrease in birthweights in the early 2000's but we could not assess a possible impact on the increase before 2000. We lacked information on paternal factors such as smoking, educational attainment and own perinatal outcomes, that could have influenced the time trend.

Some predictors of birthweight that we could have studied but did not include in the paper, were the proportion of multiple births and ART-conceived pregnancies as well as other measures of the size of the newborn. Excluding multiple births and ART births showed the same hump pattern within term births (Figure 8A). This is not surprising given that most multiple births end in preterm deliveries [74], so a change in the rate of multiple births would be unlikely to influence mean birthweight among term births. Mean length (cm) in term births showed the same hump shape, but again increased somewhat from 2008 through 2014, though not to the same level as the zenith of the hump period (Figure 8B). Mean head circumference (cm) among term births also showed the same hump pattern but started on a higher level before the hump than it ended on in the post-hump period (Figure 8C). This suggests an increased fetal size at birth, not just "fatter" babies relative to their length, although we have not studied ponderal index directly. Additionally, given that we did not find any co-occurring trends in adverse outcomes and the trend was apparent among term births only, this could suggest that any underlying cause(s) of the change led to altered fetal growth via non-pathological mechanisms.

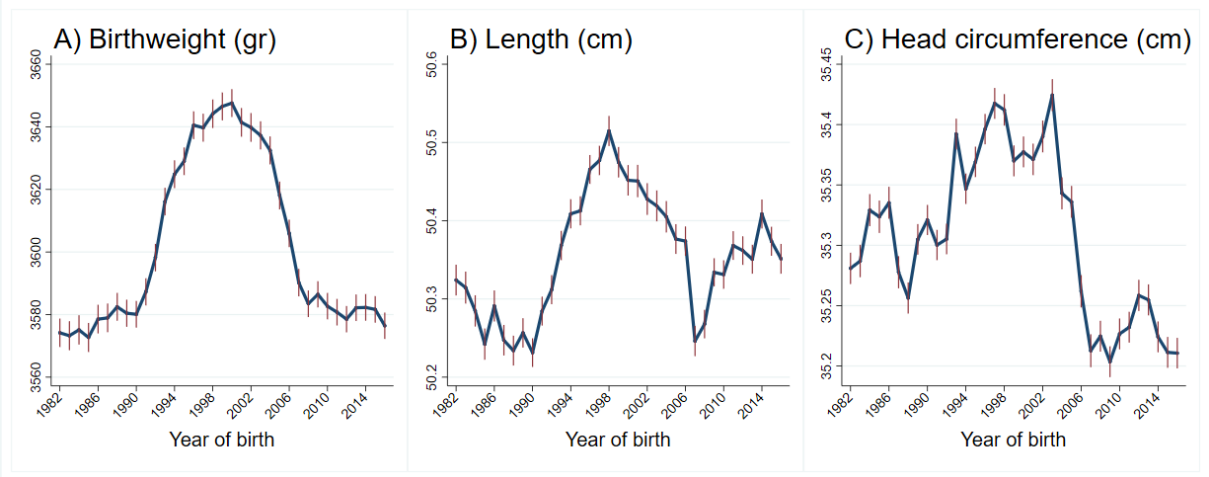

Figure 8. Trends in Norway of anthropometric measures in live-born term births: A) Mean birthweight (grams) after excluding multiples and those conceived by ART; B) Mean length (cm); C) Mean head circumference (cm). (My own estimates from the Medical Birth Registry of Norway.)

5.2.2 Maternal HbA1c levels and perinatal outcomes (paper II)

In paper II, we examined an important determinant of perinatal outcomes in a contemporary Western setting, namely maternal blood sugar regulation during pregnancy. As already discussed, it is well established that poorly controlled maternal DM and GDM during pregnancy poses a risk of adverse maternal and infant outcomes. Diagnosis of GDM is currently based on fasting glucose and glucose tolerance testing measures, and less attention is paid to values in the upper "normal" range [252]. After screening guidelines for GDM changed in Norway in 2017, an increasing number of pregnant women are eligible for screening, though adherence to screening is low, and the usefulness of the increased screening rates has been questioned [319]. The use of HbA1c testing in pregnancy has been less studied and is currently not recommended [252,260].

We found a linear relationship between HbA1c levels and fetal anthropometrics, suggesting a dose-response relationship between maternal glucose levels, here represented by HbA1c, and the rate of fetal growth. Furthermore, HbA1c levels were associated with GA and risk of preeclampsia, though mainly in the upper range of the "normal" HbA1c values. This is in line with both earlier and more recent studies on HbA1c levels at other timepoints in pregnancy and subsequent perinatal outcomes [264,272-274,330], and in contrast with some other studies where no associations were observed [255,275,276].

Some other studies have indicated that although early HbA1c levels are not specific enough to substitute screening for GDM with an OGTT at weeks 24-28, those with higher HbA1c levels may be at increased risk of adverse pregnancy outcomes than those with lower levels even when restricting to only women with GDM [268,269]. One study has shown increased risks of adverse pregnancy outcomes including LGA newborns, preeclampsia, preterm delivery, perinatal death and GDM diagnosis in women with HbA1c levels within the "pre-diabetic" and lower diabetic range (41-49 mmol/mol [5.9-6.6%]) measured before week 20, also when women with GDM who were treated were included in the study population [331]. A study in Chinese women found that HbA1c levels in the normal range at the time of OGTT-testing (weeks 24-28) were associated with risk of preeclampsia, macrosomia and preterm delivery among women both with and without GDM, with a stronger association in non-GDM women, possibly due to no intervention in this group [332]. All these studies support that measuring HbA1c levels in pregnancy could be of clinical value.

We did not find a strong association between HbA1c levels and the risk of delivering an SGA infant. However, the usefulness of studying SGA as a clinical outcome has been debated [333].

Using a hard cut-off at 1500 grams or 2500 grams to study the risk of "low birthweight", may lead to a study of primarily preterm infants [334]. Using SGA as an outcome aims to take possible effects of GA into account but raises the problem of its constant prevalence of 10% among all births. This might lead to a failure to identify infants with "true" growth restriction, as the smallest 10% at each GA are not necessarily those with restricted growth. However, given the association between GA and HbA1c levels, we assumed that any association between HbA1c and SGA might be more biologically interesting than birthweight below a given cut-off. The same arguments might be even stronger in the case of LGA versus macrosomia as an outcome, given that the shorter pregnancy duration at higher HbA1c levels would give the fetus limited chance of obtaining a weight of 4000 or 4500 grams, even with an increased growth rate *in utero*, thus leading to a failure to detect an increased risk of high fetal growth velocity.

The studies on HbA1c, including paper II in this thesis, suggest that continuous blood glucose levels during pregnancy, and not just the impaired post-prandial control of glucose spikes identified by OGTT, is an important regulator of several pregnancy processes. Furthermore, a study identified an association between the development of HbA1c levels by trimesters and birthweight [276], which may indicate both that early maternal blood glucose regulation and maintaining this regulation over time with the increased metabolic stress of an ongoing pregnancy are important for fetal growth. Additionally, fetal growth, pregnancy duration and possibly also risk of HDPs are affected on a continuous scale, not just at upper or lower extremes of an exposure [253]. Exercise in pregnancy has been shown to lower both glucose levels and the risk of developing GDM [153,335] as well as the risk of delivering a macrosomic newborn [147]. Consequently, it would not be surprising if interventions that are successful in improving perinatal outcomes among women with GDM, also would improve outcomes in women with high-normal HbA1c levels.

Furthermore, we found an association between maternal HbA1c levels and both maternal age and pre-pregnancy BMI. The ongoing shifts in characteristics of pregnant women in developed countries with higher maternal age at birth and increased BMI, could suggest that an increasing number of pregnancies will be to women within the upper "normal" range of blood glucose regulation. This could affect the proportion of pregnancies complicated by adverse outcomes, as well as mean birthweight and gestational length, and might necessitate a shift in antenatal management and screening practices, potentially using other tools than those

used today. Further studies are required to identify the optimal management strategy on a national level.

5.2.3 Perinatal outcomes in pregnancies to parents who were conceived by assisted reproductive technologies (paper III)

In paper III, we examined a possible determinant of perinatal outcomes, namely parental mode of conception. As discussed in the Introduction, there is a substantial amount of literature supporting an increased risk of adverse outcomes in pregnancies conceived by ART, and the offspring may be at increased risk of later cardiometabolic disease. It has also been shown that pregnancies conceived by infertile couples have an increased risk of adverse outcomes [97-100]. Furthermore, perinatal outcomes have a tendency of recurrence in the subsequent generation [132-134], and there are indications that those who are born preterm may experience lower fertility rates [2,291].

If women and men conceived by ART experienced more adverse outcomes in the pregnancies they mothered or fathered, this could be driven by inherited risk factors from their own parents linked to infertility, induced by the procedures inflicted on themselves at the fertilization and embryo stages, and possibly also later life effects of being born at lower birthweights, shorter gestation, and increased risks of having been born to a pregnancy complicated by an HDP [40]. However, we did not find convincing evidence that the studied perinatal outcomes differed between those who were conceived by ART or not. However, we found a difference in sex ratio between women conceived by ART and not, and although a true difference cannot be excluded, we deem it more likely to be a false positive finding [336].

Below, I speculate on the possible mechanisms for a lack of difference in perinatal outcomes aside from those already mentioned regarding selection bias into pregnancy in chapter 5.1.2.

Firstly, “externally” induced low birthweight and shorter gestations may not be “transferred” to the next generation, such as is the case for twins [337,338] and possibly for smoking [339]. If so, this would support the notion that gestational age and birthweight are mostly determined by genetic components in the mother and fetus unless an external factor impacts these outcomes. Similarly, it has not been found that twins are of increased risk of cardiovascular disease and cancer in adult life compared to singletons [340], suggesting that the mechanisms leading to impaired fetal growth may differ according to plurality. The same could be true for ART-conceived women and men, which could indicate that the adverse outcomes experienced in

ART-conceived pregnancies are mainly induced by the ART procedures and not genetic and other factors leading to use of ART to conceive.

Secondly, we have not done recurrence analyses, due to low numbers among the ART-conceived women and men with a registered pregnancy. We have therefore not formally assessed the risk of experiencing for instance an HDP given that you were born to a pregnancy complicated by an HDP, and whether this risk differed according to mode of conception.

Thirdly, it is possible that adverse outcomes associated with infertility may only be clinically relevant, or differ enough to be detectable between groups of the sizes included in this study, at older ages at the time of pregnancy. The cohorts analysed in paper III were on average younger than the current mean age at first birth in Norway [15], which is associated with more favourable perinatal outcomes, at least for women. However, we cannot draw definite conclusions, as the whole reproductive period of even the first cohorts born after conception from ART is not completed. Indeed, studies have indicated that adolescents born after conception by ART are at increased risks of puberty disorders [341], which could indicate that they might also be at increased risk of infertility [283], and we cannot exclude the possibility of selection of the healthiest ART offspring, possibly those who did not inherit genes predisposing them to infertility, into pregnancy, at this point in time. Additionally, we cannot distinguish possible biological from social selection mechanisms into pregnancy with the available information in the registries used in paper III.

Furthermore, the majority (~80%) of the study participants who had a registered pregnancy as the mother or father were conceived by IVF without ICSI with fresh embryo transfer. Therefore, we cannot deduce whether adults conceived with other ART methods, including frozen embryo transfer and IVF with ICSI, experience similar fertility and perinatal outcomes when they endeavour to become parents. Additionally, we cannot exclude the possibility of low numbers limiting our ability to detect “true” differences between ART-conceived and non-ART-conceived parents. These questions need to be explored further in later studies.

6 CONCLUSIONS

In the work included in this thesis, I studied determinants of perinatal outcomes in contemporary Norway, between 1982 and 2020, with special focus on gestational length and birthweight. I exploited existing Norwegian population registries to capture all births in Norway (paper I and III), and used data from a large pregnancy cohort with more detailed information to study a possible determinant for which information is not available in the registries (paper II). The studied determinants are all closely linked to contemporary lifestyle factors and recent changes in reproductive trends and interventions.

The temporal trend of mean birthweights among live births in Norway with an increase in the late 1990's followed by a decrease in the beginning of the 2000's was seen only among term births to Scandinavian-born women. It was not accompanied by concurrent trends in neonatal mortality, preterm rates, or low Apgar scores. The trend was not explained by offspring sex, parity, onset of delivery, marital status, seasonality, maternal age, maternal year of birth, gestational length, or newborns with congenital anomalies. Preeclampsia and maternal smoking could not explain the birthweight decline. This suggests that there might be unknown determinants of birthweight that are not detrimental to infant health, the discovery of which could help understand fetal growth processes beyond those by pathological mechanisms.

We found that maternal HbA1c levels within the normal range in gestational week 18 were associated with birthweight, pregnancy duration, risk of preeclampsia and delivery of a large-for-gestational-age infant, with less strong associations with head circumference, length at birth and preterm delivery. This suggests that HbA1c levels reflect glycaemic control also in pregnancy, even with an altered physiological state and increased turnover of red blood cells. Furthermore, maternal glucose levels seem to impact perinatal outcomes on the whole continuum of the scale, not just in the extreme levels of impaired glucose control.

Except for the odds of having a boy, which was lower among ART-conceived women, perinatal outcomes did not differ substantially by parental mode of conception. However, women and men conceived by ART had fewer pregnancies by the end of follow-up compared to their peers. These findings are in contrast with studies on transgenerational recurrence of perinatal outcomes, suggesting that ART procedures might play a more important role in the adverse perinatal outcomes experienced in ART-conceived pregnancies than genetic factors leading to use of ART.

7 FUTURE RESEARCH

The studies included in this thesis raise some questions that can lay the foundation for some interesting future research projects.

Birthweight may impact long-term health, although the potential mechanisms this could work through are complicated, debated and to some extent unclear. The rise and fall in mean birthweights in Norway between 1991 and 2007 were not explained by known determinants, pointing to unrecognised factors influencing the whole population over a few decades. This could potentially mean that there might be a difference in the future health among adolescents and adults who were born during this period. It would be interesting to assess whether adult height, weight and BMI follow the same hump-pattern as that of birthweight for the corresponding birth cohorts, and later to study the risk of cardiovascular disease and cancers. If there is any changes in trends (or no change in trends) according to birth cohorts in the hump period, this might lead to improved understanding of the importance or unimportance of birthweight for later health.

The findings in paper II on the associations between maternal HbA1c levels in gestational week 18 and perinatal outcomes suggest that there could be clinical value in measuring HbA1c in pregnancy at an earlier timepoint than the current screening for GDM done with OGTT. To determine the potential usefulness of measuring HbA1c levels routinely in the second trimester of pregnancy would require an RCT on HbA1c measurement around gestational week 18 versus conventional GDM screening, and the predictive power of detecting risk of adverse perinatal outcomes. Additionally, to assess the usefulness of potential earlier identification of at-risk individuals would require an RCT of lifestyle intervention to those within the highest "normal" range of HbA1c levels and assess whether this improves pregnancy outcomes. This would have to be compared to the effect of lifestyle interventions started after GDM screening around week 24. Furthermore, it would be interesting to estimate the risk of development of type 2 DM in the women studied in paper II later in life. This outcome could be assessed in the two interventional studies suggested above, with type 2 DM as a later endpoint (that ultimately requires a much longer follow-up) in addition to the perinatal outcomes.

Although the findings in paper III on reproductive outcomes in women and men conceived by ART are reassuring, longer follow-up time is required to obtain information on their complete reproductive period. This would capture potential delayed childbearing and total number of

children in this group, and be required to conduct a more comprehensive study on both fertility and perinatal outcomes. Furthermore, as the majority of study participants were conceived with IVF and fresh embryo transfer, our findings of similar perinatal outcomes may not extend to cohorts born after other procedures, such as IVF with ICSI and/or frozen embryo transfer. Additionally, younger cohorts born after ART may have been exposed to other culture media, hormonal stimulation protocols of the mothers, time of culturing and different biological and social selection into the use of ART. For more reliable estimates for subtypes of ART procedures, a Nordic collaboration using additional data from one or more Nordic countries such as has been established through CoNARTaS (the Committee of Nordic Assisted Reproductive Technology and Safety), could be helpful.

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9 PAPERS I-III

Paper I:

I

Stumped by the Hump: The Curious Rise and Fall of Norwegian Birthweights, 1991-2007.

Carlsen EØ, Magnus MC, Omsland TK, Magnus PM, Håberg SE, Wilcox AJ. Epidemiology. 2020 Jul;31(4):587-594. PMID: 32427635. DOI: 10.1097/EDE.0000000000001211

MS# EDE19-0710

Original Research Article

Stumped by the Hump:

The Curious Rise and Fall of Norwegian birthweights, 1991-2007

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Running head: The Norwegian Birthweight Hump

Conflicts of interest: None.

Source of Funding: This work was funded by the Norwegian Research Council's Centres of Excellence Funding Scheme (no. 262700). MCM works at Medical Research Council Integrative Epidemiology Unit at the University of Bristol, which receives infrastructure funding from the UK MRC (MC_UU_00011/3 and MC_UU_00011/6).

Description of process to obtain data and codes to replicate findings: Data is available from the Medical Birth Registry of Norway, after approval by a Norwegian Regional Committee for Medical and Health Ethics.

Abstract

Background: There was a distinct rise in mean birthweights in Norway starting in 1991 that plateaued in 1996-2002 and then declined to previous levels. We investigated whether these changes corresponded to trends in neonatal mortality or other birthweight-associated pregnancy outcomes. We also explored known predictors of birthweight and examined whether these could explain the birthweight trends.

Methods: We calculated mean birthweight for all live births in Norway in each year from 1982 to 2016, together with annual neonatal mortality and proportion of infants born preterm, or with low Apgar score. We stratified mean birthweight over time by factors including parity, gestational age, and Scandinavian versus non-Scandinavian origin of mother, to test robustness of the pattern. In addition, we used multivariable linear regression to obtain adjusted estimates for mean birthweight per year.

Results: A 50-gram rise and fall of mean birthweights during a 25-year period was not accompanied by corresponding changes in neonatal mortality, preterm births, or Apgar scores. The distinct hump pattern was restricted to term births and was not apparent among infants of mothers born outside Scandinavia. We saw a similar pattern for Sweden but not Finland. Known predictors of birthweight (such as parity, mode of onset of delivery, and marital status) did not explain the hump.

Conclusions: A distinct temporal hump in mean birthweight among Norwegian term births had no obvious explanations. Furthermore, these fluctuations in birthweight were not associated indirectly with adverse outcomes in measures of infant health.

Keywords: birthweight; Norway; ethnicity; neonatal mortality; preterm

Introduction

Birthweight is the most studied birth characteristic, and both high and low birthweights have been linked to adverse short- and long-term outcomes in the child, including infant mortality,[1] cardiometabolic diseases[2,3] and cancers.[4,5] Known predictors of infant birthweight include maternal body mass index (BMI), gestational weight gain, height, cigarette smoking, blood glucose and insulin levels during pregnancy, ethnicity, and parity.[6-8]

Despite the strong association between birthweight and perinatal outcomes, the shift in birthweight distributions seen with altitude and with maternal characteristics such as ethnicity and parity are not necessarily mirrored by similar shifts in neonatal mortality or other birth outcomes. Previous assumptions regarding birthweight as a causal factor for later disease have been questioned both methodologically[9,10] and by identification of common genetic determinants for both birthweight and later adult chronic diseases.[2,11] Regardless of underlying etiologies, birthweight is an important predictor of later health outcomes, and a better understanding of influencers of birthweight is valuable. Abrupt fluctuations in population birthweight may suggest a role for environmental factors that could be of importance for later health.

Mean birthweight in Norway has been relatively stable over the last 5 decades, with a striking exception: mean birthweights increased by approximately 50 grams between 1990 and 2000,[12] remained at this higher level for several years, and then fell back to previous levels in the following ten years,[13] making a distinctive hump-like pattern (Figure 1).

The objective of the current study was to explore whether the birthweight hump was accompanied by similar trends in adverse perinatal outcomes, and whether the birthweight hump itself could be explained by changes in gestational age, maternal parity, ethnic background, or other known predictors.

Methods

Data sources

This study included all live births in Norway between 1982 and 2016 registered in the Medical Birth Registry of Norway. We excluded live-born children with implausible or missing registrations, such as missing birthweight or birthweight below 500 grams (n=1966), and live-born children with either missing gestational length or a gestational length less than 154 days or >315 days (n=172,711).

We obtained data on Swedish birthweights by year of birth (1982-2016) from the Swedish Medical Birth Registry online database,[14] and Finnish birthweight data (1987-2016) from a Nordic report on perinatal statistics.[15]

Norwegian legislation does not require consent from registered individuals to conduct research using the national health registries. The Regional Committee for Medical and Health Ethics of South/East Norway approved the study (No. 2014/404).

Birthweight and potential explanatory factors for the changes in birthweight

Information on birthweight in grams was available in the Norwegian registry. We also obtained information on factors known to predict birthweight, including gestational length (in days) based on last menstrual period, parity (primipara versus multipara), onset of labor (spontaneous, medically induced or by cesarean section), marital status (recorded as either married, cohabiting, non-married/single, divorced/separated/widowed or other/unknown), the presence of a congenital anomaly (yes or no) and maternal country of birth as either Scandinavian (Norway, Sweden, and Denmark) or other (as a proxy for ethnicity). Furthermore, for sub-analyses, we obtained information on maternal year of birth, smoking at the end of pregnancy (daily/occasional versus never), diagnosis of preeclampsia, eclampsia or HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count), diabetes mellitus, and pre-pregnancy body mass index. Information on maternal smoking during pregnancy was available only after 1998, while information on maternal pre-pregnancy height and weight (for calculating BMI) was available only after 2006.

Term birth was defined as delivery after 37-45 completed gestational weeks (gestational days 259-315), and preterm births as delivery before 37 completed weeks (258 days or less).

We defined neonatal mortality as death of a live-born child within the first 28 days, and low Apgar-score as a score less than seven at 5 minutes. Births in June, July, or August were classified as summer births; September, October, and November as fall births; December, January, and February as winter births; and March, April, and May as spring births.

Information about maternal diabetes mellitus is registered in the birth registry as either type 1, type 2, gestational onset, use of diabetes medication during pregnancy, or diabetes before pregnancy but of unknown type. Screening, diagnosis and registration of diabetes during pregnancy have changed several times during the study period,[16-18] so we defined maternal diabetes broadly to include any registration of diabetes versus no registration. In addition, we analyzed births by the county the mother was registered as living in at time of delivery (19 counties in Norway).

Statistical methods

We described the hump by calculating the mean birthweight and 95% confidence intervals (CIs) for all live births in each year. For some subsequent analyses we grouped year of birth according to the various periods in the hump (Table 1), namely the years before the rise (1982-1990), the years of rising weights (1991-1995), the zenith (1996-2002), the decline (2003-2006), and the remaining years (2007-2016). We described child and maternal characteristics as well as other birth outcomes for each period (Table 1). We calculated standard deviation of birthweight and the birthweight corresponding to the 90^{th}, 10^{th}, 5^{th} and 2^{nd} percentiles for each time period (Table 1). For sub-analyses, we grouped the zenith period into two (zenith I, 1996-1998, and zenith II, 1999-2002). This was done to accommodate the lack of registration of preeclampsia, maternal BMI, and smoking for the earlier part of the study. We described child and maternal characteristics for each period (eTable 1).

We explored time trends in other outcomes among all births. Annual proportions of neonatal mortality, low Apgar score, and preterm birth were calculated by dividing all observations with each

outcome during a given year (nominator) by all births during that year having available information about the outcome (denominator).

Further, we calculated mean birthweight separately for term and preterm births in each year. Since the birthweight hump was apparent only among term births, we conducted subsequent analyses on term births only. Among term births, we estimated mean birthweight each year within strata of primipara and multipara, seasons of birth, and Scandinavian and non-Scandinavian mothers. We performed sub-analyses in the same way, stratifying on boys and girls, diagnosis of diabetes mellitus, the various onset of deliveries, maternal county, and each gestational week.

We used multivariable linear regression to adjust for the change in mean birthweight over time. Adjustment factors included maternal age and maternal age squared (continuous variables); parity (primipara versus multipara); gestational age in days, and gestational age in days squared within term births (as continuous variables); onset of delivery as a categorical variable (spontaneous onset of labour, caesarean section and induction); congenital anomalies (yes versus no); and marital status as a categorical variable (married, cohabiting, non-married/single, divorced/separated/widowed and other/unknown). We set 1982 as the reference value and used year of birth in 1-year intervals from 1983 to 2016 as the main exposure. We restricted this multivariable analysis to term births born to Scandinavian mothers.

Registration of preeclampsia in the Birth Registry was improved substantially in 1999, making adjustment for this variable uncertain before 1999. We therefore performed a sub-analysis with linear regression as described above from 1999-2016 with additional adjustment for preeclampsia, which we defined as any diagnosis of preeclampsia, eclampsia or HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count) (n=731,219). Information on smoking in pregnancy was collected in the birth registry from 1998, partway through the birthweight hump. We performed a sub-analysis with linear regression as described above, including preeclampsia, from 1999-2016 with additional adjustment for smoking (n=631,733), and a separate sub-analysis with additional adjustment for maternal BMI, which was available from 2007 (n=189,992). For all these sub-analyses, the year 2007 was set as reference value, and year of birth in one-year intervals was the main exposure.

To explore possible maternal cohort effects, we performed an additional sub-analysis with linear regression as described above for the whole period, adding maternal year of birth in 10-year categories (<1950, 1950-1959, … , 1980-1989, >=1990) as a covariate (n=1,410,142), again setting the year 2007 as reference value.

All analyses were performed using Stata (Statacorp, College Station, TX), version 15.0.

Results

Among 1,859,312 live births in Norway between 1982 and 2016, mean birthweight increased by about 50 grams from 1991 to 1997, and decreased with a similar magnitude between 2002 and 2007 (Figure 1, Table 1). There was no evidence of similar patterns for neonatal mortality, preterm delivery or low Apgar score (Figure 2).

The distinctive pattern of rise and fall in birthweight was present only among term births (Figure 3, Table 1), among whom birthweights increased and decreased by about 60 grams. During the same time period, the standard deviation of birthweights increased by about 20 grams as birthweights rose, and then decreased with decreasing birthweight (Table 1). The pattern of rising and falling birthweights was not present among preterm births. There was a general decline in preterm birthweights during most of the study period, with a hint of an upward trend after 2004 (Figure 3). After stratifying term births by gestational week, the hump pattern remained virtually unchanged for the various weeks, with some slight modifications (eFigure 1).

Birthweights at the 10^{th} percentile and above generally followed the same hump pattern as mean birthweight. However, at lower percentiles, the hump pattern flattened, and disappeared completely below the fifth percentile (Table 1).

In line with increased rates of induction for post-term pregnancies, the overall lengths of gestation decreased steadily during the study period (albeit with a slight increase between 1991 and 1994) (Table 1).

The proportion of deliveries by primipara women first decreased and then increased during the study period (Table 1). However, this did not explain the birthweight hump, which was present within strata of both first and later births (Figure 4A). The birthweight hump was also consistent across all seasons (Figure 4B) and in both sexes (eFigure 2). The birthweight hump persisted after excluding women with a diagnosis of diabetes mellitus (eFigure 3). The hump persisted in births with spontaneous and medical induction onset of delivery, but is less distinct for births with onset as

caesarean section (eFigure 4). The pattern was similar across all regions (19 counties) in Norway (eFigure 5).

The birthweight of term infants born in Norway to any Scandinavian mother (those born in Norway, Sweden or Denmark) showed approximately the same hump (Figure 4C), with an increase in mean birthweight between 1982 and 1997 of about 80 grams and a subsequent decrease of about 50 grams. The mean birthweight of infants of women born outside of Scandinavia were generally around 100 grams lower and increased slightly in the study period (Figure 4C).

Adjusted change in mean birthweight showed similar time trends as the unadjusted, although the post-hump level did not fully return to pre-hump levels (Figure 5). The more limited time frames (eTable 1) available for data on preeclampsia, maternal smoking, and maternal BMI showed similar birthweight trends as in the main analysis (eFigure 6). Similarly, adjustment for maternal birth cohort yielded a pattern like that in the main analysis (eFigure 6). In summary, none of the known predictors examined were able to explain the hump pattern.

We sought to explore the extent of this distinctive birthweight pattern among neighboring countries. Sweden showed a birthweight hump similar to Norway's, while birthweights in Finland followed a quite different pattern (Figure 6).

Discussion

Norway experienced a distinct temporal increase in mean birthweight of about 50 grams between 1991 and 1995, with a subsequent decrease from 2003-2006. This hump in mean birthweight was restricted to term births and was seen only among offspring born to Scandinavian women. The hump could not be explained by trends of maternal parity, infant sex, mode of onset of delivery, county, smoking, or season of birth. Moreover, indicators of perinatal health known to be associated with birthweight (neonatal mortality, 5-minute Apgar score less than seven, and preterm delivery) did not show similar or reciprocal patterns. Further, there was no evidence of a maternal cohort effect, although the separation of a possible cohort effect when exploring a period effect is challenging.

In the same period, a similar hump was evident in Sweden, but not in Finland. An increase in mean birthweight during similar time periods was also observed in Canada (1978-1998), the United States (1985-1998), Denmark (1990-1999), and the north of England (1982-2000), with a subsequent decrease in Canada and the US, leading to a hump-like pattern not dissimilar to the one in Norway.[7,19-25] As in Norway, the increase in birthweight in the US and Canada was observed mainly for term infants and not for preterm.[19]

Known determinants of birthweight did not explain the Norwegian birthweight hump. Recent increases in birthweight in other countries have been attributed to cessation of smoking, healthier lifestyle habits, and an increase in maternal BMI.[7] These factors did not explain the Norwegian pattern. In fact, the decline in Norwegian birthweights after 2002 paradoxically occurred during a time when maternal smoking levels were steadily decreasing and BMI among fertile women was increasing.[26,27] Furthermore, our sub-analyses adjusting for maternal BMI and smoking in the periods for which we had information showed no evidence of their influence on the birthweight trend.

During the study period, induction practices for pregnancies progressing beyond 40 weeks shifted towards more intervention,[28-30] which may explain the decrease in average gestational length. However, adjustment for gestational length in term births in our regression analysis did not explain the observed temporal birthweight changes. Primiparous women have children with lower birthweight as well as an increased risk of many adverse pregnancy outcomes, including preterm birth and neonatal

mortality.[31-33] The hump was present in births by both primiparous and multiparous women in our study.

Another predictor of birthweight is ethnicity or country of mother's birth.[8] This was not consistently reported in the earlier years of the study period; mother's country of origin was unrecorded for 15% of births in 1982, and fell to less than 1% in 2016. Among mothers with recorded country of birth, the proportion of deliveries to mothers born in Norway decreased from about 95% in 1982 to 71% in 2016. The temporal change in birthweight was not present when restricting to pregnancies by mothers born outside the Scandinavian countries. Lifestyle and cultural factors may have played a role in the observed birthweight trend, as women of Scandinavian heritage would be expected to be similar in their habits. The fact that Sweden experienced a similar birthweight hump supports this conjecture.

A previous study on the rise and fall of birthweights in a regional cohort in Norway speculated on the possible role of sugar-sweetened carbonated soft drinks as a factor leading to maternal weight gain and higher birthweights followed by decreasing intake and lower birthweights.[34] However, a subsequent study found that increased consumption of soft drinks during pregnancy was associated with lower birthweight, thus not supporting soft drinks as an explanation for the birthweight hump.[35]

A single factor that could shift the birthweight distribution in the pattern observed (or two different factors at work for the increase and decrease) would either have to be very common or have a very strong influence on the birthweight. We explored this idea further with a simple simulation. Assuming a mean birthweight of 3600 grams and a standard deviation of 500 grams (close to the situation in this study), a risk factor with a prevalence of 5% would have to decrease birthweight by 1000 grams to lead to an overall population decrease of 50 grams. Even with a prevalence of 10%, exposed individuals would have to decrease their birthweight by 500 grams. With a more realistic magnitude of effect, such as 100 grams, the risk factor would need to have a prevalence of 50% in the population, and an effect of 200 grams corresponds to a prevalence of 25%. We were not able to identify any factors that come close to those parameters, with the possible exception of a shift in the

proportion of foreign-born mothers. There are apparently important determinants of birthweight that have yet to be identified.

Strengths and limitations

This study was based on registry data comprising all registered live births in Norway between 1982 and 2016, giving valid estimates of birthweight and other perinatal measures in the complete population of Norway. Some procedures for registration in the birth registry were revised in 1999, but for most measures in this study, the recordings were similar across the study period.

There have been no major changes since the 1970s in the type of scales used to measure birthweights. Standardization procedures for the measurement of birthweight from 1993 are still in use (*personal communication, Nils Magnar Thomassen at the Norwegian Metrology Service, 17.10.19, e-mail*), leaving measurement error as an unlikely explanation for the observed time trend.

Although we have information on maternal country of birth, we lack specific information on maternal race/ethnicity, a factor known to influence birthweight in the offspring.[19] Second-generation immigrants born in Norway were recorded as having Scandinavian origin, possibly blurring the distinction between Scandinavian and non-Scandinavian origin of mother in later years of the study period. However, given that Norwegian-born mothers contributed 93% of births in 1982-1990, it is unlikely that the distribution would have shifted enough to affect the observed downward change in mean birthweight starting already in 1999.

Furthermore, we did not have access to maternal BMI or smoking habits during pregnancy for the earlier part of the study period or reliable diagnosis of preeclampsia, which limited our ability to adjust for these factors. However, our sub-analyses for the years for which we had these data did not indicate that the decreasing smoking trend changed the decreasing segment of the hump. We saw no effect of maternal diabetes on the birthweight hump pattern, although the diagnosis of gestational diabetes mellitus has changed markedly during the study period,[16-18] limiting the interpretation of this adjustment.

Conclusions

A distinct rise and fall in mean birthweight occurred in Norway from 1992 to 2007. We could not explain this unusual rise and fall of birthweight by known predictors of birthweight. We acknowledge that our explorations to evaluate the effects of changes in birthweight are indirect. Even so, it is clear that the changes in birthweight over a relatively short period had no apparent consequences for either neonatal death or Apgar scores.

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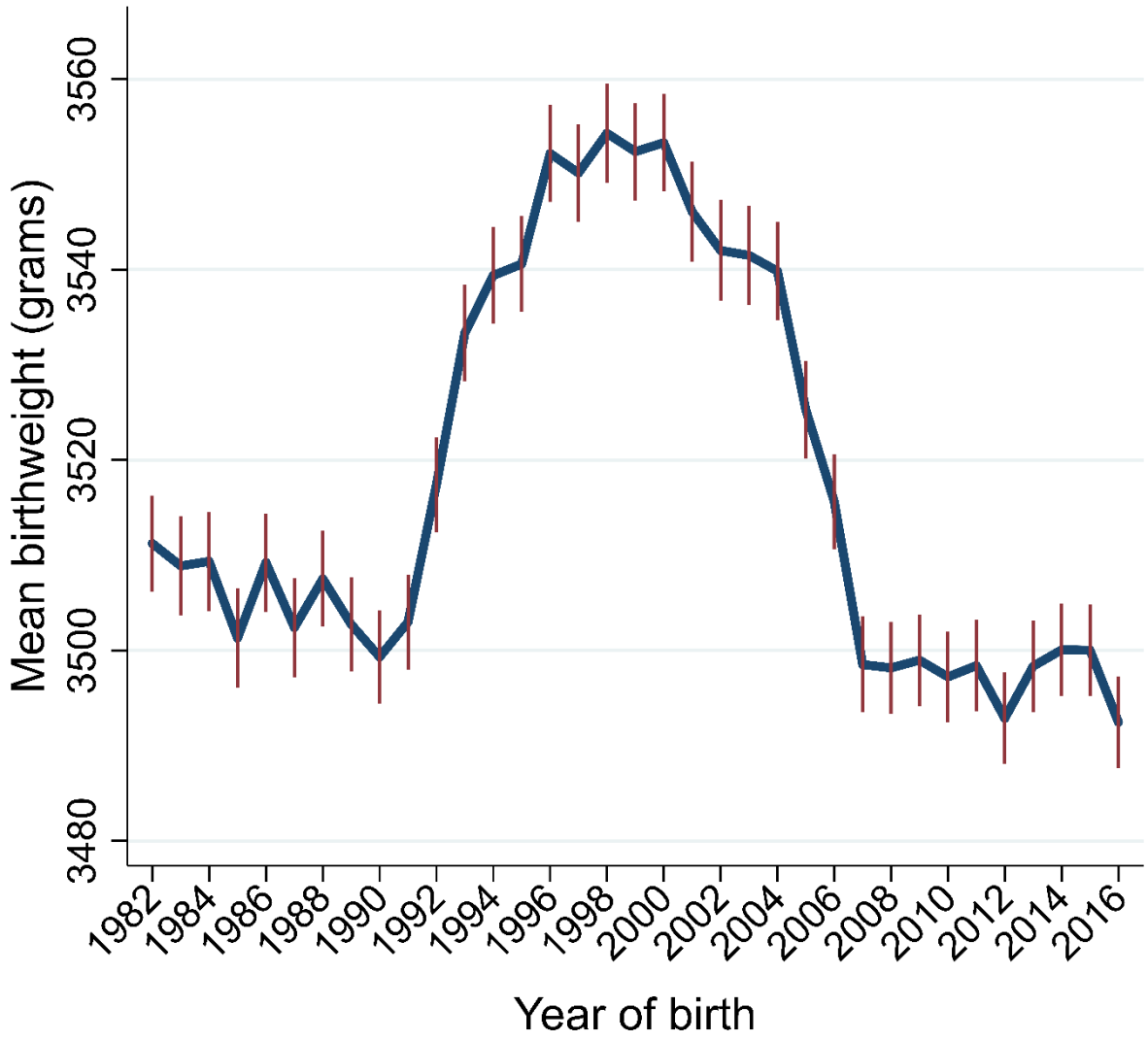


Figure 1: Mean birthweight for all live born children in Norway by year of birth from 1982 to 2016. Vertical lines show the 95% confidence intervals.

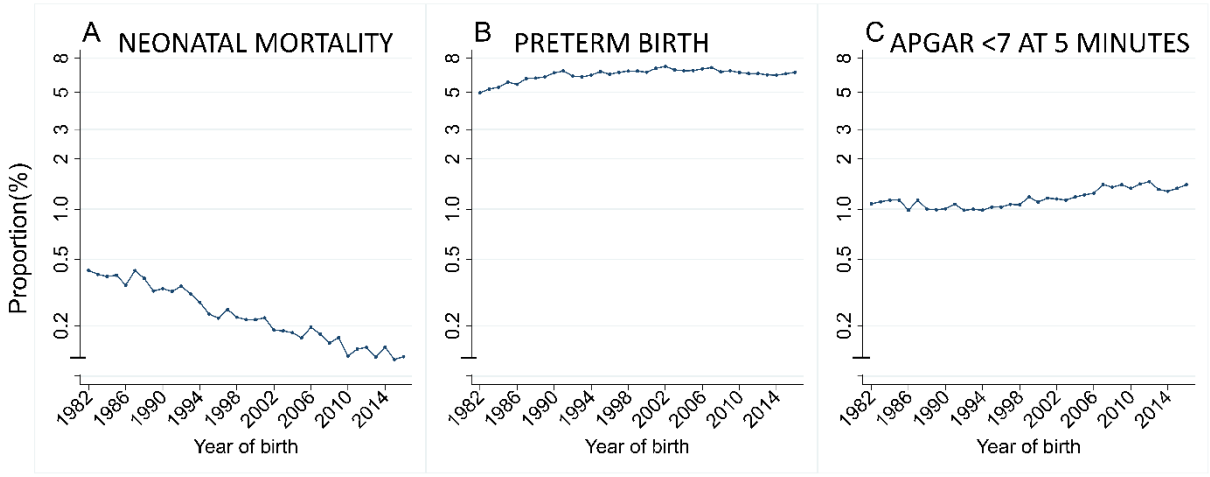


Figure 2: Proportion (% of all live born children) with A: neonatal mortality, B: preterm births, and C: newborns with Apgar score less than seven at five minutes, in Norway in the years 1982 to 2016. Note the use of log-scale for the y-axes.

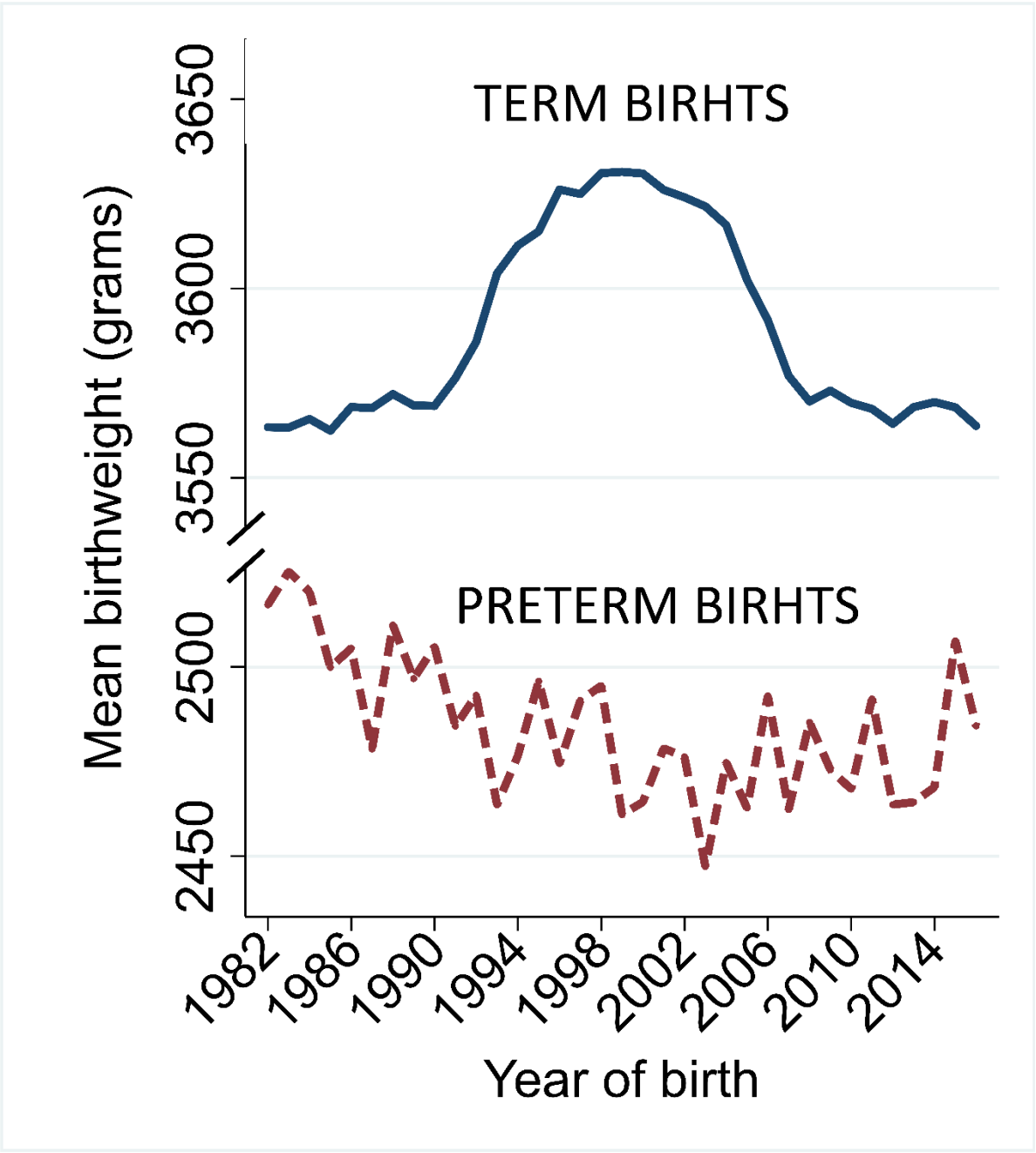


Figure 3: Mean birthweight by year of birth in Norway from 1982 to 2016. For preterm births (<37 gestational weeks and term live born children by year of birth.

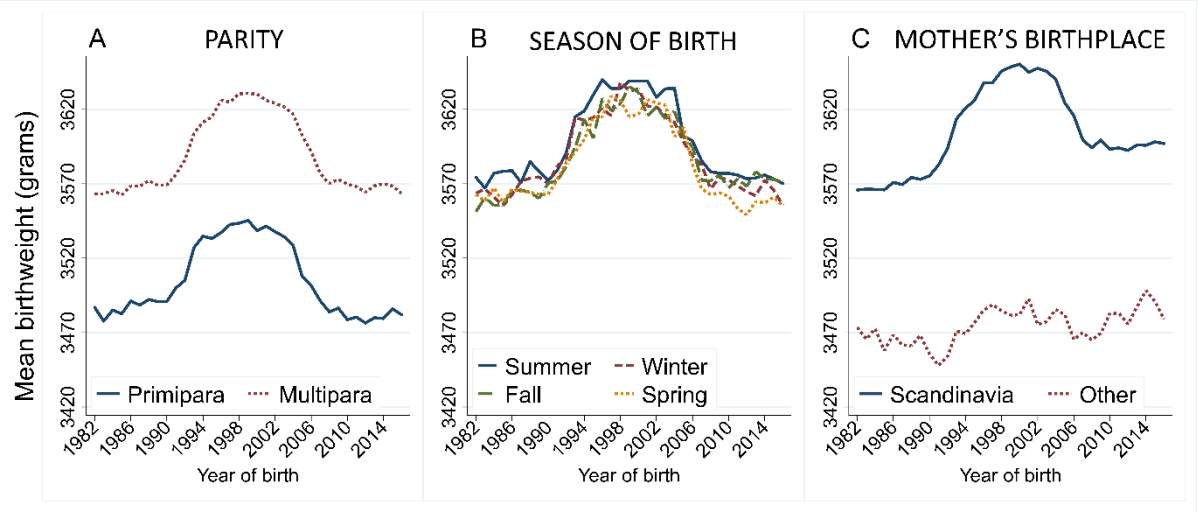


Figure 4. Mean birthweight (in grams) in live born term births by year of birth by following groups: A: parity; B: season of birth (Summer: June, July, August; Winter: December, January, February; Spring: March, April, May; Fall: September, October, November); and C: mother born in Scandinavia or other country.

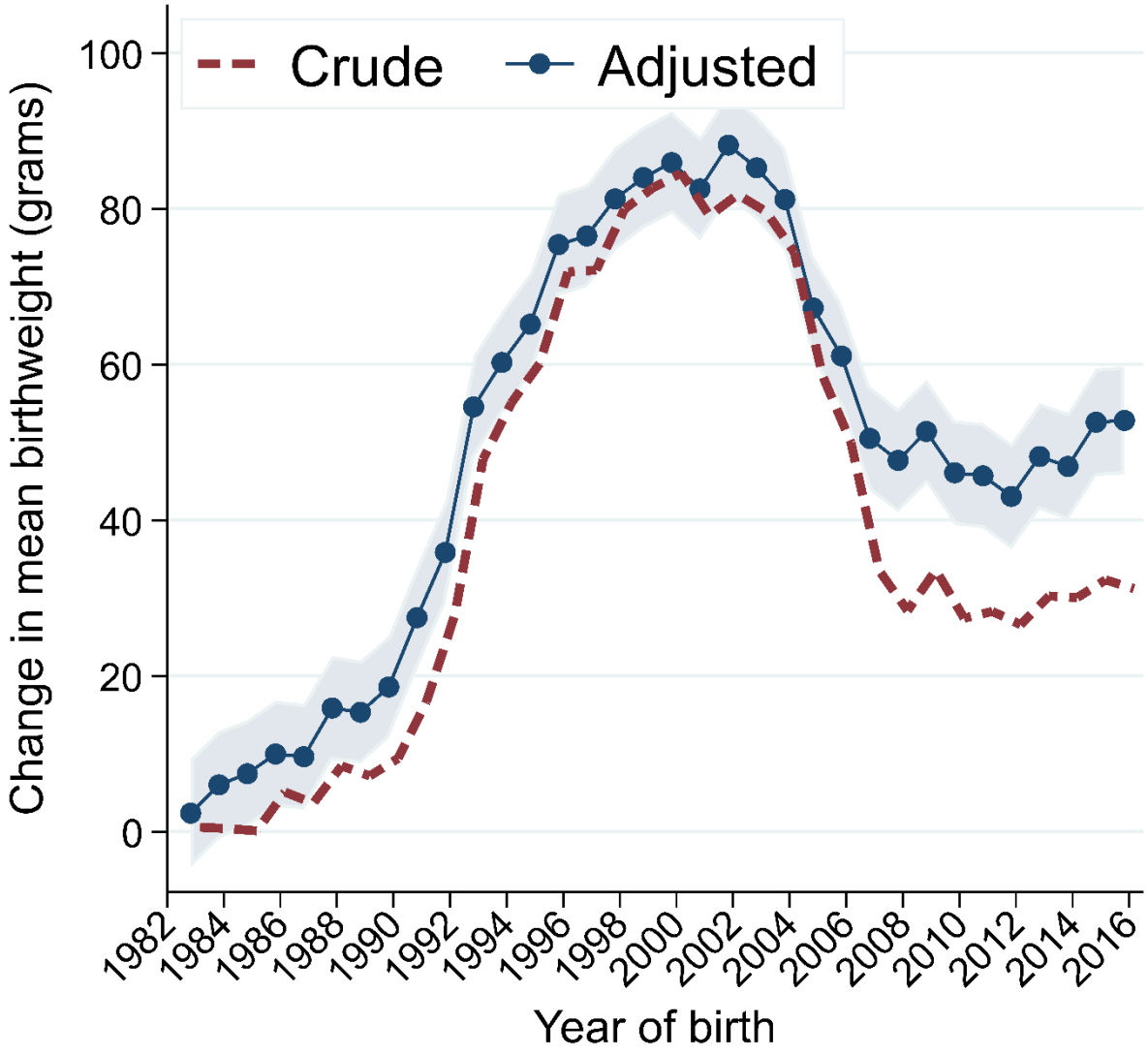


Figure 5: Crude and adjusted change in mean birthweight in live-born children born at term to Scandinavian-born mothers by year of birth (1982 as reference). Adjusted estimates obtained by linear regression were adjusted for parity (primipara versus multipara), maternal age continuous and squared, gestational age continuos and squared, onset of delivery (spontaneous versus medically induced versus caesarean section), marital status (married, cohabiting, non-married/single, divorced/separated/widowed and other/unknown), and congenital anomalies (no versus yes). Shaded band showing 95% confidence intervals for adjusted estimates.

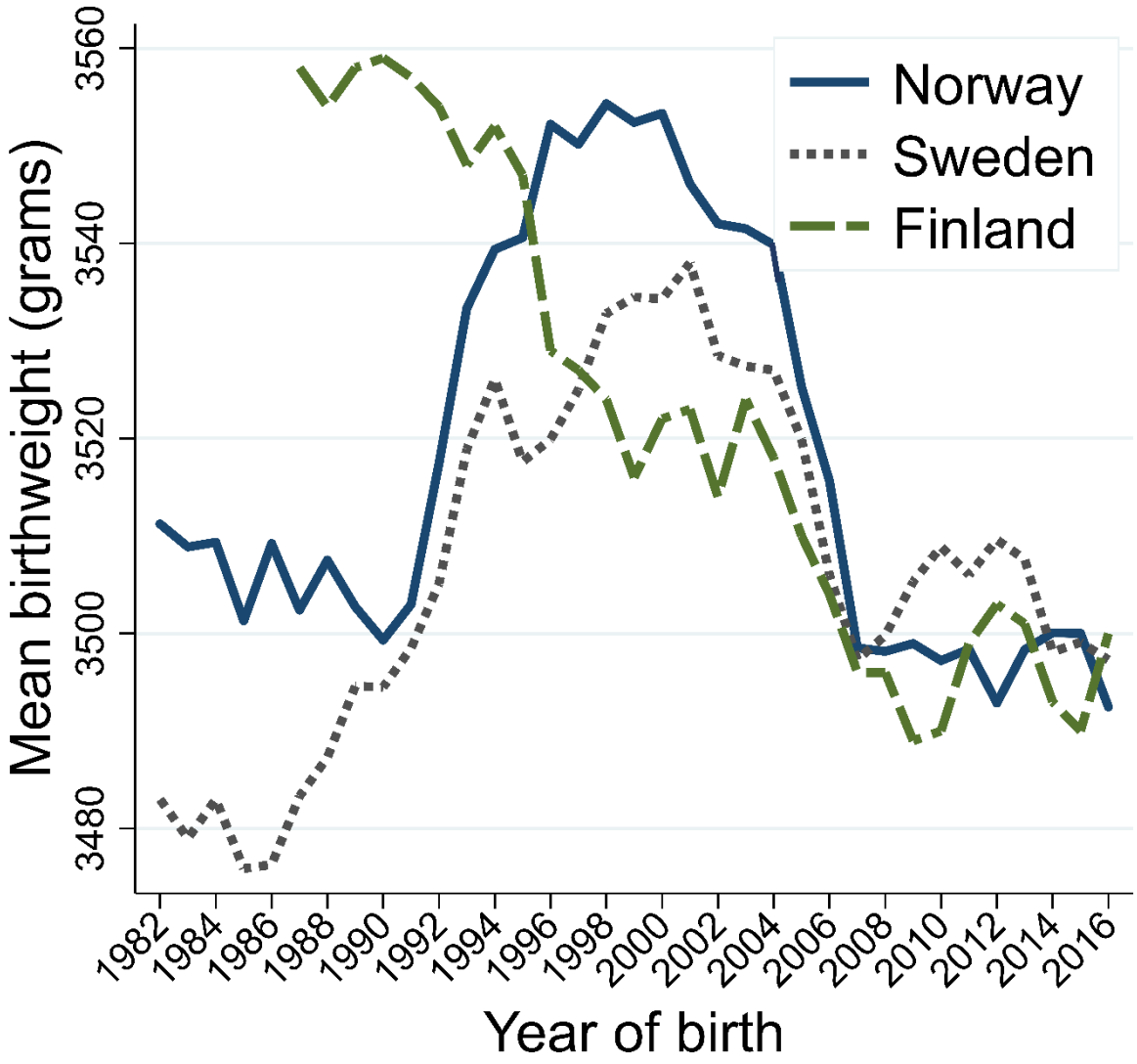


Figure 6: Mean birthweight for live born children in Norway (full line) and Finland (long dashes), all births in Sweden (short dashes), by year of birth. Data from Finland available from 1987 onwards (The Finnish Medical Birth Registry was established in 1987).

Table 1: Characteristics of children born in Norway from 1982 to 2016.

	Year of birth					
	1982-1990	**1991-1995**	**1996-2002**	**2003-2006**	**2007-2016**	**Total**
Part of hump:	Before	Increase	Zenith	Decrease	After	
Number of live births:	486 024	300 287	409 975	230 871	606 480	2 033 637
Missing/implausible birthweight (%)	0.14	0.09	0.14	0.07	0.05	0.10
Missing/implausible gestational age (%)	8.5	9.8	7.4	7.5	8.9	8.5
Study population (n)	444 060	270 506	379 124	213 528	552 094	1 859 312
Preterm births[a] (%)	5.8	6.4	6.7	6.8	6.6	6.4
Term births (n) [b]	418 370	253 093	353 587	198 984	515 801	1 739 835
Mean birthweight (grams)	3567	3598	3628	3608	3569	3589
SD birthweight (grams) [c]	500	508	518	508	491	504
90th percentile (grams) [d]	4200	4250	4290	4260	4200	4230
5th percentile (grams) [d]	2770	2790	2800	2790	2780	2780
2nd percentile (grams) [d]	2550	2560	2560	2570	2570	2560
Mean gestational age (days)	283.3	282.9	282.9	282.6	281.9	282.7
Primipara (%)	42.7	41.1	39.8	40.8	42.2	41.5
Mean maternal age (years)	27.6	28.6	29.5	30.2	30.4	29.3
Mothers without known country of birth (%)	15.3	7.9	3.7	1.7	1.4	6.2
Norwegian-born mothers if known country of birth (%)	93.2	90.3	87.2	82.9	75.2	84.7
Congenital anomalies (%)	2.9	3.1	3.2	4.7	3.8	3.4
Neonatal mortality (%)	0.14	0.12	0.09	0.08	0.05	0.10
Apgar-score at 5 min <7 (%)	0.69	0.70	0.81	0.93	1.01	0.84
Summer births (%)[e]	25.2	25.5	25.8	26.3	26.9	26.0
Start of delivery:						
Spontaneous start of delivery (%)	82.6	81.6	82.0	79.5	74.5	79.6
Induced start delivery (%)	15.3	14.0	12.6	13.4	18.8	15.4
C-section start delivery (%)	2.2	4.4	5.4	7.1	6.7	5.1
Marital status:						
Married (%)	72.6	56.6	50.7	49.4	46.2	55.4
Cohabiting (%)	14.2	34.8	41.2	44.4	47.0	35.9
Unmarried/single (%)	11.7	7.7	5.8	5.0	5.7	7.4
Divorced/widowed (%)	1.28	0.74	0.59	0.50	0.43	0.72
Other/unknown (%)[f]	0.18	0.17	1.73	0.66	0.71	0.71

[a] Born before 37 completed weeks.
[b] Born in gestational weeks 37-45.
[c] SD: standard deviation
[d] Birthweight percentile in term births

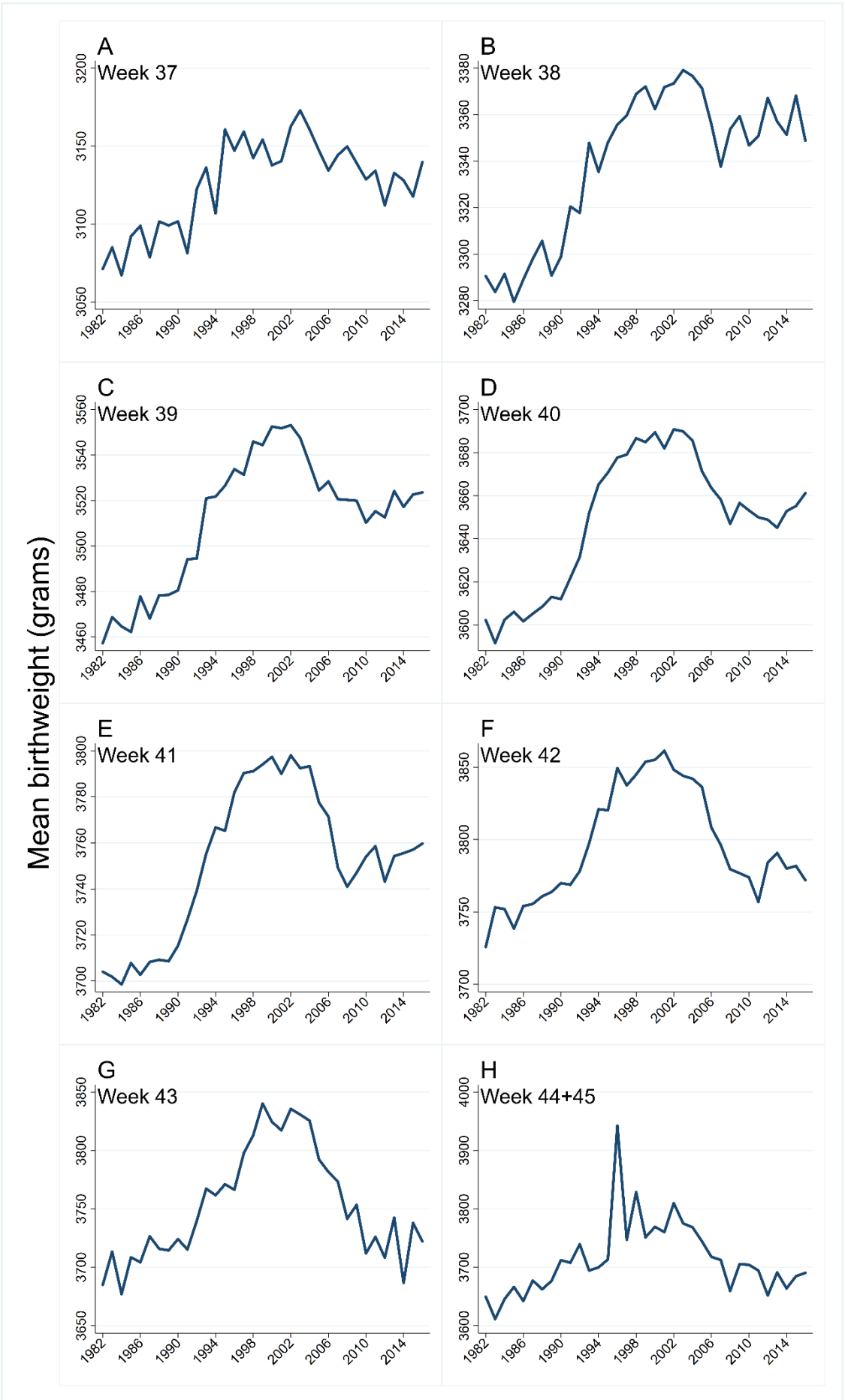
[e] Summer births: births in June, July or August.
[f] Other or unknown marital status.

Supplementary material (paper I)

eTable 1: Characteristics of term children born in Norway from 1982 to 2016.

	Year of birth						
	1982-1990	**1991-1995**	**1996-1998**	**1999-2002**	**2003-2006**	**2007-2016**	**Total**
Part of hump:	Before	Increase	Zenith I	Zenith II	Decrease	After	
Term births (n):	418 370	253 093	150 806	202 781	198 984	515 801	1 739 835
Boys (%)	51.2	51.2	51.2	51.1	51.0	51.1	51.1
Preeclampsia (%)	-	-	-	3.6	3.1	2.4	-
Missing info on smoking (%)	100	100	100	14.7	18.1	13.7	-
Smoking of non-missing (%)	-	-	-	23.4	17.0	9.1	-
Diagnosis of diabetes (%)[a]	0.4	0.8	1.0	1.3	1.5	3.3	1.6
Missing maternal BMI [b] (%)	100	100	100	100	100	47.4	-
Mean maternal BMI [b]	-	-	-	-	-	24.3	-

[a] Diagnosis of any form of diabetes mellitus or use of diabetes medication during pregnancy.
[b] BMI; Body-mass index.


Mean birthweight (grams)
A
Week 37
3050
3100
3150
3200
B
Week 38
3280
3300
3320
3340
3360
3380
C
Week 39
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3500
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D
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E
Week 41
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3720
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3780
3800
F
Week 42
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3850
G
Week 43
3650
3700
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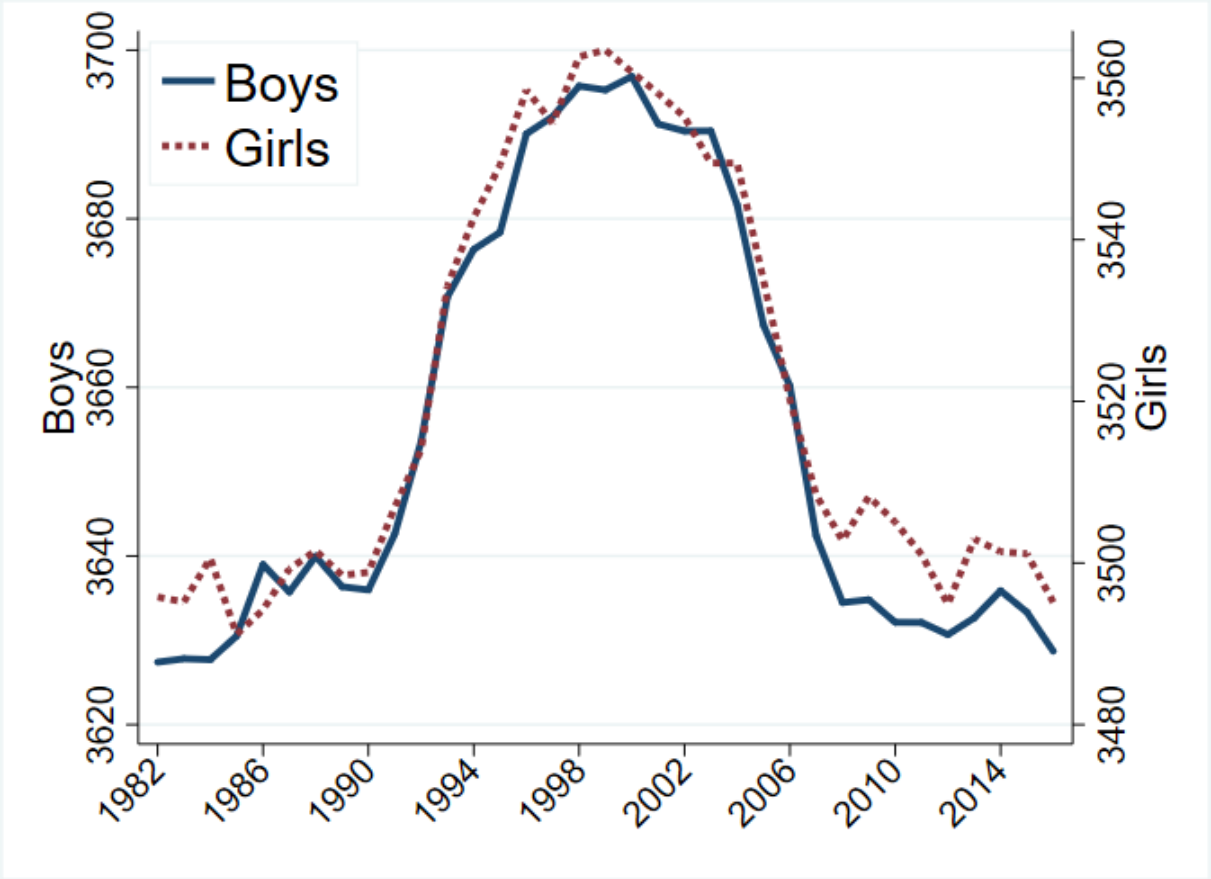
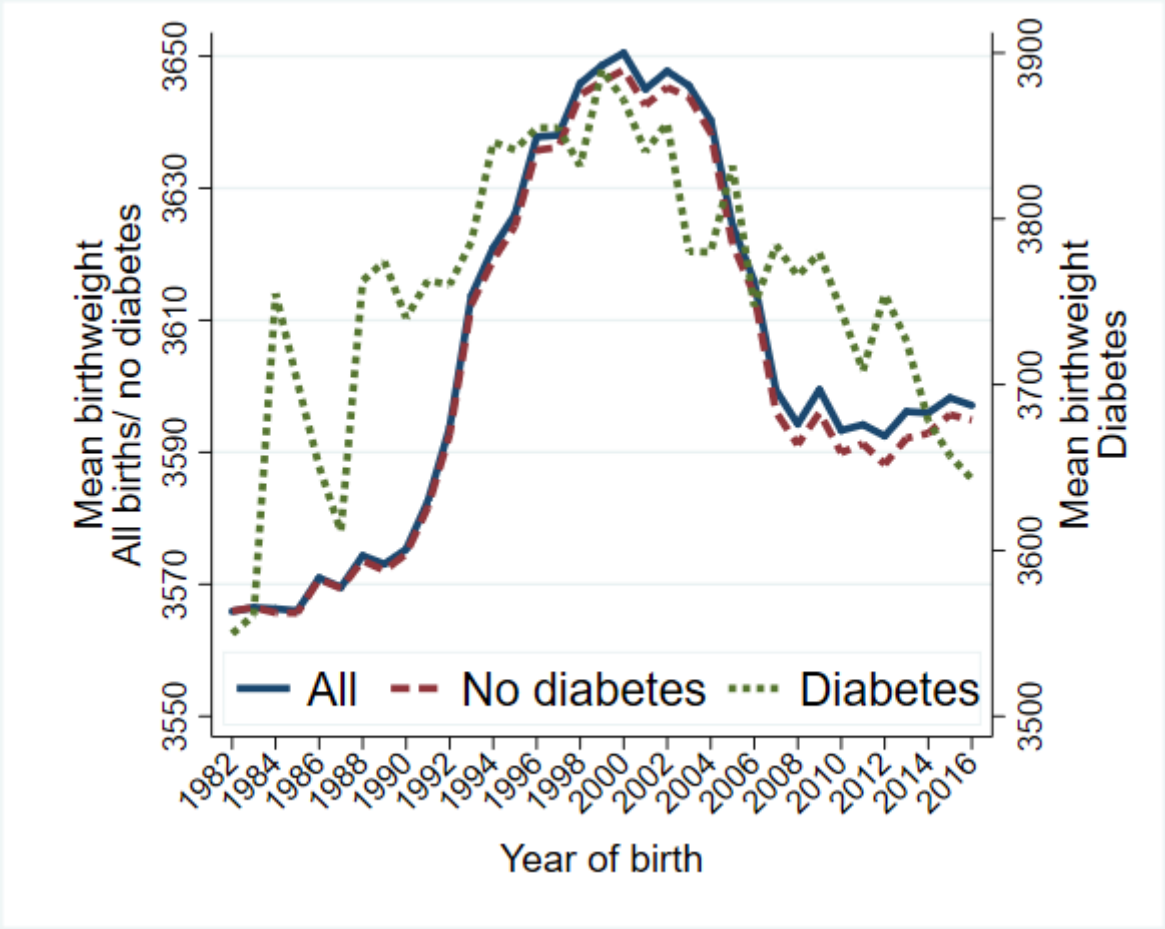
3800
3850
H
Week 44+45
3600
3700
3800
3900
4000
1982
1986
1990
1994
1998
2002
2006
2010
2014

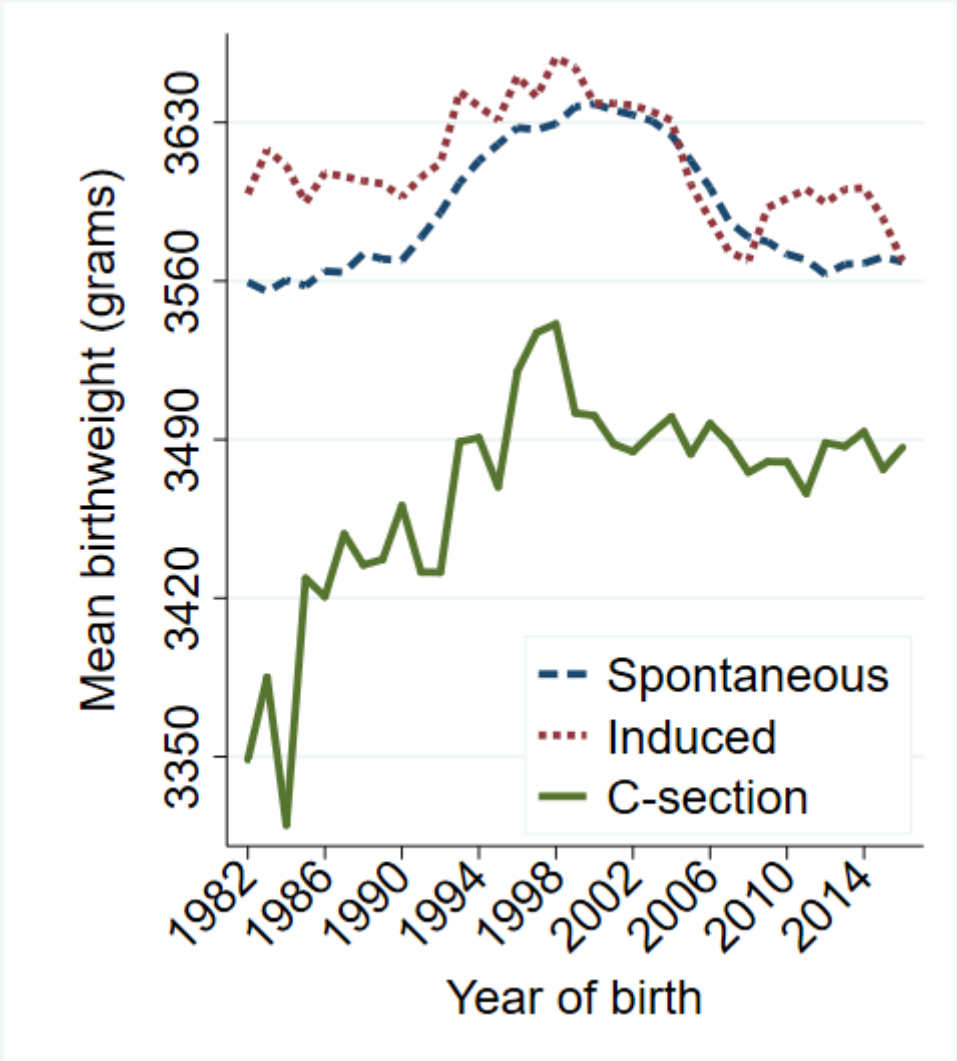
eFigure 1: Mean birthweight for term live births born to Scandinavian women in Norway by year of birth between 1982 and 2016, stratified by gestational age in weeks. A: gestational week 37; B: gestational week 38; C: gestational week 39; D: gestational week 40; E: gestational week 41; F: gestational week 42; G: gestational week 43; H: combined gestational weeks 44 and 45 (combined due to low numbers). Note different scales on y-axes. X-axes showing year of birth.



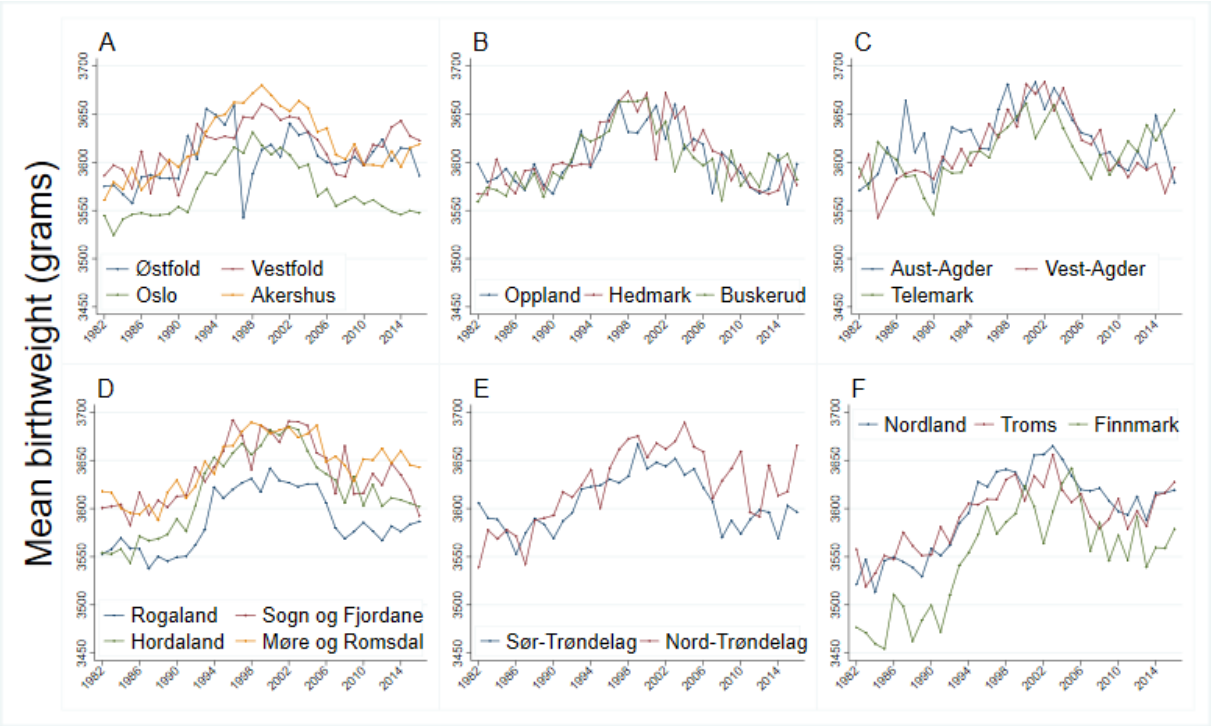
eFigure 2: Mean birthweight (in grams) in live-born term births by year of birth, stratified by sex. Note different scales on the y-axes.



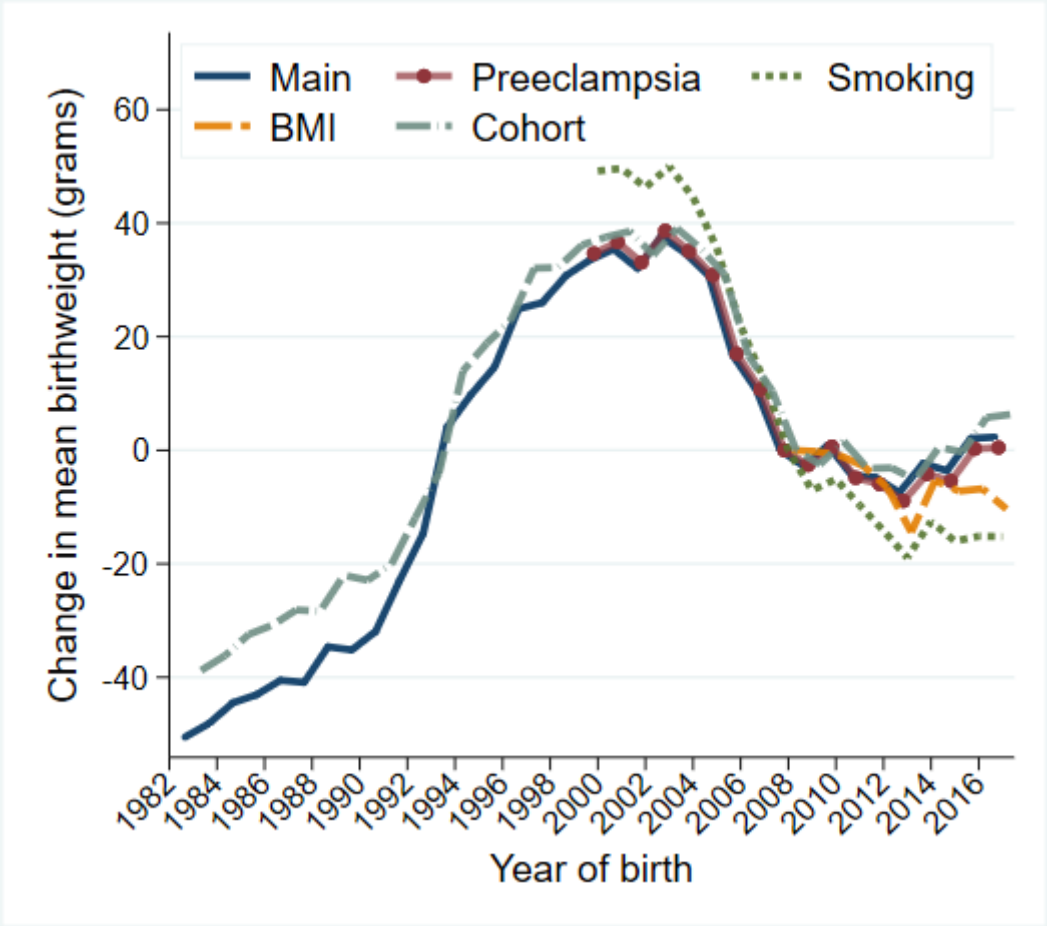
eFigure 3: Mean birthweight for term live births born to Scandinavian women in Norway by year of birth between 1982 and 2016, stratified by diagnosis of diabetes mellitus vs no diagnosis of diabetes mellitus and all births regardless of diagnosis. Note different scales on y-axes, with right y-axis showing mean birthweight for offspring from a pregnancy with any diagnosis of diabetes mellitus.



eFigure 4: Mean birthweight (in grams) in live-born term births by year of birth, stratified by mode of onset of delivery.



eFigure 5: Mean birthweight for term live births born to Scandinavian women in Norway by year of birth between 1982 and 2016, stratified by county of residence at time of birth. A: Østfold, Vestfold, Oslo and Akershus; B: Oppland, Hedmark and Buskerud; C: Aust-Agder, Vest-Agder and Telemark; D: Rogaland, Sogn og Fjordane, Hordaland, and Møre og Romsdal; E: Sør-Trøndelag and Nord-Trøndelag; E: Nordland, Troms and Finnmark. X-axes showing year of birth.



eFigure 6: Adjusted estimated change in mean birthweight in live-born children born at term to Scandinavian-born mothers by year of birth compared to year 2007. Estimates obtained by linear regression adjusted for parity (primipara versus multipara), maternal age continuous and squared, gestational age continuos and squared, onset of delivery (spontaneous versus medically induced versus caesarean section), marital status (married, cohabiting, non-married/single, divorced/separated/widowed and other/unknown), congenital anomalies (no versus yes) with 2007 as reference year.
The different models: Main: the main adjusted model, currently shown in Figure 5 (n=1,410,916) with the covariates listed above. Preeclampsia: added preeclampsia (yes/no) to the main model (n=731,219). Smoking: added smoking (yes/no) to the preeclampsia-model (n=631,733). BMI: added BMI (continuous) to the smoking-model (n=189,992). Cohort: main model (figure 5), adjusting for maternal year of birth in 10-year categories (<1950, 1950-1959, … , 1980-1989, >=1990) (n=1,410,142).

Paper II:

Glycated haemoglobin (HbA1c) in mid-pregnancy and perinatal outcomes. Carlsen EØ, Harmon Q, Magnus MC, Meltzer HM, Erlund I, Stene LC, Håberg SE, Wilcox AJ. Int J Epidemiol. 2022 Jun 13;51(3):759-768. PMID: 34993542. DOI: 10.1093/ije/dyab270.

II

International Epidemiological Association

International Journal of Epidemiology, 2022, 1–10
https://doi.org/10.1093/ije/dyab270
Original article

Original article

Glycated haemoglobin (HbA1c) in mid-pregnancy and perinatal outcomes

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Received 16 February 2021; Editorial decision 8 November 2021; Accepted 20 December 2021

Abstract

Background: Maternal diabetes is a well-known risk factor for pregnancy complications. Possible links between long-term maternal blood sugar in the normal range and pregnancy complications are less well described.

Methods: We assayed glycated haemoglobin (HbA1c) in blood samples collected around the 18th week of pregnancy for 2937 singleton pregnancies in the Norwegian Mother, Father and Child Cohort Study (2000–09). Perinatal outcomes (gestational length, birthweight, birth length and head circumference, large-for-gestational age, small-for-gestational age, congenital malformations, preterm delivery and preeclampsia) were obtained from medical records. We tested associations using linear and log-binomial regression, adjusting for maternal age, body mass index (BMI) and smoking.

Results: Size at birth increased modestly but linearly with HbA1c. Birthweight rose 0.10 standard deviations [95% confidence interval (CI): 0.03, 0.16], for each 5-mmol/mol unit increase in HbA1c, corresponding to about 40 g at 40 weeks of gestation. Large-for-gestational age rose 23% (95% CI: 1%, 50%) per five-unit increase. Other pregnancy complications increased in non-linear fashion, with strongest associations within the top quartile of HbA1c (>35 mmol/mol or >5.4%). Per unit HbA1c within the top quartile, preterm delivery increased by 14% (95% CI: 1%, 31%), preeclampsia increased by 20% (95% CI: 5%, 37%) and gestational duration decreased by 0.7 days (95% CI: −1.0, −0.3).

Conclusions: Among women with no recorded diabetes, higher HbA1c levels at 18 gestational weeks were associated with important perinatal outcomes independent of mother's age, smoking or BMI.

Key words: Birthweight, diabetes, glucose, HbA1c, preeclampsia, pregnancy, pregnancy complications, preterm, MoBa

Key messages

- High maternal glucose is a well-known risk factor for pregnancy complications.
- We find that normal variations in long-term maternal blood sugar, as measured by glycated haemoglobin (HbA1c), are related to pregnancy complications even among pregnant women without diabetes.
- Glycated haemoglobin (HbA1c) levels are linearly related to infant size at birth.
- Pregnant women with glycated haemoglobin (HbA1c) levels in the upper quartile (but still within the generally accepted normal range) are at increased risk of preterm delivery and preeclampsia.

Introduction

Glycated haemoglobin (HbA1c) provides an integrated measure of blood glucose levels across the previous 90–120 days. HbA1c assays are standard for monitoring glycaemic control in patients with diabetes.[1,2] Although monitoring blood glucose is particularly important during pregnancy (because hyperglycaemia is a recognized risk factor in pregnancy),[3–9] HbA1c is not the preferred tool for pregnancy glucose monitoring. This is because increased haemoglobin turnover during pregnancy can slightly reduce HbA1c levels, and also because as an integrated measure of glucose, HbA1c may not adequately capture the short-term fluctuations regarded as important to diabetes management during pregnancy.[1,2,10–16]

For epidemiological purposes, however, the study of HbA1c in pregnancy has decided advantages. Not only is HbA1c simple to measure, but also the fact that it integrates maternal glucose levels across long periods of fetal development may make it a superior predictor of pregnancy complications. It is plausible that even within the normal range of maternal glucose, HbA1c may be linked with pregnancy outcomes. Until very recently, there has been little epidemiological research to explore this possibility.[2,17] Only a handful of epidemiological studies have studied HbA1c in pregnancy in women without diabetes.[7,16–21]

Our purpose was to explore the normal variation in long-term maternal glucose levels measured at 18 weeks of gestation in pregnancies without diabetes, and to describe possible links with outcomes of pregnancy. We chose outcomes that have previously been associated with diabetes-related hyperglycaemia, including fetal growth, gestational duration, preeclampsia, preterm delivery and congenital malformations. We controlled for known risk factors such as age, body mass index (BMI) and smoking, which could potentially confound the associations with HbA1c.

Methods

This project was approved by the Regional Committees for Medical and Health Research Ethics South East Norway (2014/434 and 2014/404). Data collection in MoBa was licensed by the Norwegian Data Protection Agency and approved by the Regional Committees for Medical and Health Research Ethics. The MoBa cohort is now regulated by the Norwegian Health Registry Act.

Study design

Our study was based on a subsample of the Norwegian Mother, Father and Child Cohort Study (MoBa). MoBa recruited pregnant women across the nation between 1999 and 2008 at their routine ultrasound screening (at approximately 18 weeks).[22] Participation rate was 41%. The cohort includes 95 200 women and 114 500 offspring (with some women contributing more than one pregnancy). In 2014, maternal HbA1c was measured in a random sample of 2979 singleton pregnancies with relatively complete data (Supplementary Methods S1, available as Supplementary data at *IJE* online).[23] To avoid confounding by interventions for diabetes mellitus, we excluded 42 women (1.4%) registered with diabetes in the Medical Birth Registry[24] (either pre-pregnancy, unspecified or gestational diabetes), leaving 2937 pregnancies for analysis. The prevalence of diabetes in this sample is slightly lower than the prevalence recorded among all pregnancies at

birth during the same period (1.5–2.2%),[25] reflecting the selection of relatively healthy women into MoBa.[23]

Outcomes

The outcomes of interest were those that have been associated with frank hyperglycaemia: birthweight, birth length and head circumference, decreased gestational length and higher risk of preeclampsia, preterm birth and congenital malformations. Using unique personal identification numbers, pregnancies were linked to the Medical Birth Registry to obtain information on gestational age at delivery (days), birthweight (grams), birth length (centimetres), head circumference (centimetres), congenital malformations (any registered malformation) and preeclampsia [defined as a registration of preeclampsia, eclampsia or HELLP syndrome (haemolysis, elevated liver enzymes, low platelet count)]. Gestational age was based on routine ultrasound scan around week 18 ($n = 2876$) or on last menstrual period when ultrasound dating was missing ($n = 51$); 10 were missing both.

We calculated z-scores for birthweight, length and head circumference to allow standardization by sex, gestational age (whole weeks) and parity (0 or 1+). Singleton births in Norway between 1999 and 2017 were the reference population. Small-for-gestational age (SGA) was defined as birthweight less than the 10th percentile, and large-for-gestational age (LGA) as birthweight above the 90th percentile in the reference population. Preterm delivery was defined as delivery before 37 completed weeks.

HbA1c assay

At a routine ultrasound visit around 18 gestational weeks, venous blood was collected and sent to the MoBa biobank for storage at −20°C.[26] We have no direct evidence comparing fresh samples with samples stored at −20°C; HbA1c has been shown to be relatively stable in samples frozen at −80°C.[27] HbA1c was measured with an immunoturbidimetric method at the Biochemistry Laboratory, Forensic Toxicology Unit, Finnish Institute for Health and Welfare, Helsinki, Finland. The laboratory is accredited by the Finnish Accreditation Service (FINAS, Helsinki). HbA1c levels are reported as mmol/mol, and as corresponding values in % calculated with the formula: number in mmol/mol*0.0915) + 2.15.[28,29] The samples were analyzed in two batches, December 2014–January 2015 and July–October 2015. The between-series precision expressed as coefficient of variation (CV) [mean ± standard deviation (SD)] was 2.0% ± 0.3 in the first and 1.8% ± 0.2 in the second batch. The between-series precision for the entire study (between-batch CV) was 1.9% ± 0.3. The laboratory took part in the HbA1c external quality assessment scheme organized by Lab quality (Helsinki, Finland). Trueness of the method was evaluated by using samples from the proficiency testing, with values assigned by the European Reference Laboratory for Glycohemoglobin. Systematic error (BIAS% ± SD) was 3.0% ± 1.4 and 4.1% ± 3.1, respectively, in the two batches (time periods). Further details of storage and measurements of HbA1c are provided in the Supplementary Methods S2 (available as Supplementary data at IJE online).

Covariates

The Medical Birth Registry provided data on maternal age at delivery (whole years), parity, use of assisted reproductive technologies and child sex. MoBa cohort questionnaires provided information on self-reported height and pre-pregnancy weight [used to calculate body mass index (BMI, kg/m^2)], smoking during pregnancy, educational level (less than high school, high school, college ≤4 years, college >4 years), weight gain during pregnancy and native language of the mother and her parents (Norwegian or other).

A challenge in an exploratory analysis of HbA1c during pregnancy is the limited knowledge about causal factors that determine it. Previous studies have varied widely in the selection of variables for adjustment.[7,17] Given this uncertainty, we explored univariate and multivariate associations between HbA1c and a number of possible confounders, chosen for their plausible association with HbA1c and their known association with pregnancy outcomes. We saw few associations in our data; just three factors met criteria for confounding: maternal age, BMI and sustained smoking at time of recruitment (Supplementary Table S1 and Supplementary Figure S1, available as Supplementary data at *IJE* online). All our analyses adjust for these three factors.

We did not adjust for variables that are either related to prior pregnancy outcomes or associated only with HbA1c,[30] as these variables cannot confound our associations. In the analysis of gestational age, we additionally adjusted for parity (primiparous, multiparous) in order to improve precision of the estimate. We do not necessarily assume a causal association between maternal HbA1c levels and pregnancy outcomes and we provide a directed acyclic graph (DAG) to describe our decisions with regard to adjustment for confounding factors (Supplementary Figure S2, available as Supplementary data at *IJE* online).

Statistical analysis

Given the exploratory nature of this analysis, we chose a parsimonious multivariable analysis, adjusting for maternal age (continuous), BMI (continuous) and current smoking at

Table 1 Descriptive characteristics of the study population ($n = 2937$ singleton pregnancies). Norway, 2002–09

Characteristic	Total
Study sample/pregnancies, no.	2937
HbA1c (mmol/mol), mean (SD) [conversion to % Hb, (SD)]	32.7 (2.9) (5.1 (0.26))
Maternal age at delivery (years), mean (SD)	30.3 (4.2)
Maternal pre-pregnancy BMI (kg/m^2), mean (SD)	23.9 (3.9)
Missing information on BMI, no. (%)	46 (1.6)
Primipara, no. (%)	1505 (51)
Smokers at time of recruitment to MoBa (daily or occasional), no. (%)	176 (6)
Quit smoking before recruitment (daily or occasional), no. (%)	361 (12)
Missing information on smoking, no. (%)	7 (0.2)
Gestational age at blood sampling (weeks), mean (SD)	18.5 (1.3)
Missing information on time of blood sampling, no. (%)	12 (0.4)
Gestational age at birth (weeks), mean (SD)	40.1 (1.4)
Missing information on gestational age, no. (%)	10 (0.3)
Preterm births, no. (%)	87 (3.0)
Preeclampsia, no. (%)	87 (3.0)
Any congenital anomaly, no. (%)	141 (4.8)
Birth length (cm), mean (SD)	50.5 (2.1)
Missing information on length, no. (%)	1 (0.03)
Head circumference (cm), mean (SD)	35.4 (1.6)
Missing information on head circumference, no. (%)	31 (1.1)
Birthweight (grams), mean (SD)	3653 (505)
Large-for-gestational age, no. (%)	369 (12.6)
Small-for-gestational age, no. (%)	188 (6.4)

Large-for-gestational age was defined as birthweight >90th percentile for gestational age in weeks, sex and parity (0 and 1+). Small-for-gestational age was defined as birthweight <10th percentile for the same parameters.

HbA1c, glycated haemoglobin; SD, standard deviation; BMI, body mass index; MoBa, the Norwegian Mother, Father and Child Cohort Study.

week 18 (yes or no; smokers who quit before recruitment were included in the non-smoking category) and parity (0 versus 1+).

We used linear regression for continuous outcomes and log-binomial regression to estimate relative risks for binary outcomes. Initially, we explored relationships between HbA1c and outcomes in a flexible way, with a four-knot restricted cubic spline model. The relationship between HbA1c and standardized birthweight, birth length, head circumference and LGA were all approximately linear. There was no indication that restricted cubic splines improved the model fit over that with HbA1c as a linear continuous variable, and we used simple linear models in all further analyses (Supplementary Table S2, available as Supplementary data at *IJE* online). However, in our graphical displays of the regression analyses, we have used the four-knot restricted cubic spline model for transparency while using the simpler models for interpretable coefficients in the tables showing regression analyses.

The remaining outcomes—SGA, gestational age, preterm birth, preeclampsia and congenital malformations—appeared nonlinear in their association with HbA1c. Using likelihood ratio testing, we confirmed that a single-knot linear spline provided adequate model fit to all outcomes compared with a model using cubic splines with four knots (Supplementary Table S2). The location of the knot was determined by inspection of graphs with various knot locations. A knot between 34 and 35 mmol/mol (5.3% and 5.4%) adequately captured the inflection point for these outcomes, with the further advantage of dividing the study population into the top quartile ($n = 710$) and lower three quartiles ($n = 2227$). A recent paper[31] identified impaired glucose metabolism in pregnancy at 5.7% or above (about 37 mmol/mol). We did not have sufficient power to examine associations at this extreme value.

Results are reported as follows. For outcomes showing a linear dose-response relationship with HbA1c, we estimated mean difference (for continuous outcomes) or relative risks (for binary outcomes) per five-unit increase in HbA1c (~0.4% unit). For outcomes showing nonlinear dose-response relationships with HbA1c, we expressed estimates per one-unit increase in HbA1c. This more narrow interval was chosen to acknowledge the restricted range of HbA1c values above the knot (≥35 mmol/mol).

We explored the robustness of gestational age results by excluding preterm deliveries and pregnancies complicated by

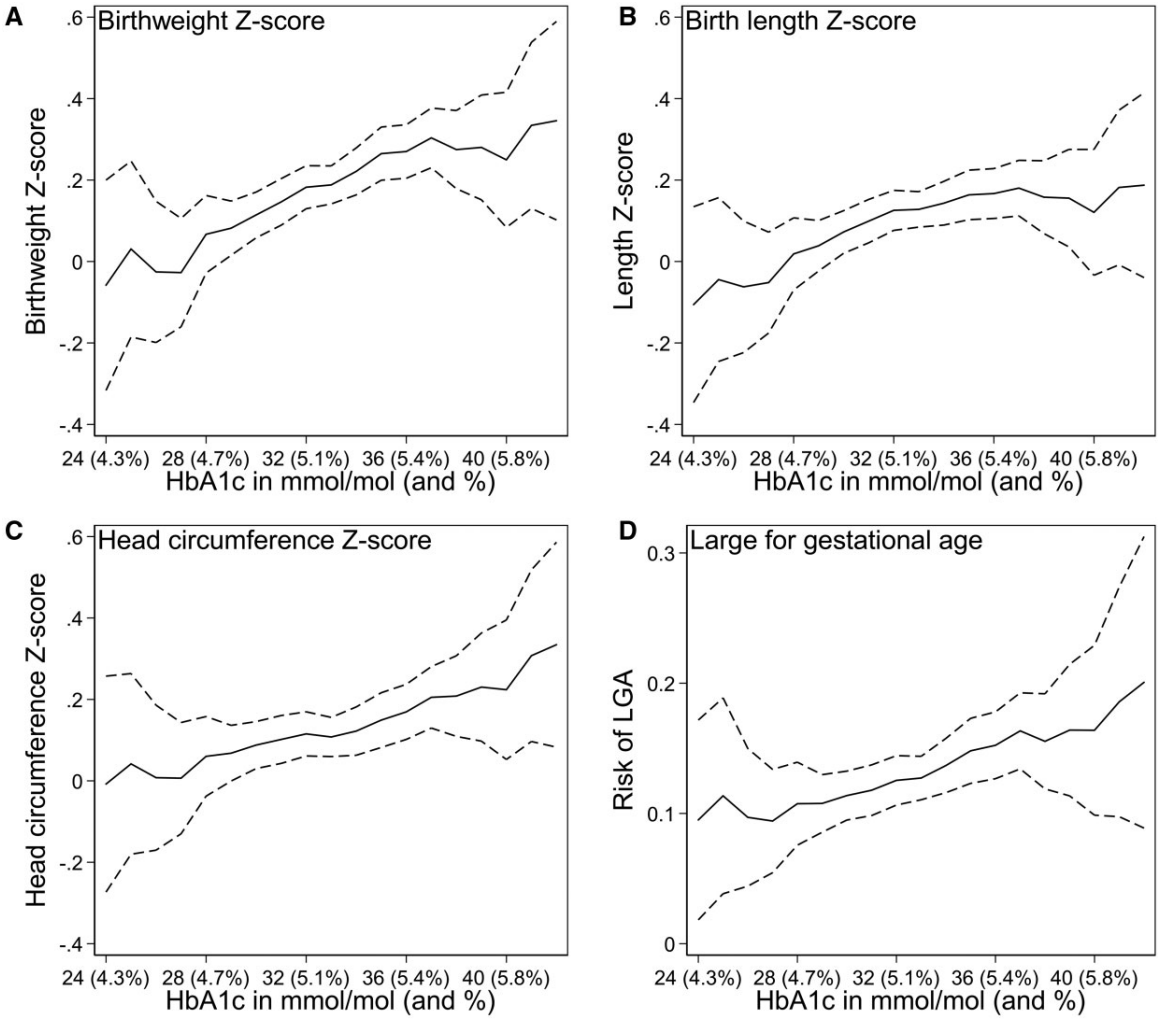

Figure 1 Birth size in relation to 18-week glycated haemoglobin (HbA1c) levels among up to 2874 singleton pregnancies, Norway, 2002–09. Panel A) Predicted birthweight z-score for HbA1c level. Panel B) Predicted birth length z-score for HbA1c level. Panel C) Predicted head circumference z-score for HbA1c level. Panel D) Predicted risk of large-for-gestational age (>90th percentile birthweight for sex, parity and gestational age in weeks) compared with normal-for-gestational age (10–90th percentiles). Solid lines show the predicted values, with dashed lines showing the 95% confidence intervals. Z-scores standardized for gestational age in weeks, parity and sex. All predicted outcomes are adjusted for body mass index (BMI) (set to mean), maternal age (set to mean) and smoking (set to non-smokers). The predictions are modelled using restricted cubic splines for HbA1c with four knots (placed at 28, 32, 34 and 38 mmol/mol). HbA1c levels were grouped at the extremes, with 42+ mmol/mol (6%) as the highest value (16 observations) and 24 mmol/mol (4.3%) as the lowest value (five observations).

preeclampsia. In additional sensitivity analyses, we restricted to deliveries with a spontaneous onset of labour (as reported in the Medical Birth Registry) and separately conducted analyses stratified by parity. All analyses were performed using Stata (Statacorp, College Station, TX), version 15.0.

Results

Characteristics of the 2937 mothers and newborns are shown in Table 1. Blood was sampled at a mean of 18.5 gestational weeks [standard deviation (SD) 1.3]. HbA1c values had a strongly Gaussian distribution (Supplementary Figure S3, available as Supplementary data at *IJE* online), with a mean of 32.7 mmol/mol (5.1%), an SD of 2.9 mmol/mol (0.26%) and a range of 22 to 47 mmol/mol (4.2–6.5%).

Fetal growth

Infant size, expressed in z-scores, increased linearly with higher HbA1c (Figure 1). Adjusted birthweight z-score increased 0.10 SD per 5-mmol/mol unit (~0.4% unit) increase in HbA1c [95% confidence interval (CI): 0.03, 0.16, P for trend = 0.003] (Table 2). This is equivalent to about a 40-g increase at 40 weeks of gestation. Smaller increases were also present for head circumference (0.05 SD per five units of HbA1c; 95% CI: −0.01 to 0.12) and length (0.05 SD; 95% CI: −0.01 to 0.11) (Figure 1,

Table 2 Linear and single-knot spline regression analyses for glycated haemoglobin (HbA1c) measured at 18 gestational weeks and multiple perinatal outcomes. Singleton pregnancies, Norway, 2002–09

Birth outcome[a]	HbA1c continuous, estimate per 5 units (95% CI)	HbA1c level ≤34 mmol/mol,[b] estimate per unit (95% CI)	HbA1c level ≥35 mmol/mol,[b] estimate per unit (95% CI)	Number of observations for each model, no. (% of study sample)
Birthweight (z-score)	0.10 (0.03, 0.16) $P = 0.003$	NA	NA	2874 (97.9)
Length (z-score)	0.05 (−0.01, 0.11) $P = 0.09$	NA	NA	2873 (97.8)
Head circumference (z-score)	0.05 (−0.01, 0.12) $P = 0.10$	NA	NA	2843 (96.8)
Large-for-gestational age[c]	1.23 (1.01, 1.50) $P = 0.04$	NA	NA	2874 (97.9)
Gestational age (days)	−0.81 (−1.47, −0.16) $P = 0.02$	0.07 (−0.12, 0.26) $P = 0.49$	−0.66 (−0.99, −0.33) $P < 0.001$	2874 (97.9)
Small-for-gestational age[c]	0.93 (0.72, 1.22) $P = 0.62$	0.95 (0.88, 1.02) $P = 0.17$	1.07 (0.95, 1.21) $P = 0.27$	2874 (97.9)
Preterm birth[c]	1.36 (0.95, 1.95) $P = 0.09$	1.01 (0.90, 1.13) $P = 0.86$	1.14 (1.00, 1.31) $P = 0.05$	2874 (97.9)
Preeclampsia[c]	1.07 (0.74, 1.54) $P = 0.73$	0.92 (0.83, 1.02) $P = 0.12$	1.20 (1.05, 1.37) $P = 0.007$	2884 (98.2)
Any congenital malformation[c]	0.93 (0.69, 1.24) $P = 0.61$	0.94 (0.86, 1.01) $P = 0.10$	1.10 (0.97, 1.25) $P = 0.13$	2884 (98.2)

[a]Adjusted for: maternal age (whole years), maternal pre-pregnancy body mass index (BMI) (kg/m^2), smoking in pregnancy (yes versus no). Also adjusted for parity (0 versus 1+) for gestational age. Z-scores (birthweight, length, head circumference) and small- and large-for-gestational age standardized for gestational age in whole weeks, parity (0 vs 1+) and sex. Large-for-gestational age was defined as >90th percentile, and small-for-gestational age as <10th percentile.

[b]Showing the single-knot linear spline regression coefficients for the linear slope of HbA1c level up to and including 34 mmol/mol, and from 35 mmol/mol and up, respectively.

[c]Estimated risk ratio from multinomial regression model assessing risk of small-for-gestational age and large-for-gestational age compared with normal-for-gestational age. Estimated relative risk from binomial regression models for outcomes preterm birth, preeclampsia and any congenital malformation.

Table 2). The relative risk (RR) of delivering an LGA baby increased 23% per five-unit increase in HbA1c (95% CI: 1%, 50%) (Figure 1, Table 2). The risk of SGA was elevated at low levels of HbA1c and then again at higher levels (Table 2; and Supplementary Figure S4, available as Supplementary data at *IJE* online). Since SGA babies may comprise both normal and pathologically small infants,[32] our finding could suggest normal small growth at low levels of maternal blood glucose and abnormal growth restriction at high levels, although no firm interpretation is possible given the weak pattern.

Gestational age at birth

In a simple linear analysis, there was an association between HbA1c levels and decreasing gestational age (−0.8 days per five units of HbA1c; 95% CI: −1.5, −0.2). Model fit for this variable was improved with a spline model. The spline model suggests a weak association between HbA1c and gestational duration in the lower three quartiles of HbA1c but a marked decline within the top quartile (−0.7 days per one unit of HbA1c; 95% CI: −1.0, −0.3) (Table 2, Figure 2). Within the top quartile, this decline corresponds to a decrease of 3.3 days per five-unit increase in HbA1c (95% CI: −5.0, −1.7). The shortening of pregnancy within the upper quartile was robust in sensitivity analyses, including stratified analyses of births without preterm deliveries or preeclampsia (Supplementary Figure S5 and Supplementary Table S3, available as Supplementary data at *IJE* online).

Preeclampsia, preterm birth and congenital malformations

The risks of preterm delivery and preeclampsia were both increased within the highest quartile of HbA1c. Preeclampsia increased 20% per unit increase of HbA1c (95% CI: 5%, 37%) and preterm delivery increased 14% per unit increase (95% CI: 0%, 31%) (Table 2, Figure 3). There was the suggestion of a slight increase in risk of congenital malformations in the upper quartile of HbA1c (RR 1.10; 95% CI: 0.97, 1.25) (Table 2, Figure 3).

Discussion

In this population sample, maternal HbA1c at 18 gestational weeks was linearly associated with infant size at birth (weight, length, head circumference and LGA) independent of the mother's BMI, age or smoking. Within the highest quartile of HbA1c (35 mmol/mol or greater), increasing HbA1c was related to shorter pregnancy duration and an increased risk of preeclampsia and preterm

delivery. These observations were despite the fact that women with a diagnosis of diabetes had been excluded, and HbA1c values were in a range considered as normal.

Previous literature

The types of adverse outcomes we observed are the same as (but less severe than) outcomes in pregnancies with gestational diabetes.[4,9,33,34] Although there are few epidemiological studies on HbA1c in pregnant women without diabetes, the past studies generally support our results. The only large study of HbA1c very early in pregnancy (around 7 gestational weeks) found an increased risk of LGA, preterm delivery, preeclampsia and major congenital malformations among women in the extreme upper 3rd percentile of HbA1c concentrations.[7] One previous study measured HbA1c late in pregnancy, around week 28. The study was designed primarily to compare HbA1c with other glucose measures, but the authors reported associations of HbA1c as a continuous measure with birthweight, preterm delivery and preeclampsia.[20] Two recent studies have assessed HbA1c and pregnancy outcomes. Chen *et al.*[21] considered the predictive power of HbA1c in the high range of normal (5.7%–6.4%, or approximately 38–47 mmol/mol) in early pregnancy, although they did not exclude pregnancies that developed gestational diabetes mellitus (GDM). Bi *et al.*[17] assessed HbA1c continuously across quintiles, and addressed three of the outcomes in our analysis (birthweight, LGA and preterm delivery).

Strengths and limitations

Our study participants came from a national pregnancy cohort, with the inevitable selection that occurs in volunteer studies. The impact of selection in this cohort has been explored for a few key aetiological associations, with only minor bias apparent.[35] Further selection may have occurred because women with relatively complete data were chosen for HbA1c assay. However, our study was presumably less selective than studies that required women to submit to an oral glucose tolerance test.

We did not have information on maternal ethnicity, which has been a confounder in studies outside Norway.[2,36] Our surrogate measure (native tongue other than Norwegian for the woman or her parents) was unrelated to HbA1c.

Our z-score measures of fetal size included gestational age as an adjustment variable. Adjusting for this mediating variable raises the theoretical possibility of collider stratification bias.[35] However, not to adjust would raise the practical problem of failing to account for reduced birth size due to the shortened pregnancies associated with high HbA1c.

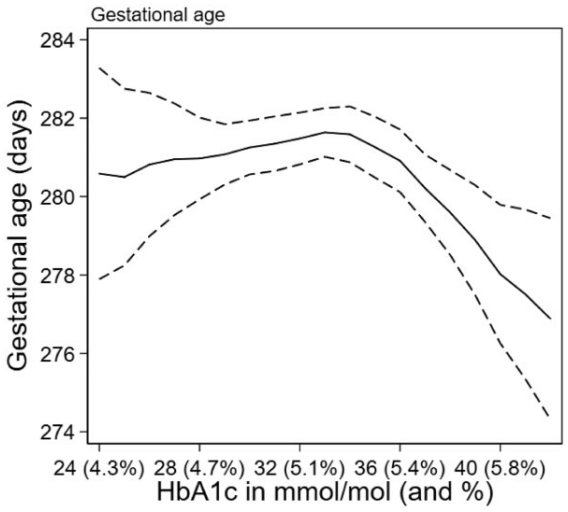

Figure 2 Gestational age at birth in relation to 18-week glycated haemoglobin (HbA1c) levels among 2874 singleton pregnancies, Norway, 2002–09. Predicted gestational age for each value of HbA1c (solid line), with 95% confidence intervals (dashed lines). The predictions were made using a linear regression model with restricted cubic splines for HbA1c with four knots (placed at 28, 32, 34 and 38 mmol/mol). Predictions were estimated using the adjusted models with the following covariates: maternal age (set to mean), body mass index (BMI) (set to mean), smoking (set to non-smokers) and parity (set to primipara). Note that HbA1c levels were grouped at the extremes, with 42+ mmol/mol (6%) as the highest value (16 observations) and 24 mmol/mol (4.3%) as the lowest value (five observations).

HbA1c can be affected by levels of blood haemoglobin, and we lacked information on this variable. Pathologies of haemoglobin are relatively rare. The anaemia associated with pregnancy could be a potential confounding factor, although this is expected to be relatively minor.[16]

Our analysis of congenital malformations was limited by lack of data on specific defects. It is possible that the weak trend we observed towards increased total malformations in the upper quartile of HbA1c reflects stronger increases of specific defects that have been associated with frank diabetes (e.g. cardiac defects).[37] More detailed studies can test this hypothesis.

Information on diagnosis of preeclampsia was obtained from the Medical Birth Registry of Norway. The validity of this diagnosis has been described, indicating a high specificity but lower sensitivity, particularly among milder cases of preeclampsia.[38] We cannot exclude the possibility that misclassification might have muted the association between HbA1c and preeclampsia in this study.

Interpretation

The linear associations of HbA1c with infant size supports the hypothesis that fetal growth is a continuous function of maternal glucose levels, with no obvious threshold.[39]

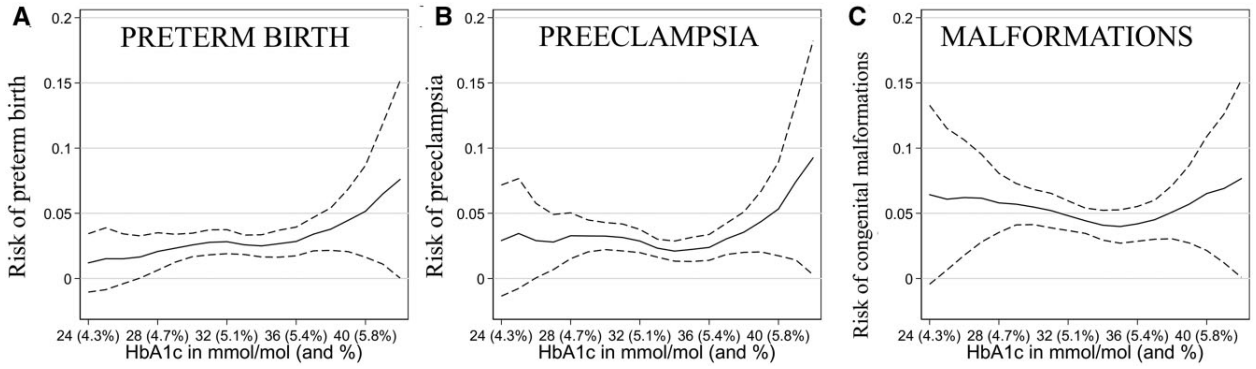

Figure 3 Pregnancy outcomes in relation to 18-week glycated haemoglobin (HbA1c) among up to 2874 singleton pregnancies, Norway, 2002–09. Panel A) Predicted risk of preterm birth. Panel B) Predicted risk of preeclampsia. Panel C) Predicted risk of any congenital malformation. Solid lines show the predicted values, with dashed lines showing the 95% confidence intervals. The predictions were made using a binomial regression model with restricted cubic splines for HbA1c with four knots (placed at 28, 32, 34 and 38 mmol/mol). Adjusted for maternal age (set to mean), body mass index (BMI) (set to mean), and smoking (set to non-smokers). Note that HbA1c levels were grouped at the extremes, with 42+ mmol/mol (6%) as the highest value (16 observations) and 24 mmol/mol (4.3%) as the lowest value (five observations).

Given that physiological changes of pregnancy can slightly reduce the apparent level of HbA1c at a given level of blood glucose, we did not rely on pre-specified criteria for high HbA1c. After excluding women with diabetes, we based our analysis on variation within the observed distribution of HbA1c, particularly within the upper quartile. Whereas this approach lacks the rigour of a pre-specified hypothesis, it is suitable for an exploratory description of a topic with limited prior history. Our nonlinear analyses are *post hoc*, and we give less weight to the exact magnitude of risk or the location of an HbA1c threshold than to the general observation that pregnancy complications apparently increase within the upper range of HbA1c in women without diabetes. Our results provide explicit hypotheses for testing in future studies.

It remains to be seen whether the increased risks we have identified with high-normal levels of HbA1c at week 18 can be mitigated by the dietary and exercise recommendations routinely provided later in pregnancy to women with gestational diabetes.[36] It would also be of interest in future studies to explore whether these variations in normal HbA1c during pregnancy might be associated with outcomes in childhood, such as body size or neurodevelopment.

In conclusion, higher HbA1c levels in mid-pregnancy among women without diabetes were associated with larger infant size at birth and shorter pregnancy duration. Within the highest quartile of HbA1c, risk of preterm birth and preeclampsia also increased. Natural variation in long-term maternal glucose in women without diabetes, and independent of maternal BMI, smoking or age, may be a useful clinical predictor of pregnancy complications.

The individual-level data used in this study can only be given after approval by the Norwegian ethical committees approving that the applications are consistent with the consent provided. Access to the study dataset is available by application to the Norwegian Institute of Public Health using a form available on the English language portion of their website at [http://www.fhi.no/eway/].

Supplementary Data

Supplementary data are available at *IJE* online.

Funding

The Norwegian Mother, Father and Child Cohort Study is supported by the Norwegian Ministry of Health and Care Services and the Ministry of Education and Research. This work was funded in part by the Norwegian Research Council's Centres of Excellence Funding Scheme (no. 262700). M.C.M works at the Medical Research Council Integrative Epidemiology Unit at the University of Bristol, which receives infrastructure funding from the UK MRC (MC_UU_00011/3 and MC_UU_00011/6). Support was also provided by the intramural programme of the National Institute of Environmental Health Sciences, National Institutes of Health, USA.

Acknowledgements

We are grateful to the families in Norway who have contributed to this ongoing cohort study. We thank Drs Cuilin Zhang and Anne Marie Jukic for helpful suggestions on an earlier draft of the paper.

Author Contributions

This study was conceptualized and supervised by A.J.W. H.M.M. and I.E. were responsible for data collection. E.O.C. carried out data analysis and prepared the first draft of the manuscript. M.C.M. provided technical supervision of the analysis and participated in interpretation. Q.H., L.C.S. and S.E.H. participated in design of the analysis and interpretation of the results. All authors made critical contributions to the final interpretation and presentation of data,

and all have approved this submitted version. All accept responsibility for the paper as submitted.

Conflict of Interest

None declared.

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Supplementary Material (paper II)

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Supplementary Methods S1: Details of data sources and linkages

The Medical Birth Registry of Norway
The Medical Birth Registry of Norway was established in 1967 as the first National birth registry in the world.[1] It is mandatory for midwives or the obstetrician to complete a birth record for all deliveries after 12 gestational weeks. Information on the birth record includes maternal demographic characteristics, parity, pre-existing chronic diseases and pregnancy complications. Information on child characteristics include sex, birthweight, length at birth, head circumference and gestational age at birth. Each woman has a unique personal identification number (PIN) used to link the information of the Norwegian Mother, Father and Child Cohort study.

The Norwegian Mother, Father and Child Cohort Study (MoBa)
Questionnaires were administered to mothers at 15 and 30 gestational weeks during pregnancy and at regular intervals after birth throughout childhood. Data collection after age 7 is ongoing. The questionnaires gathered a broad range of information, including socio-economic factors (marital status, education and income), lifestyle factors (smoking, body-mass index etc.) health status (chronic and infectious diseases) and anthropometric measures (height and weight). We used data available in November 2015 (V.9 of the quality assured data files).

The Norwegian Mother, Father and Child Cohort Study is supported by the Norwegian Ministry of Health and Care Services and the Ministry of Education and Research. We are grateful to all the participating families in Norway who take part in this on-going cohort study.

The present study is embedded within the first phase of the Norwegian Environmental Biobank, a sub-study of the Norwegian Mother, Father and Child Cohort study
The Norwegian Environmental Biobank was given permission to draw biological material from the MoBa biobank, including blood samples drawn in week 17-18 of pregnancy.[2-5] For that study, participants were selected based on availability of all biological samples from mid-pregnancy and at birth, and only women who had answered all questionnaires up until 3 years after giving birth were eligible. Only singleton pregnancies were included, and pregnancies with children with autism, suspected autism, or symptoms of severe language delay were excluded. A total of n=2999 pregnancies were included in the study.[5] For our study exploring HbA1c in mid-pregnancy, we included all pregnancies within the Norwegian Environmental Biobank study with a valid measurement of HbA1c, leaving a study population of n=2979 singleton pregnancies. For our main analyses, we excluded women with any diagnosis of diabetes mellitus (Type 1, n=8; Type 2, n=6; Unspecified, n=1; Gestational, n=24; registration of diabetic medication during pregnancy, n=3) as registered in the Medical Birth Registry of Norway, leaving a main study population for analyses consisting of n=2937 singleton pregnancies.

Supplementary Methods S2: glycated haemoglobin (HbA1c) measurements

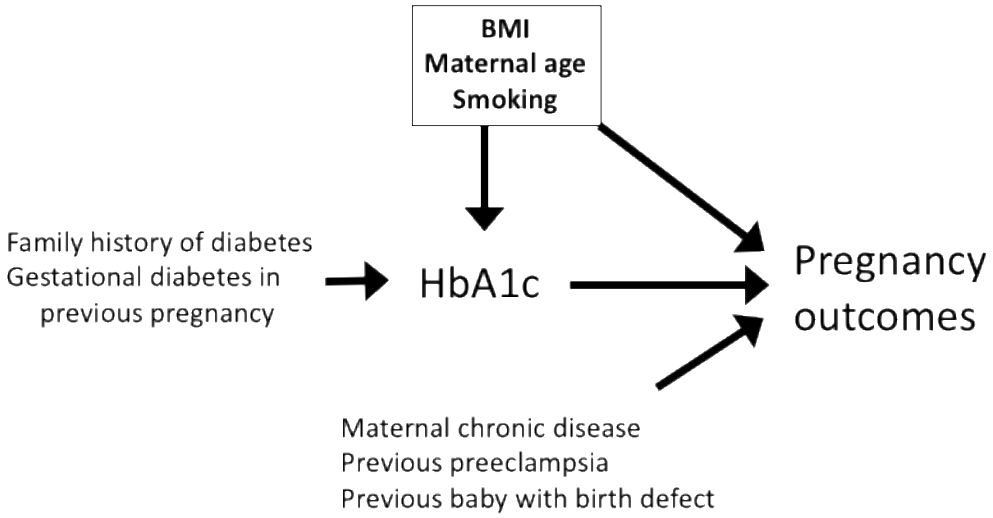
Samples were stored at -20 ºC until analysis, for a range of 5-12 years. Glycated haemoglobin (HbA1c) is relatively stable in frozen samples.[6,7] HbA1c was measured with an immunoturbidimetric method using an Architect c8000 analyzer (Abbott Laboratories, Abbott Park, Illinois, USA) at the Biochemistry Laboratory, Forensic Toxicology Unit, Finnish Institute for Health and Welfare, Helsinki, Finland. The laboratory is accredited by the Finnish Accreditation Service (FINAS, Helsinki) and fulfills the requirements of the standard SFS-EN ISO/IEC 17025:2005. The scope of accreditation (T077) covers the HbA1C assay. The samples were analyzed in two batches, December 2014-January 2015 and July-October 2015. The between-series precision expressed as coefficient of variation (CV) [mean ± standard deviation (SD)] was 2.0% ±0.3 in the first and 1.8% ±0.2 in the second batch. The between-series precision for the entire study (between-batch CV) was 1.9 % ±0.3. The laboratory took part in the HbA1c external quality assessment scheme organized by Lab quality (Helsinki, Finland). Trueness of the method was evaluated by using samples from the proficiency testing, with values assigned by the European Reference Laboratory for Glycohemoglobin. Systematic error (BIAS% ±SD) was 3.0 % ±1.4 and 4.1 % ±3.1, respectively, during the time periods.

A method comparison between the immunoturbidimetric method and an enzymatic method by Abbott Laboratories was also conducted at the Biochemistry Laboratory.[8] Samples obtained from 100 subjects of four different nationalities (Finnish, Somali, Russian and Kurdish, n=25 of each) were analyzed by both methods. Very good agreement was observed.[8] The regression equation of the immunoturbidimetric versus enzymatic method was $y = 0{,}988x - 1{,}026$, $R^2 = 0{,}983$.

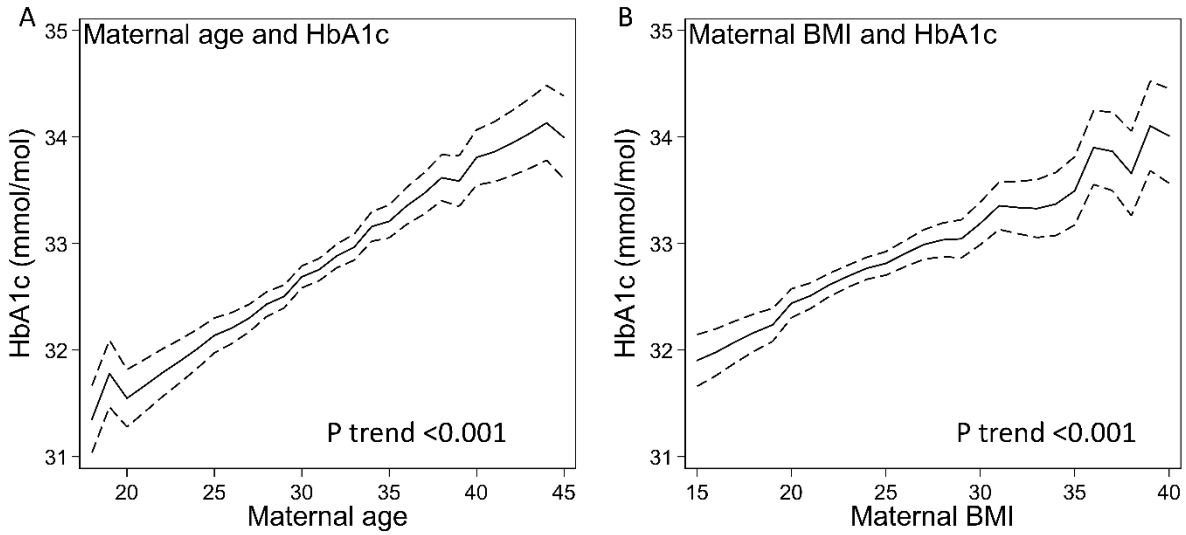
We did not have information about factors interfering with HbA1c measurements, such as hemoglobinopathies,[9-11] which could have biased the results due to unmeasured confounding. However, considering the fact that the genetic variants of hemoglobin shown to affect the accuracy of the method used in our study i.e., the HbS and HbC traits,[9] are very uncommon in the Norwegian population,[12] we do not expect this to affect our results.

The use of HbA1c to diagnose diabetes in pregnancy is controversial. The World Health Organization (WHO) and the International Association of Diabetes and Pregnancy Study Groups (IADPSG) recommend that HbA1c equal to or greater than 48 mmol/mol (6.5%) can be used to diagnose overt diabetes mellitus in pregnancy.[13,14] The American Diabetes Association and IADPSG advise against the use of HbA1c for diagnosis in the second and third trimesters of pregnancy.[15,16] No women in our study had an HbA1c concentration ≥48 mmol/mol (6.5%) after excluding those with a registered diagnosis of diabetes.

Supplementary Figure S1: Directed acyclic graph showing the selection of variables as potential confounding variables (in box) and other non-confounding variables of the associations between glycated haemoglobin (HbA1c) and selected pregnancy outcomes.

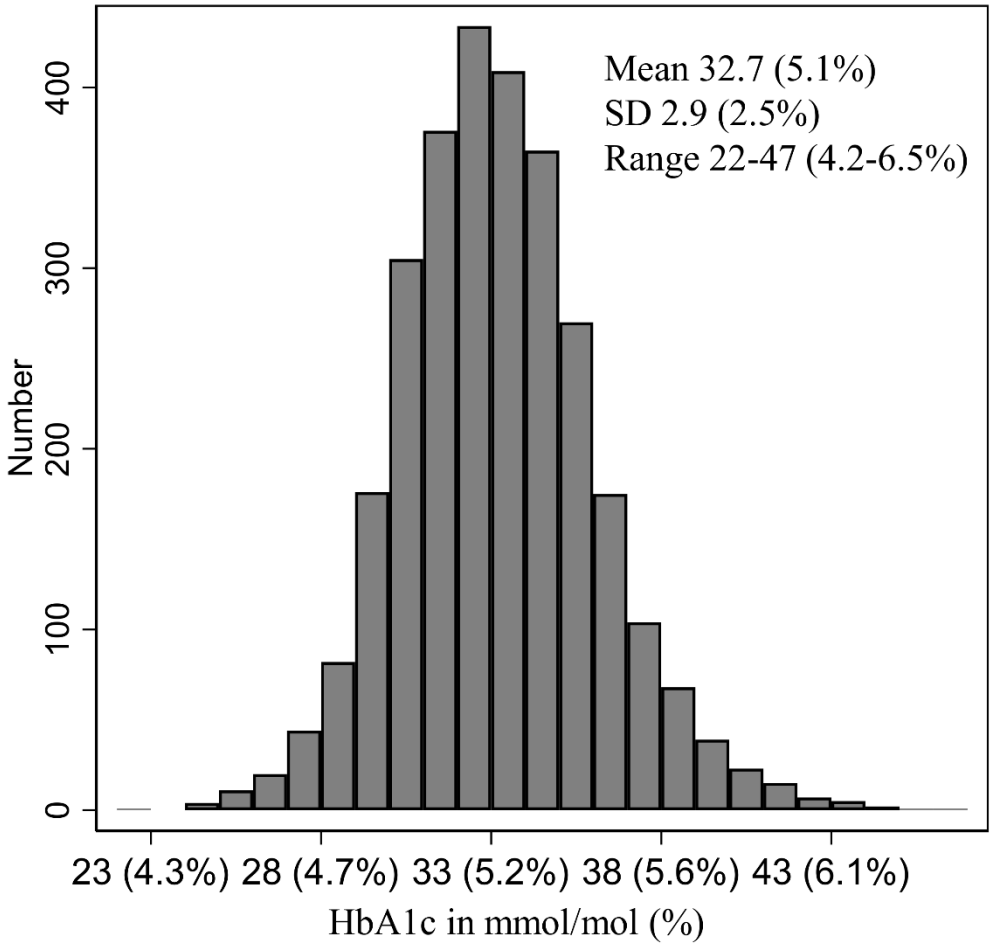


Supplementary Figure S2: Predicted levels of glycated haemoglobin (HbA1c) (mmol/mol) for maternal age and pre-pregnancy body mass index (BMI, kg/m^2), Norway, 2002-2009, in n=2891 singleton pregnancies in mothers without diabetes mellitus, 98.4% of the study population.



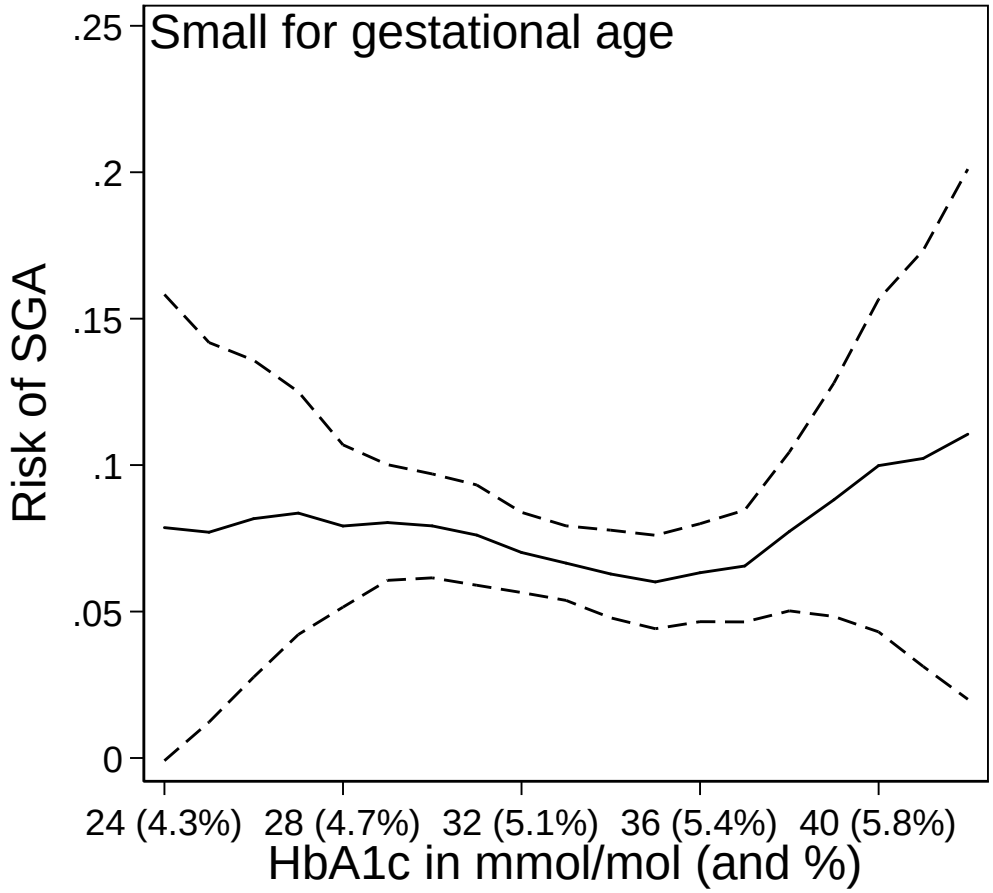
A) maternal age adjusted for maternal body mass index (BMI) (set to mean) and B) maternal pre-pregnancy BMI adjusted for maternal age (set to mean). Solid lines show predicted values; dashed lines show the 95% confidence intervals. Prediction based on a linear regression model with only the covariates HbA1c, maternal age and maternal BMI included. Excluded from the analyses if unavailable information on either of the covariates.

Supplementary Figure S3: Distribution of glycated haemoglobin (HbA1c) levels[a] among 2937 women without registered diabetes mellitus at 18 weeks of pregnancy. Singleton pregnancies in Norway, 2002-2009.



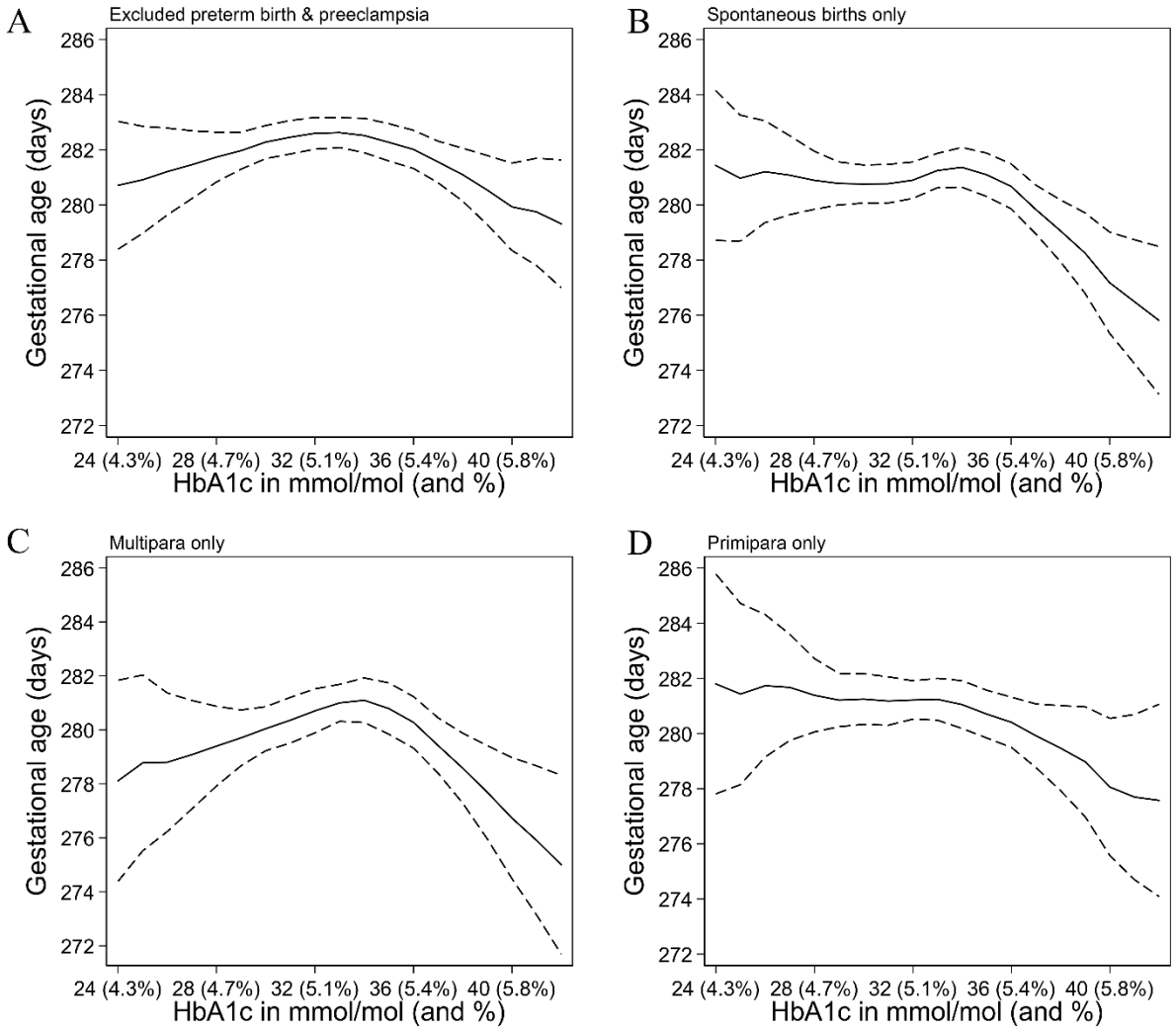
[a] Results in parentheses show the corresponding HbA1c value in %-units, calculated using the formula "(*number in mmol/mol*0.0915)+2.15".*

Supplementary Figure S4: Predicted risk[a] of giving birth to a small-for-gestational age (SGA) infant in relation to 18-week glycated haemoglobin (HbA1c) levels. Solid line show the predicted values, and dashed lines show the 95% confidence intervals.



[a] The predictions were made using a logistic regression model with restricted cubic splines for HbA1c with 4 knots (placed at 28, 31, 34 and 38 mmol/mol). Predictions were estimated using the adjusted models with the following covariates: maternal age (set to mean), body mass index (BMI) (set to mean) and smoking (set to non-smokers). Small-for-gestational age (SGA) was defined as a birthweight <10th percentile for gestational age in weeks, sex and parity (0 or 1+). The model compared odds of SGA versus normal for gestational age (a birthweight at the 10-90th percentiles for the given parameters).
Note that HbA1c levels were grouped at the extremes, with 42+ mmol/mol (6%) as the highest value (16 observations) and 24 mmol/mol (4.3%) as the lowest value (5 observations).

Supplementary Figure S5: Gestational age in relation to 18-week glycated haemoglobin (HbA1c) levels, sensitivity analyses. Panel A) removed preterm births and pregnancies with preeclampsia.[a] Panel B) included only births with a reported spontaneous start of delivery. Panel C) included multiparous women only. Panel D) included primiparous women only. The graphs show the predicted gestational duration for each value of HbA1c (solid line), with 95% confidence intervals (between dashed lines). [b]



[a] Removed n=87 preterm births and n=71 term births with preeclampsia.
[b] The predictions were made using a linear regression model with restricted cubic splines for HbA1c with 4 knots (placed at 28, 31, 34 and 38 mmol/mol for panel A, B and 29, 32, 43 and 38 for panel C and 28, 31, 33 and 37 for panel D). Predictions were estimated using the adjusted models with the following covariates: maternal age (set to mean), body mass index (BMI) (set to mean), smoking (set to non-smokers), and parity (set to primipara, not included in panels C and D). Note that HbA1c levels were grouped at the extremes, with 42+ mmol/mol (6%) as the highest value (16 observations) and 24 mmol/mol (4.3%) as the lowest value (5 observations).

Supplementary Table S1: Associations between different covariates and glycated haemoglobin (HbA1c) levels. Unadjusted associations with 95% confidence intervals and associated p-values, adjusted for maternal age and adjusted for maternal body mass index (BMI; kg/m^2), and lastly adjusted for both maternal age and BMI.

Variable	**Unadjusted model,** estimated change in HbA1c (95% confidence interval)	**p-value**	**Adjusted for maternal age,** estimated change in HbA1c (95% confidence interval)	**p-value**	**Adjusted for maternal BMI,** estimated change in HbA1c (95% confidence interval)	**p-value**	**Adjusted for maternal age and BMI,** estimated change in HbA1c (95% confidence interval)	**p-value**
Maternal age	0.11 (0.09;0.14)	<0.001	-		0.11 (0.09;0.14)	<0.001	-	
Maternal body mass index (BMI)	0.08 (0.05;0.11)	<0.001	0.08 (0.05;0.10)	<0.001	-		-	
Parity								
Multipara vs primipara	0.30 (0.09; 0.51)	0.005	-0.04 (-0.26;0.18)	0.75	0.30 (0.09;0.51)	0.004	-0.3 (-0.25;0.19)	0.82
Smoking at 18 weeks								
Smoker vs non-smoker	0.56 (0.12;0.99)	0.01	0.58 (0.15;1.01)	0.009	0.52 (0.08;0.97)	0.02	0.54 (0.11;0.97)	0.02
Smoker or quitter vs non-smoker	0.17 (-0.10;0.44)	0.22	0.23 (-0.04;0.50)	0.09	0.16 (-0.11;0.43)	0.26	0.22 (-0.05;0.48)	0.11
Quitter vs non-smoker	-0.03 (-0.35;0.29)	0.86	0.04 (-0.27;0.35)	0.80	-0.03 (-0.35;0.28)	0.83	0.04 (-0.28;0.35)	0.81
Maternal education								
< High school	0.13 (-0.39;0.65)	0.63	0.28 (-0.23;0.79)	0.29	0.07 (-0.45;0.60)	0.79	0.21 (-0.31;0.73)	0.42
High school	0.01 (-0.24;0.27)	0.93	0.16 (-0.09;0.41)	0.21	-0.01 (-0.27;0.24)	0.92	0.14 (-0.11;0.39)	0.28
College ≤ 4 years (reference)	1		1		1		1	
>4 years college	0.00 (-0.27;0.27)	0.99	-0.22 (-0.49;0.05)	0.11	0.03 (-0.24;0.30)	0.81	-0.18 (-0.45;0.09)	0.19
Native language								
Foreign-speaker vs Norwegian	0.18 (-0.21;0.57)	0.37	0.12 (-0.27;0.50)	0.55	0.20 (-0.19;0.60)	0.31	0.14 (-0.24;0.53)	0.47
Sex								
Girl vs boy	-0.03 (-0.24;0.18)	0.76	-0.04 (-0.24;0.17)	0.72	-0.03 (-0.23;0.18)	0.81	-0.03 (-0.24;0.18)	0.78
Weight gain before week 18	-0.001 (-0.04;0.04)	0.94	-0.02 (-0.06;0.01)	0.20	0.03 (-0.01;0.07)	0.17	0.00 (-0.04;0.04)	0.91
Maternal height	0.94 (-0.86;2.75)	0.31	0.60 (-1.18;2.38)	0.51	1.29 (-0.52;3.10)	0.16	0.95 (-0.84;2.73)	0.30
Use of assisted reproductive technologies (ART)								
ART versus non-ART	-0.07 (-0.82; 0.69)	0.86	-0.42 (-1.17; 0.33)	0.28	-0.08 (-0.84; 0.67)	0.83	-0.43 (-1.17; 0.32)	0.26

Supplementary Table S2: Comparisons of model fits by likelihood ratio tests of regression analyses of glycated haemoglobin (HbA1c) measured at 18 gestational weeks and multiple perinatal outcomes. Singleton pregnancies, Norway, 2002-2009.

Outcome [a]	P-value for likelihood-ratio-test of HbA1c as restricted cubic splines (4 knots) vs linear continuous	P-value for likelihood-ratio-test of HbA1c as restricted cubic splines (4 knots) vs linear spline 1 knot
Birthweight Z-score	0.69	NA [b]
Length Z-score	0.43	NA [b]
Head circumference Z-score	0.70	NA [b]
Large-for-gestational age	0.99	NA [b]
Small-for-gestational age	NA [b]	0.67
Preterm birth	NA [b]	0.27
Preeclampsia	NA [b]	0.13
Congenital malformations	NA [b]	0.71
Gestational age	NA [b]	0.59

[a] Adjusted for: maternal age (whole years), maternal pre-pregnancy body mass index (BMI) (kg/m^2), smoking in pregnancy (yes versus no). Also adjusted for parity (0 versus 1+) for gestational age. Z-score and small and large-for-gestational-age standardized for gestational age in whole weeks, parity (0 vs 1+) and sex. Large-for-gestational age was defined as >90th percentile, and small-for-gestational age as <10th percentile.

[b] NA: Not assessed

Supplementary Table S3: Linear single-knot spline regression analyses for glycated haemoglobin (HbA1c) measured at 18 gestational weeks and gestational age. Sensitivity analyses. Singleton pregnancies, Norway, 2002-2009.

Model for gestational age in days [a]	**HbA1c level ≤34 mmol/mol**, [b] estimate per unit (95% CI)	**HbA1c level ≥35 mmol/mol,** [b] estimate per unit (95% CI)	**Number of observations for each model** no. (% of study sample)
Excluded preterm births and pregnancies with preeclampsia	0.08 (-0.09 to 0.25) p=0.35	-0.52 (-0.83 to -0.22) p=0.001	2719 (92.6)
Term births only	0.08 (-0.08 to 0.25) p=0.32	-0.53 (-0.83 to -0.24) p<0.001	2789 (95.0)
Non-preeclamptic births only	0.02 (-0.17 to 0.21) p=0.86	-0.65 (-0.99 to -0.30) p<0.001	2789 (95.0)
Spontaneous onset of delivery only	0.08 (-0.12 to 0.27) p=0.43	-0.71 (-1.06 to -0.35) p<0.001	2391 (81.4)
Multipara only	0.26 (-0.02 to 0.53) p=0.07	-0.79 (-1.24 to -0.34) p=0.001	1397 (47.6)
Primipara only	-0.11 (-0.38 to 0.16) p=0.43	-0.54 (-1.07 to -0.02) p=0.04	1477 (50.3)

[a] Adjusted for: maternal age (whole years), maternal pre-pregnancy body mass index (BMI) (kg/m^2), smoking in pregnancy (yes versus no). Also adjusted for parity (0 versus 1+) in the models excluding preterm births and preeclampsia and in the model only looking at spontaneous onset of delivery.

[b] Showing the single-knot linear spline regression coefficients for the linear slope of HbA1c level up to and including 34 mmol/mol and from 35 mmol/mol and up, respectively.

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Paper III:

Reproductive outcomes in women and men conceived by assisted reproductive technologies.

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Submitted manuscript, under review.

III

Reproductive outcomes in women and men conceived by assisted reproductive technologies

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Abstract

Objectives: To determine whether perinatal outcomes differ in pregnancies to women or men conceived by ART compared to their naturally conceived peers.

Design: Prospective register-based study.

Setting: Medical Birth Registry of Norway.

Participants: Persons born in Norway between 1984 and 2002 with a registered pregnancy by the end of 2020.

Exposure: Mothers and fathers who were themselves conceived by ART.

Main outcome measures: Comparing the pregnancies of ART-conceived to naturally conceived women and men, we assess mean birthweight, gestational age, and placental weight by linear regression, and the odds of low or high birthweight, congenital malformations, low 5-minute Apgar score, use of ART, hypertensive disorders of pregnancy and preeclampsia, preterm birth, and offspring sex, by logistic regression. The occurrence of any registered pregnancy in ART-conceived and naturally conceived persons from age 14 until end of follow-up was assessed using Cox proportional regression.

Results: Among 1,092,151 persons born in Norway from 1984 to 2002, 163,427 were registered at least once as mothers, and 121,883 as fathers. Of these, 318 men and 448 women were themselves conceived by ART. Those conceived by ART had little evidence of increased risk of adverse outcomes in their own pregnancies, use of ART, or any difference in mean birthweight, placental weight or gestational age. There was a slightly decreased risk of having a boy among mothers conceived by ART (odds ratio 0.79, 95% confidence interval [CI] 0.66 to 0.95). ART-conceived persons were slightly less likely to have a registered pregnancy within the follow-up period (women: adjusted hazard ratio (HR) 0.87; 95% CI 0.79 to 0.95, men: 0.91, 0.82 to 1.02).

Conclusions: Persons conceived by ART were not at increased risk of obstetric or perinatal complications when becoming parents. The proportions of ART-conceived women and men with a registered pregnancy was lower than among naturally conceived, but longer follow-up time is required to fully assess their fertility and reproductive history.

Introduction

The first child conceived by assisted reproductive technologies (ART) in Norway was born in 1984. Since then, more than 50,000 infants have been conceived by ART in Norway, and more than 10 million born worldwide (1). ART-conceived pregnancies carry increased risk of complications, including hypertensive disorders of pregnancy, preterm birth and low birthweight, with some variation by ART method (2-8). A high proportion of early cohorts of ART-conceived persons were part of a multiple birth, which explains some of the obstetric and perinatal outcomes, but not all (2-4). It is still debated whether these risks are increased by causes of subfertility, or by the ART treatments (9). ART-conceived persons may themselves be at increased risk of later health problems, including cancers and cardiometabolic conditions, but findings are inconsistent (10-15).

Some adverse pregnancy outcomes recur from mother to daughter (16-23) or sons (17, 20, 22). We might expect increased reproductive problems among ART offspring, due to the higher risk of perinatal complications in ART pregnancies and the tendency for perinatal complications in general to recur across generations. Furthermore, ART procedures themselves might have long-term effects on offspring reproductive health. Some studies indicate no differences in hormonal status or pubertal development in ART-conceived females, and some studies indicate differences in hormonal status and sperm quality among ART-conceived males, but results are conflicting (24-30). To the best of our knowledge, no studies have examined pregnancy outcomes or fertility rates in ART-conceived and their naturally conceived peers.

In the current study, we describe registered pregnancies and perinatal outcomes of all ART-conceived and naturally conceived persons born in Norway between 1984 and 2002.

Methods

Data sources

We conducted a prospective population-based study of all men and women born in Norway between 1984 and 2002 who were alive and living in Norway at the age of 14. 1984 was the year of the first birth following ART in Norway. Our primary data source was the Medical Birth Registry of Norway (31). The birth registry contains information on all Norwegian deliveries and fetal losses after 12 gestational weeks. We used personal identification numbers to link information from a person's own birth record, to pregnancies where that person was registered as a mother or father by the end of 2020.

Included pregnancies

Miscarriage was defined as a fetal loss between gestational weeks 12 and 22 or, if gestational age was missing, a birthweight <500 grams. Miscarriages and late induced abortions (12 – 22 gestational weeks) were included as a registered pregnancy in the analyses of ever having a registered pregnancy. However, they were excluded when analysing perinatal outcomes (restricted to births only).

Conception by assisted reproductive technologies

It is mandatory for fertility clinics in Norway to report the use of ART to the birth registry. We defined ART as any use of ART (including fresh and frozen embryo transfer, and in vitro fertilization [IVF] with and without intracytoplasmic sperm injection [ICSI]). Intrauterine inseminations were not defined as ART. Oocyte donation became legal in Norway in January, 2021 (32) (after our study period), while sperm donation has been allowed during the whole study period.

Obstetric and perinatal outcomes

Information on birthweight was analysed as a continuous variable or grouped into three categories; low birthweight, <2500 grams; normal birthweight, 2500-4499 grams; and high

birthweight, ≥4500 grams. Information on placental weight in grams was treated as a continuous outcome.

We calculated gestational age from birth registry data using ultrasound dating if the estimated gestational age at birth was between 22 and 45 completed weeks. If ultrasound data were not available, we used date of last menstrual period (LMP) with the same time restriction, and for ART-conceived pregnancies, LMP was estimated from date for embryo-insertion adjusted to supposed LMP. Preterm delivery was defined as a delivery before 37 completed gestational weeks.

The birth registry also provided information on plurality and on Apgar scores after 5 minutes, which we defined as low if below 7. Congenital malformations were grouped into a single category (any versus none). We defined hypertensive disorders of pregnancy (HDP) as any record of preeclampsia, eclampsia, HELLP (hemolysis, elevated liver enzymes and low platelets) or hypertension discovered during pregnancy.

Characteristics of women and men conceived by ART or naturally

Characteristics of women and men who were registered as parents in the birth registry included age at delivery (in whole years), parity (grouped it into 0 or 1+), smoking status at the beginning of pregnancy (women only, grouped into none, daily, occasional or "not consenting to give smoking status"). Women who reported smoking at end of pregnancy were categorized as daily smokers. In regression analyses, smoking was included in two categories: (no versus smoking/no consent). Maternal marital status was defined as a combined group of "married/cohabiting/registered partner" versus "other" (including single, widowed, separated and unknown). Maternal folic acid supplement was categorised as "yes" or "no". Maternal body mass index (kg/m^2, BMI) was available from 2006 (70% of index pregnancies among women and 75% of partners to the men).

From the parents' own birth records we extracted the following information: birthweight, gestational age at birth, maternal country of birth (Norwegian or other/missing), whether or not the parent was part of a plural birth (yes or no), county of birth, or whether the pregnancy was complicated by a hypertensive disorder of pregnancy.

Statistical analyses

We compared the likelihood of having a pregnancy among men and women according to whether or not they were ART-conceived by using Cox proportional hazards regression to estimate the hazard ratio (HR) of having a registered pregnancy during follow-up. We used age of the women and men (in years) as the underlying time-scale, and followed each person from age 14, either until age at conception for a registered pregnancy, emigration, death, or December 31st, 2020 for those residing in Norway who had not experienced a pregnancy. For miscarriages or stillbirths missing gestational age (0.4% of first pregnancies for women and 0.2% for men), we calculated mother's and father's age at conception assuming a gestational age of 14 weeks for miscarriages and 22 weeks for stillbirths. All estimates were adjusted for parents' birth year in intervals (1984-1989, 1990-1993, 1994-1997, 1998-2002), and further adjusted for information from the parents' birth records on their mothers' country of birth (Norwegian or other), and county within Norway (11 counties, a variable associated with availability of ART procedures).

Obstetrical and perinatal outcomes

Our main analysis of pregnancy outcomes was restricted to the first registered pregnancy ("index pregnancy") born ≥22 gestational weeks for parents born after 1984. In a secondary analysis we included all registered pregnancies ≥22 weeks for these parents.

We grouped birth characteristics for mothers, fathers, and their offspring according to whether the parent had been conceived by ART, and calculated means for continuous characteristics and proportions for categorical or binary characteristics.

Each newborn was counted as a separate entry for newborn outcomes, while each pregnancy was counted once for parental characteristics, regardless of number of fetuses. Specifically, the outcomes of preeclampsia, hypertensive disorders of pregnancy, and preterm birth were counted only once for pregnancies with multiples (twins, triplets).

We used linear regression to compare the mean birthweight, gestational age, and placental weight according to whether the parents were conceived by ART. We used logistic regression to calculate odds ratios according to parental ART status of undergoing a preterm delivery, developing preeclampsia or another hypertensive disorder of pregnancy, having an infant with any congenital malformation, having a low 5-minute Apgar score, having high or low birthweight, or having a boy. We also considered the odds of themselves conceiving with ART.

ART procedures have not been practiced long enough to observe the whole reproductive period for these persons born in 1984 and later. Moreover, the proportion of ART-conceived persons among all births has risen steadily over time, which might produce differences between the groups that could confound associations. We analysed the first registered pregnancy for persons born after 1984, and present results from two regression models: 1) a crude analysis not adjusting for any covariates; and 2) a model adjusting for offspring sex and for variables with time trends or variations during the study period, as potential mediators. These included the following variables at the time of the first pregnancy produced by ART-conceived parents and their peers: age at conception (<25 years, 25-29 years, ≥30 years), year of pregnancy (<2011, 2011-2015, 2016-2018, 2019-2020), smoking, folic acid supplement, grandmaternal country of birth (Norwegian or other). In the analyses of men, both maternal and paternal age at conception were included as covariates. Both regression models were subsequently performed with all registered pregnancies, where we further adjusted for parity (0 or ≥1).

All analyses were conducted using Stata version 16.0 SE (Statacorp, College Station, TX).

Patient and public involvement

No patients were involved in setting the research question or the outcome measures, nor were they involved in developing plans for recruitment, design, or implementation of the study. No patients were asked to advise on interpretation or writing up of results. Results of this study will be disseminated to study participants through our public websites, public media and publicly available newsletters, and through obstetricians and fertility clinicians reaching the relevant patient community.

Results

Women

531,015 liveborn girls were registered between 1984 and 2002 (after excluding neonatal deaths within the first 24 hours), of which 526,455 were living in Norway at age 14 and with no prior pregnancy (eFigure 1a in Supplement). Of these, 4763 were ART-conceived and 521,692 were naturally conceived. After censoring those who moved out of the country, at least one pregnancy was registered for 448 (9%) of the ART-conceived women and 162,979 (31%) of the naturally conceived women. 383 (83%) of the ART-conceived women who had at least one registered pregnancy were conceived by the use of IVF without ICSI. The likelihood of having a registered pregnancy before the end of 2020 was lower in the ART-conceived group than the naturally conceived group, with a minimally adjusted HR of 0.69 (95% confidence interval, 0.62 to 0.75) and a further adjusted HR of 0.87 (0.79 to 0.95) (Table 1). There was no indication of non-proportionality in the adjusted model (p-value Schoenfeld residuals of 0.99).

Table 1. **Incidence of a first registered pregnancy by mode of conception.**

Sex	Mode of conception	Number of study participants	Analysis time at risk, years	One registered pregnancy in MBRN, No. (%)	Hazard ratio (95% CI)	
					Minimally adjusted[1,2]	Fully adjusted[1,3]
Women	**Naturally conceived**	521692	5787856.8	162,979 (31.2)	1 (Reference)	1 (Reference)
	ART-conceived	4763	42188.1	448 (9.4)	0.69 (0.63 to 0.75)	0.87 (0.79 to 0.95)
Men	**Naturally conceived**	550,402	6545492.0	121,565 (22.1)	1 (Reference)	1 (Reference)
	ART-conceived	5083	46217.6	318 (6.3)	0.77 (0.69 to 0.86)	0.91 (0.82 to 1.02)

[1] Hazards ratios of having a pregnancy before 2020 in women and men born after 1984. Fetal losses/stillbirths after gestational week 12 are included in the analysis. Persons entered the risk set at age 14 and were censored at age of death or emigration, or age at the end of the study period (December 31, 2020).

[2] The hazard ratios were adjusted for year of birth categorically (1984-1989, 1990-1993, 1994-1997, 1998-2002).

[3] The hazard ratios were adjusted for year of birth (categorically), grandmaternal country of birth (Norwegian or other/missing), grandmaternal age at birth (continuous) and county of birth (categorically).

Characteristics at time of the index pregnancy of mothers who were conceived with ART were similar to naturally conceived mothers (Table 2). The ART-conceived mothers were more likely to have been part of a multiple birth, to have been born after a pregnancy complicated by a hypertensive disorder and to have had lower birthweight or gestational age at birth than naturally conceived mothers (Table 2).

Table 2. Characteristics of mothers born between 1984 and 2002 at the time of their first registered pregnancy. [1]

Characteristic	Naturally conceived mothers	ART-conceived mothers
Pregnancies, No.	162,991	449
Children, No.	164,027	457
Age at conception, mean (SD)	25.3 (4.0)	24.5 (3.4)
Parity		
0, No. (%)	160,288 (98.3)	441 (98.2)
≥1, No. (%)	2703 (1.7)	8 (1.8)
Smoking at beginning of pregnancy		
No, No. (%)	124,671 (76.5)	384 (85.5)
Daily or occasional, No. (%)	24,389 (15.0)	32 (7.1)
Missing, No. (%)	13,931 (8.5)	26 (5.8)
Pre-pregnancy BMI, mean (SD)	24.4 (4.9)	24.3 (4.8)
Missing BMI, No. (%)	49,103 (30.1)	72 (16.0)
Folic acid intake during pregnancy, No. (%)	129,097 (79.2)	375 (83.5)
Marital status		
Married/registered/cohabiting, No. (%)	143,920 (88.3)	394 (87.8)
Other, No. (%)	19,071 (11.7)	55 (12.2)
Multiple birth, No. (%)	2036 (1.2)	7 (1.6)
Stillbirth index pregnancy, No. (%)	559 (0.3)	<5 (0.2)
Mother of the woman's country of birth		
Norway, No. (%)	134,669 (82.6)	382 (85.1)
Other, No. (%)	28,322 (17.4)	67 (14.9)
Hypertensive disorder of pregnancy in mother of the woman, No. (%)	7418 (4.6)	40 (8.9)
Woman's own birthweight in gr, mean (SD)	3457 (541)	2935 (779)
Woman's own gestational age in days, mean (SD)	281.7 (13.7)	269.3 (21.6)
Themselves part of multiple birth, No. (%)	3212 (2.0)	187 (41.6)

[1] Miscarriages and induced abortions before week 22 are excluded from the analysis.

When assessing pregnancy outcomes of index pregnancies, we included only the first registered pregnancy. Offspring birthweight, gestational age and placental weight were similar in births to ART-conceived and naturally conceived women (Table 3). The other outcomes, including preterm delivery, low Apgar score, preeclampsia or other hypertensive disorders of pregnancy, conceiving with ART or having a newborn with a congenital malformation, low or high birth weight, were also similar in ART-conceived and naturally conceived women (Figure 1a). The associations did not change substantially after adjusting for possible confounders. The odds of having a boy were lower among the ART-conceived mothers (adjusted OR 0.79, 95% CI 0.66, 0.95).

Table 3. **Linear regression models comparing birthweight, gestational age, and placental weight by parental mode of conception.**

Sex	Outcome	Naturally conceived, mean (SD)	ART-conceived, mean (SD)	Difference between ART-conceived and naturally conceived (95 % CI)	
				Unadjusted	Adjusted [1]
Women	Birthweight, grams	3425 (589)	3434 (602)	9 (-45 to 63)	5 (-49 to 59)
	Placental weight, grams	651 (154)	641 (143)	-9 (-24 to 5)	-4 (-18 to 11)
	Gestational age, days	278.1 (14.7)	278.6 (14.9)	0.0 (-1.4 to 1.4)	0.3 (-1.1 to 1.6)
Men	Birthweight, grams	3440 (586)	3451 (562)	12 (-53 to 76)	2 (-62 to 66)
	Placental weight, grams	651 (154)	636 (151)	-15 (-32 to 2)	-11 (-28 to 6)
	Gestational age, days	278.1 (14.3)	277.6 (14.0)	-0.4 (-2.0 to 1.3)	-0.4 (-2.0 to 1.2)

[1] Adjusted for maternal age at conception (<25 years, 25-29 years, 30+ years), year of birth index pregnancy (categorically, <2011, 2011-2015, 2016-2018, 2019-2020), offspring sex, smoking (none vs smoking or missing), folic acid supplement (yes vs no), grandmaternal country of birth (Norway or other). Also adjusted for paternal age at conception (<25 years, 25-29 years, 30+ years) in the analyses of men.

Results were similar when including subsequent pregnancies (eTables 1 and 2 in Supplement). There was an increased risk for a low 5-minute Apgar score among offspring of ART-conceived mothers, although this was based on small numbers and with a wide confidence interval (adjusted OR 1.65, 1.04 to 2.61).

Men

Among 561,136 liveborn boys born between 1984 and 2002, there were 555,485 registered as living in Norway at age 14 (eFigure 1b in Supplement). Of these, 5083 were conceived by ART and 550,402 were naturally conceived. Of these, 318 (6%) ART-conceived men and 121,565 (22%) naturally conceived men fathered at least one pregnancy. Of the ART-conceived men who fathered at least one pregnancy, 274 (86%) were conceived by the use of IVF without ICSI. The likelihood of contributing a registered pregnancy before the end of 2020 was lower among ART-conceived men, with an unadjusted HR of 0.77 (95% confidence interval, 0.69 to 0.86) and an adjusted HR of 0.91 (0.82 to 1.02) (Table 1). There was no indication of non-proportionality in the adjusted model (p-value Schoenfeld residuals of 0.21).

Fathers conceived by ART were more likely to have been part of a multiple birth or part of a pregnancy complicated by a hypertensive disorder, and more likely to have had lower birthweight or gestational age at birth (Table 4). At the time of their index pregnancy, the characteristics were generally similar to naturally conceived fathers (Table 4).

When assessing their first registered pregnancy in the birth registry, offspring of ART-conceived men had similar birthweight, gestational age and placental weight to the offspring of naturally conceived men (Table 3). The odds were similar for experiencing a preterm delivery, low or high birthweight, a congenital malformation, low Apgar score, preeclampsia or other hypertensive disorders of pregnancy, having a male baby, and conceiving with ART (Figure 1b). These associations did not change substantially after adjusting for possible confounders. Results were also similar when including subsequent pregnancies (eTables 1 and 3 in Supplement).

Table 4. Characteristics of fathers born between 1984 and 2002 at the time of their first registered pregnancy. [1]

Characteristic	Naturally conceived fathers	ART-conceived fathers
Pregnancies, No.	121,580	318
Children, No.	122,931	322
Man's age at conception, mean (SD)	26.6 (3.9)	25.2 (3.4)
Maternal (partner) parity		
0, No. (%)	108,425 (89.2)	296 (93.1)
≥1, No. (%)	13,155 (10.8)	22 (6.9)
Maternal (partner) smoking at beginning of pregnancy		
No, No. (%)	95,556 (78.6)	271 (85.2)
Daily or occasional, No. (%)	16,221 (13.1)	26 (8.2)
Missing, No. (%)	9803 (8.1)	21 (6.6)
Maternal (partner) pre-pregnancy BMI, mean (SD)	24.3 (4.8)	24.4 (5.0)
Missing BMI, No. (%)	30,572 (25.1)	52 (16.4)
Maternal (partner) folic acid intake during pregnancy, No. (%)	97,848 (80.5)	272 (85.5)
Marital status		
Married/registered/cohabiting, No. (%)	108,773 (89.5)	283 (89.0)
Other, No. (%)	12,807 (10.5)	35 (11.0)
Multiple birth, No. (%)	1510 (1.2)	<5 (1.3)
Stillbirth index pregnancy, No. (%)	140 (0.1)	0 (0)
Mother of the man's country of birth		
Norway, No. (%)	99,650 (82.0)	262 (82.4)
Other, No. (%)	21,930 (18.0)	56 (17.6)
Hypertensive disorder of pregnancy in mother of the man, No. (%)	5943 (4.9)	36 (11.3)
Man's own birthweight in gr, mean (SD)	3596 (571)	3043 (813)
Man's own gestational age in days, mean (SD)	280.6 (14.0)	267.7 (22.5)
Themselves part of multiple birth, No. (%)	2373 (2.0)	127 (39.9)

[1] Miscarriages and induced abortions before week 22 are excluded from the analysis.

Discussion

In this registry-based study of all pregnancies to men and women born between 1984 and 2002 in Norway, the ART-conceived and naturally conceived parents had pregnancies with similar rates of complications and outcomes. A smaller proportion of ART-conceived men and women had a registered pregnancy before the end of 2020, although this difference was strongly attenuated by adjustments, suggesting that the remaining differences may be due to residual confounding. Surprisingly, we found a lower odds of giving birth to a boy among the ART-conceived women. While false-negative results are common with sex ratios (especially in the absence of a prior hypothesis) (33), we cannot exclude a possible true difference. This puzzling finding should be addressed in future studies. We also observed an increased odds of a low 5-minute Apgar score among newborns born to ART-conceived women when including all births rather than just first births. The wide confidence intervals warrant caution in interpretation, although this finding should be followed up in later studies.

Major strengths of this study were our access to all births, including all ART-conceived births, in Norway since the earliest ART birth in 1984, and the linking of information from parents' own birth with the outcomes of pregnancies they produce, while accounting for differences in baseline characteristics.

Limitations of this study include the small number of pregnancies produced by ART-conceived persons, which provides little statistical power to examine rare outcomes or to detect small true differences, in particular for the binary outcomes. There may also be residual confounding in the analyses assessing the likelihood of having a registered pregnancy in the birth registry by end of follow-up. Specifically, we did not have information on socioeconomic status in the parent generation, which may have affected both the use of ART to conceive and the later likelihood of having a registered pregnancy among the offspring. Furthermore, given that all parents were born in Norway, this study represents a relatively

homogeneous population. While we cannot be certain of the generalizability to a more ethnically diverse population, we see no reason that possible adverse effects of ART would affect various ethnicities differently.

Since time does not yet allow for complete follow up of reproductive history for ART offspring, there remain difficult questions of selection bias. The reduced rate of first registered pregnancies for ART-conceived men and women could in theory be caused by an increased risk of unrecorded miscarriages before 12 weeks. Selection into pregnancy could be further affected by a variety of factors, such as unmeasured socio-economic differences, varying fertility wishes, or differences in chronic conditions. Furthermore, we could not separate subfecundity from social factors that determine the decision to conceive. For example, daughters of older mothers are more likely to remain childless (34), but they do not to have lower fecundability (35, 36). There may be family-based differences in the preferred age at starting a family and ART-conceived people would tend to come from parents who had started trying to reproduce at relatively old ages (37). Adjustment for their mother's age at time of birth substantially closed the gap between pregnancy rate in ART- and naturally conceived offspring. Although we can only speculate at this point, we might take further reassurance about the fertility of ART-conceived offspring from the fact that subfertile couples have an increased risk of adverse pregnancy outcomes (38, 39) and we find little evidence of increased risk of adverse pregnancy outcomes in the ART-conceived. Future studies of the fecundability of ART offspring should be able to answer this question more definitively.

Conclusions

Men and women who were conceived by ART and become parents do not appear to be at increased risk of adverse pregnancy outcomes. ART-conceived persons were less likely to conceive for reasons that are likely to be social and voluntary. Larger studies with longer

follow-up time and a more direct assessment of time to pregnancy will help complete the picture of fertility among offspring conceived by ART. Meanwhile, these early results are reassuring for the increasing number of ART-conceived adolescents and young adults entering their reproductive years.

Footnotes

Contributors

EOC, MCM, SEH and AJW conceived and designed the study. SEH obtained access to data. EOC conducted data analysis and drafted the initial version of the manuscript. AJW, SEH, MCM and HIH provided important insight during the data analysis. All authors contributed in the interpretation of the data and critically revised the manuscript. All authors had full access to the data in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis. EOC is the guarantor. The corresponding author attests that all listed authors meet authorship criteria and that no other meeting the criteria have been omitted.

Funding

This work was funded by the Research Council of Norway project number 320656 and through its Centres of Excellence funding scheme, project number 262700, and by the European Research Council grant agreement number 947684, by the Norwegian Institute of Public Health (NIPH) and by Telemark Hospital Trust. The funding agencies had no role in the conceptualization, design, data collection, analysis, decision to publish, or preparation of the manuscript.

Competing interests

All authors have completed the ICMJE uniform disclosure form at http://www.icmje.org/disclosure-of-interest/ and declare: EOC, HIH, SEH and MCM had financial support from the Norwegian Research Council, and MCM had support from the European Research Council and HIH from Telemark Hospital Trust for the submitted work, HIH has received travel reimbursements from Ferring Pharmaceuticals and Gedeon Richter Nordics; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; HIH is a member of the Norwegian biotechnology

council and Head of the Board of the Norwegian Association for assisted reproduction; no other relationships or activities that could appear to have influenced the submitted work.

Ethical approval

This study was approved by the Regional Committee for Medical and Health Ethics of South/East Norway (No. 2014/404), which waived the need of consent from participants in this registry-based study.

Data sharing

No additional data are available.

The lead author (EOC) affirms that the manuscript is an honest, accurate, and transparent account of the study being reported: that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

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Summary boxes

Section 1: What is already known on this topic.

- Use of assisted reproductive technologies (ART) is increasing worldwide.
- Pregnancies conceived by assisted reproductive technologies are at higher risk of several obstetric and perinatal complications.
- The possible pregnancy risks faced by ART offspring who start their own families has not yet been studied.

Section 2: What this study adds.

- There was no indication that women or men conceived by ART are at higher risk of pregnancy complications or adverse perinatal outcomes when compared to naturally conceived peers.
- There were fewer pregnancies among ART-conceived men and women compared to their naturally conceived peers, which may be attributable to social factors and the limited time available for follow-up.

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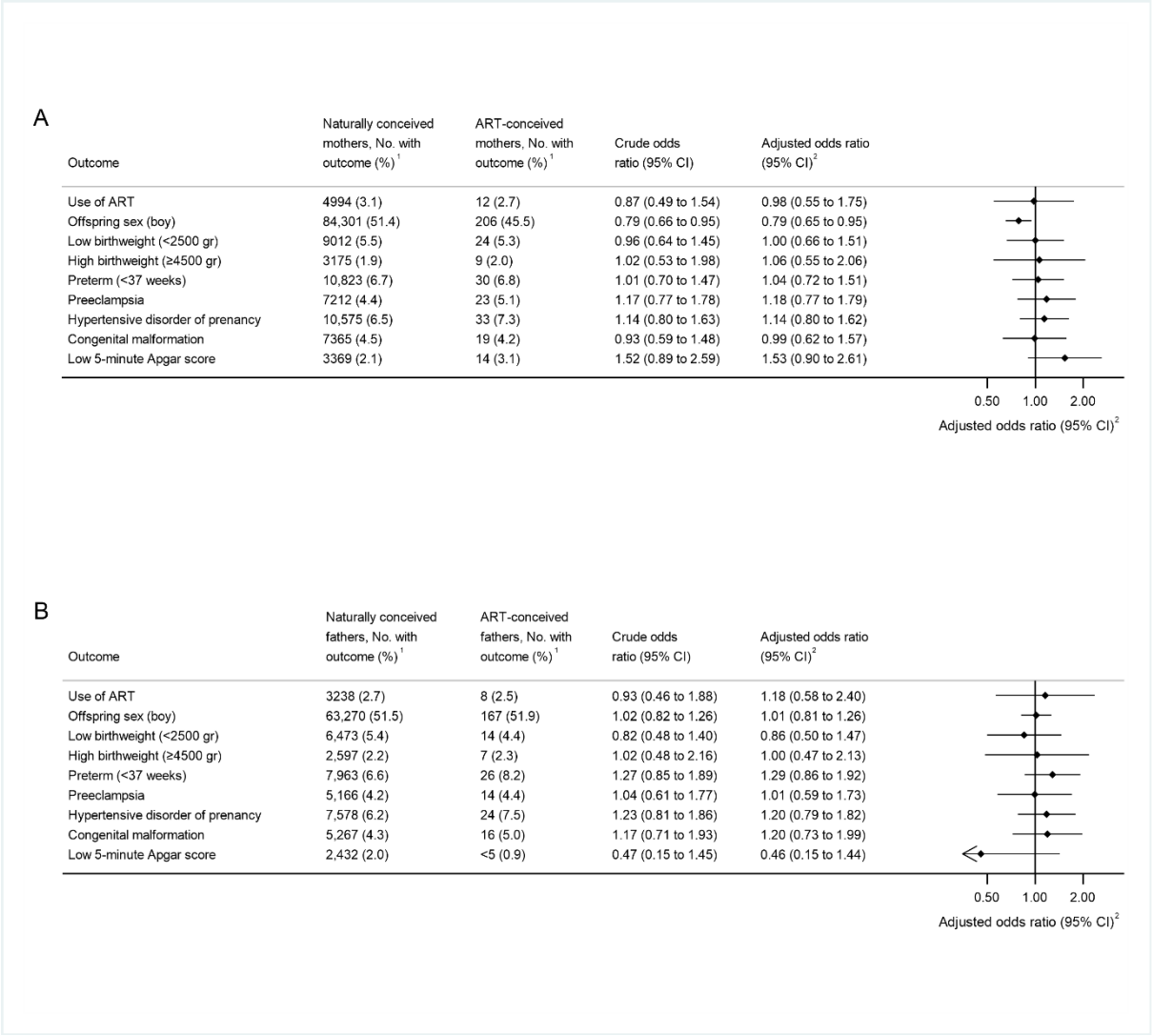
Figures

Figure 1. The odds ratios of pregnancy outcomes in pregnancies to ART-conceived and naturally conceived women and men.



A) Women, B) Men.

Diamonds correspond to point estimates, lines correspond to 95% confidence intervals.

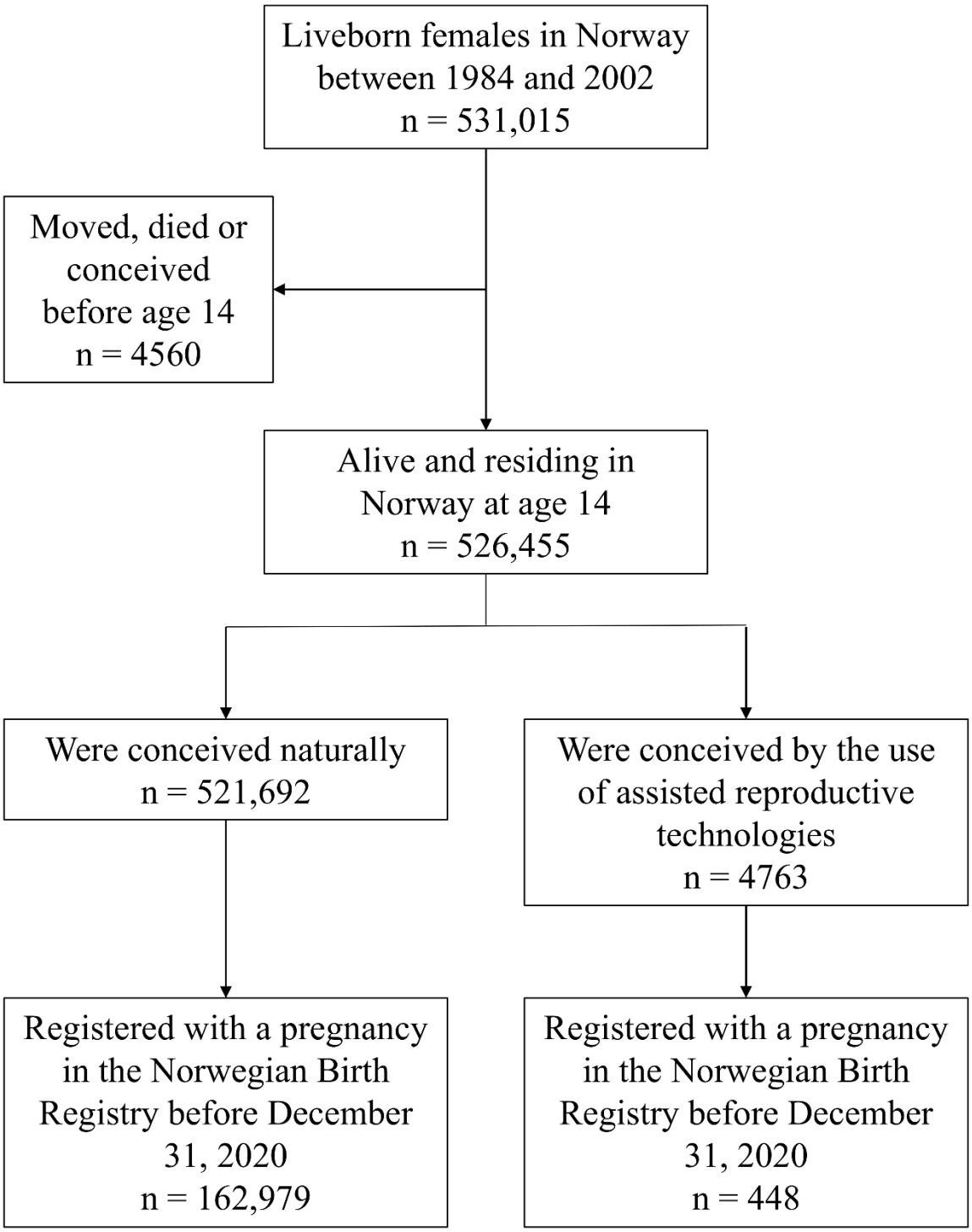
[1] For outcomes offspring sex, low and high birthweight, congenital malformations and low Apgar scores, each child is counted regardless of plurality. For outcomes ART conception, preterm birth, preeclampsia, hypertensive disorders of pregnancy, each pregnancy contributes with one count.

[2] Adjusted for maternal age at conception (<25 years, 25-29 years, 30+ years), year of birth index pregnancy (categorically, <2011, 2011-2015, 2016-2018, 2019-2020), offspring sex, smoking (none vs smoking or missing), folic acid supplement (yes vs no), grandmaternal country of birth (Norwegian or other). Also adjusted for paternal age at conception (<25 years, 25-29 years, 30+ years) in the analyses of men.

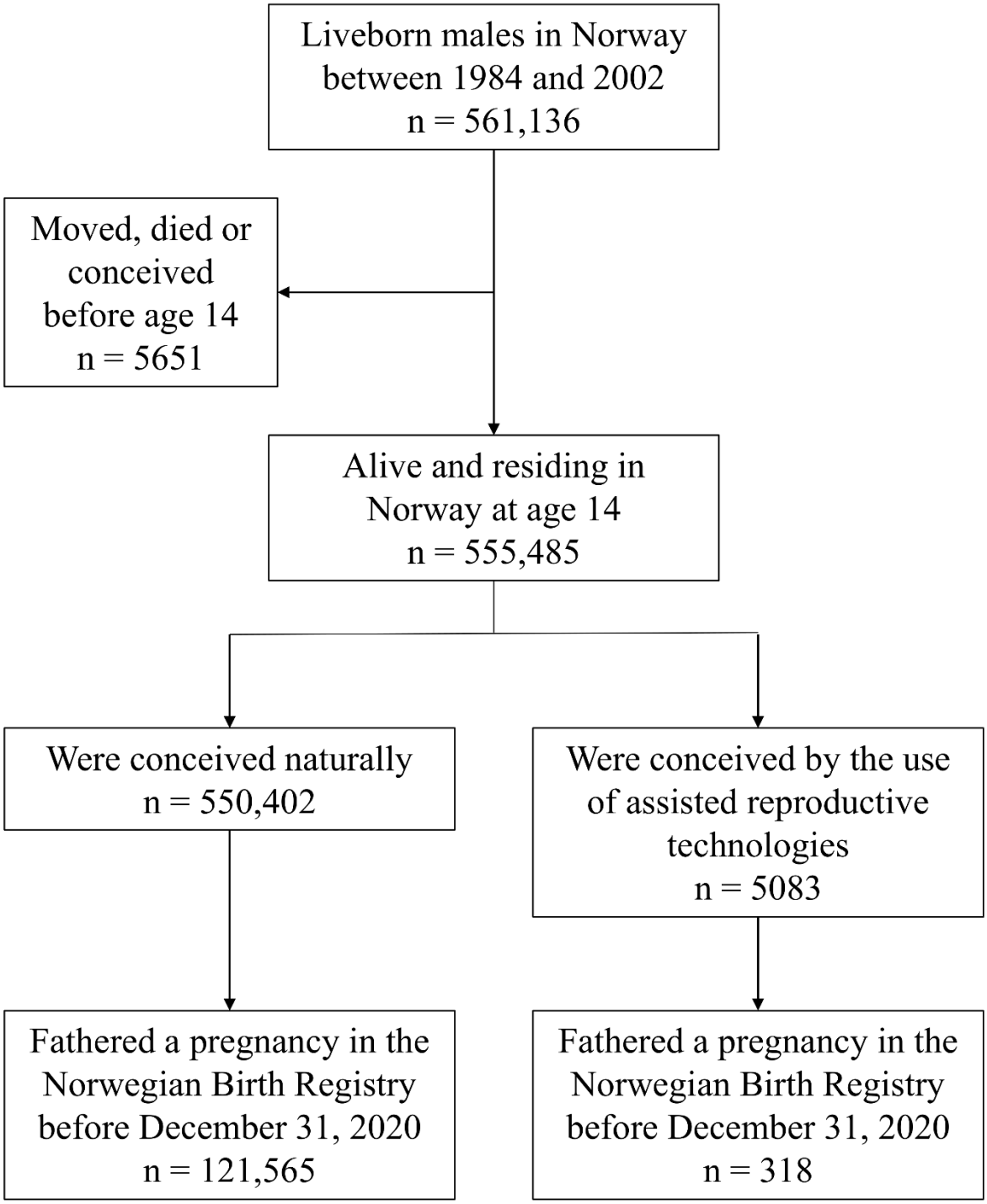
Supplementary material (paper III)

eFigure 1. Flow-chart of study participant selection. A) Women, B) Men.[1]

A)



B)



[1] Liveborn definition: exclusion of stillbirths and those dead within 24 hours.

eTable 1. Linear regression models comparing birthweight, gestational age, and placental weight by parental mode of conception.[1]

	Outcome	Naturally conceived, mean (SD)	ART-conceived, mean (SD)	Difference between ART-conceived and naturally conceived (95 % CI)	Adjusted difference between ART-conceived and naturally conceived (95 % CI)[2]
Women	Birthweight, grams	3501 (585)	3489 (604)	-11 (-55 to 33)	7 (-37 to 51)
	Placental weight, grams	663 (157)	658 (158)	-6 (-17 to 7)	4 (-8 to 15)
	Gestational age, days	278.2 (13.8)	277.9 (14.6)	-0.6 (-1.7 to 0.5)	-0.3 (-1.4 to 0.7)
Men	Birthweight, grams	3500 (582)	3490 (568)	-10 (-63 to 43)	0 (-52 to 52)
	Placental weight, grams	660 (156)	657 (171)	-3 (-17 to 12)	3 (-12 to 17)
	Gestational age, days	278.1 (13.5)	277.4 (13.6)	-0.6 (-1.9 to 0.7)	-0.5 (-1.8 to 0.8)

[1]All registered births, not just first births. Miscarriages and induced abortions before week 22 not included. Included pregnancies, women; n=287,447, included newborns, women; n=289,316. Included pregnancies, men; n=200,575, included newborns by men; n=202,772.

[2] Adjusted for maternal age at conception (<25 years, 25-29 years, 30+ years), year of birth index pregnancy (categorically, <2011, 2011-2015, 2016-2018, 2019-2020), offspring sex, smoking (none vs smoking or missing), folic acid supplement (yes vs no), grandmaternal country of birth (Norway or other) and parity. Also adjusted for paternal age at conception (<25 years, 25-29 years, 30+ years) in the analyses of men.

eTable 2. The odds ratios of pregnancy outcomes in pregnancies to ART-conceived and naturally conceived women.[1]

Outcome	Naturally conceived, No. with outcome (%)[2]	ART conceived, No. with outcome (%)[2]	Odds ratio of outcome in ART conceived compared to naturally conceived (95 % CI)	
			Crude	Adjusted[3]
Use of ART	7136 (2.5)	15 (2.3)	0.91 (0.55 to 1.52)	0.99 (0.59 to 1.67)
Offspring sex (boy)	148,389 (51.4)	310 (46.5)	0.82 (0.70 to 0.96)	0.82 (0.70 to 0.95)
Low birthweight (<2500gr)	13,026 (4.5)	36 (5.4)	1.20 (0.86 to 1.68)	1.18 (0.84 to 1.65)
High birthweight (≥4500 gr)	8,329 (2.9)	17 (2.5)	0.89 (0.55 to 1.44)	1.00 (0.62 to 1.63)
Preterm birth	16,475 (5.8)	41 (6.3)	1.09 (0.80 to 1.50)	1.06 (0.78 to 1.46)
Preeclampsia	9,249 (3.2)	28 (4.2)	1.33 (0.91 to 1.94)	1.22 (0.83 to 1.78)
Any hypertensive disorder of pregnancy	14,136 (4.9)	41 (6.2)	1.28 (0.93 to 1.75)	1.17 (0.85 to 1.61)
Congenital malformations	11,365 (3.9)	27 (4.0)	1.03 (0.70 to 1.51)	1.03 (0.70 to 1.52)
Low 5-minute Apgar score (<7)	4,782 (1.7)	19 (2.8)	1.74 (1.10 to 2.75)	1.64 (1.04 to 2.59)

[1] All births included, not just first births. Miscarriages and induced abortions before week 22 not included. Included pregnancies; n=287,447, included newborns, n=289,316.

[2] For outcomes offspring sex, low and high birthweight, congenital malformations and low Apgar scores, each child is counted regardless of plurality. For outcomes ART conception, preterm birth, preeclampsia, hypertensive disorders of pregnancy, each pregnancy contributes with one count.

[3] Adjusted for maternal age at conception (<25 years, 25-29 years, 30+ years), year of birth index pregnancy (categorically, <2011, 2011-2015, 2016-2018, 2019-2020), offspring sex, smoking (none vs smoking or missing), folic acid supplement (yes vs no), grandmaternal country of birth (Norway or other), and parity (0 or 1+).

eTable 3. The odds ratios of pregnancy outcomes in pregnancies to ART-conceived and naturally conceived men.[1]

Outcome	Naturally conceived, No. with outcome (%)[2]	ART-conceived, No. with outcome (%)[2]	Odds ratio of outcome in ART-conceived compared to naturally conceived (95 % CI)	
			Crude	Adjusted[3]
Conception by ART	4456 (2.2)	11 (2.4)	1.08 (0.59 to 1.97)	1.35 (0.74 to 2.49)
Offspring sex (boy)	104,001 (51.4)	238 (51.2)	0.99 (0.83 to 1.19)	0.99 (0.82 to 1.19)
Low birthweight (<2500gr)	9,122 (4.6)	18 (4.0)	0.86 (0.53 to 1.37)	0.85 (0.53 to 1.36)
High birthweight (≥4500 gr)	5,720 (3.0)	15 (3.4)	1.14 (0.68 to 1.91)	1.23 (0.73 to 2.07)
Preterm birth	11,683 (5.9)	30 (6.6)	1.13 (0.78 to 1.64)	1.10 (0.76 to 1.59)
Preeclampsia	6,590 (3.3)	19 (4.2)	1.27 (0.80 to 2.02)	1.15 (0.72 to 1.82)
Any hypertensive disorder of pregnancy	10,011 (5.0)	29 (6.3)	1.29 (0.88 to 1.88)	1.17 (0.80 to 1.72)
Congenital malformations	7,834 (3.9)	24 (5.2)	1.35 (0.90 to 2.04)	1.34 (0.89 to 2.03)
Low 5-minute Apgar score (<7)	3,353 (1.7)	7 (1.5)	0.91 (0.43 to 1.91)	0.84 (0.40 to 1.79)

[1] All births included, not just first births. Miscarriages and induced abortions before week 22 not included. Included pregnancies n=200,575, included newborns by men; n=202,772.

[2] For outcomes offspring sex, low and high birthweight, congenital malformations and low Apgar scores, each child is counted regardless of plurality. For outcomes ART conception, preterm birth, preeclampsia, hypertensive disorders of pregnancy, each pregnancy contributes with one count.

[3] Adjusted for maternal and paternal age at conception (<25 years, 25-29 years, 30+ years), year of birth index pregnancy (categorically, <2011, 2011-2015, 2016) , offspring sex, smoking (none vs smoking or missing), folic acid supplement (yes vs no), grandmaternal country of birth (Norway or other), and parity (0 or 1+).