Twin Pregnancies: Long-term Maternal Mortality, Birthweight in Subsequent Pregnancy and Adverse Pregnancy Outcomes in Next Generation

Prativa Basnet

Thesis for the degree of Philosophiae Doctor (PhD)
University of Bergen, Norway
2024

UNIVERSITY OF BERGEN

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Year: 2024

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Name: Prativa Basnet

Print: Skipnes Kommunikasjon / University of Bergen

Scientific environment

The work in this thesis has been carried out in the Research Group for Registry based Reproductive Epidemiology (ReproEpi) at the Department of Global Public Health and Primary Care, Faculty of Medicine, University of Bergen using the population-based national registry data from the Medical Birth Registry of Norway, Norwegian Cause of Death Registry and Statistics Norway.

The work was funded by the European Research Council- Advanced Grant by the European Union.

The main supervisor has been Associate Professor Liv Grimstvedt Kvalvik. Professor Rolv Skjærven, Associate professor Linn Marie Sørbye and Professor Nils-Halvdan Morken have been co-supervisors. Professor Kari Klungsøyr has been an integral part of the research work. During this PhD work, I also had the opportunity to collaborate with Harmon E. Quaker and Allen J. Wilcox at the National Institute of Environmental Health Sciences, North Carolina, USA.

Acknowledgements

While still living in Finland, I clearly remember the day when professor Rolv Skjærven and I had our first ever phone conversation. I did not then have the slightest of hint that it would be the very day that would mark the beginning of an exciting PhD journey in Norway. It was an unexpected start coinciding with the onset of the corona pandemic, a period when the entire country was thrown into a state of lockdown. Reflecting on those initial days, I can still feel the chaos, uncertainty, and fear that encapsulated the world. The world changed then, and I wonder if it will ever return to the familiar normalcy of the pre-corona days.

With the long summer sun, a sense of hope began to emerge. As time progressed, I had the opportunity to step into the university after all those months confined to digital meetings within the four walls of my apartment. Although the entire year and a half involved remote work and limited social interactions, I had already reached an important stage of my PhD journey – My mid-way evaluation, despite being held digitally. Amid those challenging times it was a significant milestone.

Undertaking a PhD in a new country, with unfamiliar faces, a new research group, and a multitude of new experiences, has proven to be a wonderfully fulfilling and enriching journey. All these years at UiB, I consider myself fortunate to have had the opportunity to be amongst people who have supported and motivated me over and over.

First and foremost, I want to express my deep appreciation for the valuable opportunity that Professor Rolv Skjærven has given to me. I am forever grateful to you for your unwavering support and trust in me. I am always fascinated by your research enthusiasm and novel ideas. You have a talent for saying clever things that

are usually infused with deep life lessons and wisdom. I am always inspired by your kind nature and positive attitude. I have great respect for you.

My supervisor associate professor Liv G. Kvalvik is an incredible part of this journey. I am very fortunate to have you as my main supervisor. You have patiently guided me in every stage of my PhD journey from the start to this final stage. I am forever grateful for your scientific knowledge, your patience, and your guidance, all of which contributed to our excellent work. I feel privileged to be working under your mentorship. Your professionalism, kindness and humbleness will always be an inspiration to me.

Thank you to my excellent co-supervisors. Associate professor Linn Marie Sørbye has a heart of gold. I still remember our first summer party at your home. It was one best thing and undoubtedly the most enjoyable experience post corona. Thank you for your kindness and support. I shall always remember your quality supervision. Your heart-warming emails and words of encouragement have always been a source of motivation when things got a little slower from my side. I am also very thankful to Professor Nils-Halvdan Morken for your important contribution to the research work. I am always inspired by your brief and clear communication. Thank you for sharing your wise words and research knowledge, I truly appreciate how they were always to the point.

Thank you so much Professor Kari Klungsøyr for sharing your wealth of knowledge on data and research. I am thankful for your detailed comments and valuable time on my work. Your scientific knowledge, constructive criticism and research expertise has significantly improved the quality of the research work.

Thank you, Quaker E Harmon, for your valuable time and sharing your research knowledge and expertise. I sincerely appreciate your collaboration. I have learned a lot from you and hopefully will have the opportunity to work with you in the future.

Thank you, Allen J. Wilcox, for your valuable time and comments on the research work. I am beyond grateful to have the opportunity to learn and collaborate on research projects with you.

Each of my PhD fellow colleagues Yanni, Sage and Aditi. Thank you for your friendship, smiles, dinner, and cherished memories. I value each one of you and shall never forget the time we spent together. Thank you to all EPISTAT family members, it was a pleasure to be with you all during lunch, meetings, and conferences. Thank you to the administration at IGS, I appreciate your support and kindness.

My special thanks and gratitude to my family in Nepal. You all have always been there for me, my parents, my three younger sisters Prabha, Sanjita and Ashu, and my in-laws. My niece and nephew Liva and Kabir, you have been my much-needed distraction in this journey. It has never been easy to live away from you. Our everyday conversations have kept us close and given me the strength to remain positive each day.

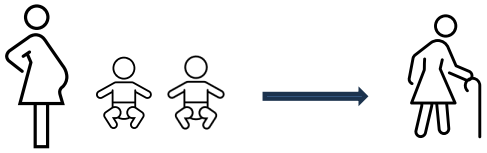
My husband Yogesh Dhakal, I am eternally grateful for your understanding, love and support throughout my PhD journey and every day. Thank you from the depths of my heart.

The universe made this POSSIBLE.

Prativa Basnet
Bergen, Norway

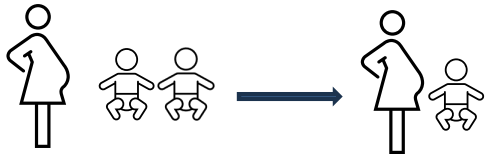
Thesis at a glance

Paper I



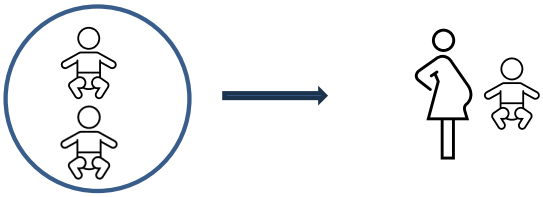
Question: Do women with twin pregnancies have increased risk of long-term cardiovascular disease mortality compared to women with singleton pregnancies?
Period: 1967-2020.
Study Population: 974 892 women with twin or singleton pregnancies registered in the Medical Birth Registry of Norway.
Exposure: Reproductive history of women.
Outcome: Cardiovascular mortality before 70 years.
Conclusion: Women with one lifetime pregnancy, twin or singleton, had an increased risk of cardiovascular mortality compared to women with three singleton pregnancies.

Paper II



Question: What is the birthweight of second singleton pregnancy after a first twin pregnancy compared to a first singleton pregnancy?
Period: 1967-2020.
Study Population: 778 975 women with twin or singleton pregnancies.
Exposure: Plurality status of the first pregnancy (twin or singleton).
Outcome: Birthweight in subsequent singleton pregnancy.
Conclusion: Offspring's birthweight in a second singleton pregnancy was similar for women with a first twin pregnancy or women with a first singleton pregnancy.

Paper III



Question: What is the risk of adverse pregnancy outcomes in twin-born women's own pregnancies compared to singleton-born women's pregnancies?
Period: 1967-2020.
Study Population: 9 184 twin-born and 492 894 singleton-born women during 1967-2005, with registered pregnancies during 1981-2020.
Exposure: Twin-born or singleton-born
Outcome: Preeclampsia, preterm delivery and perinatal loss.
Conclusion: Twin-born women had no increased risk of adverse pregnancy outcomes (preeclampsia, preterm delivery and perinatal loss) compared to singleton-born women.

Abstract in English

Background: Twin pregnancies are common but have generally been less studied compared to singleton pregnancies. Using population-based national registry data we were able to study twin pregnancies for better understanding on how their reproductive and obstetric history impact long-term mortality, birthweight in subsequent pregnancy and inter-generational association of adverse pregnancy outcomes.

Aims: The first aim was to investigate if women with twin pregnancies had increased risk of long-term cardiovascular disease mortality compared to women with singleton pregnancies (*Paper I*). The second aim was to study birthweight in subsequent singleton pregnancy after a first twin pregnancy compared to after a first singleton pregnancy (*Paper II*). Finally, the third aim was to compare the later risk of adverse pregnancy outcomes between twin-born women and singleton-born women in their own pregnancies (*Paper III*).

Material and Methods: The main data source was the Medical Birth Registry of Norway (1967-2020, *Papers I-III*) with linkage to The Cause of Death Registry (*Paper I*) and Statistics Norway (*Papers I-III*). The unique national identification number was used to link all births to a given mother, providing sibling and generational files. Cox regression proportional hazard models (*Paper I*), linear regression models (*Paper II*) and generalized linear models (*Paper III*) were used to calculate hazard ratio (HR), mean difference and relative risk (RR) with 95% Confidence Intervals (CI), adjusted for possible confounding factors. All statistical analyses were performed using STATA (StataCorp LLC, College Station, Texas).

Results: *Paper I*: Women with one lifetime pregnancy, twin or singleton, had increased risk of cardiovascular mortality (adjusted HR 1.72, 95% CI 1.21-2.43 and 1.92, 1.78-2.07, respectively), compared to women with three singleton pregnancies (reference population). However, women with a first twin pregnancy and continued reproduction did not have an increased risk of cardiovascular disease mortality (adjusted HR 0.76, 0.48-1.19) compared to the reference population. Adjusted HRs

for cardiovascular mortality in women with one lifetime pregnancy with any complications were 2.36 (1.49-3.71) and 3.56 (3.12-4.06) for twin and singleton pregnancy, respectively. *Paper II*: Mean combined birthweight of first-born twins was more than 1000 grams larger than mean birthweight of first-born singletons. When comparing mean birthweight of subsequent singleton babies following a first twin pregnancy to a first singleton pregnancy, the adjusted mean difference was just 21 grams (5.2-36.7) *Paper III*: We found no increased risk for adverse pregnancy outcomes in twin-born women compared with singleton-born women's later pregnancies adjusted RR for preeclampsia 1.00 (0.93-1.09), preterm delivery 0.96 (0.90-1.02) and perinatal loss 1.00 (0.84-1.18). Compared with singleton-born exposed to preeclampsia in utero, twin-born exposed to preeclampsia had lower risk of adverse outcomes in their own pregnancies; preeclampsia aRR 0.73 (0.58-0.91) and preterm delivery aRR 0.71 (0.56-0.90). Compared with preterm singleton-born women, preterm twin-born women did not differ in risk of preeclampsia (aRR 1.05 (0.92-1.21)), and perinatal loss (aRR 0.99 (0.71-1.37)) and had reduced risk of preterm delivery (aRR 0.83 (0.74-0.94)).

Conclusions: Despite twin pregnancies being clinically more complicated and challenging, we found that women with twin pregnancies did not have increased long-term cardiovascular disease mortality compared to women with singleton pregnancies. Women with first twin- or singleton pregnancies had offspring with comparable birthweight in the next pregnancy. Also, women born as twin did not have increased risk of adverse pregnancy outcomes in their own pregnancies.

Key words: pregnancy, twins, parity, multiples, maternal mortality, cardiovascular disease mortality, cohort data, birthweight, preeclampsia, preterm delivery, perinatal loss, inter-generation study.

Abstract in Norwegian

Bakgrunn: Tvillingsvangerskap har vært mindre studert enn svangerskap med enkeltfødte. Ved å bruke populasjonsbaserte registerdata ønsket vi å belyse sammenheng mellom tvillingsvangerskap, reproduksjon og langtidsdødelighet.

Mål: Den første målsetningen var å undersøke om kvinner med tvillingsvangerskap hadde høyere kardiovaskulær dødelighet sammenlignet med kvinner med enkeltfødsler (artikkel I). Den andre målsetningen var å studere fødselsvekt i påfølgende enkeltfødte barn etter et første svangerskap med tvillinger eller enkeltfødte (artikkel II). Den tredje målsetningen var å undersøke om kvinner som selv var født tvilling hadde økt risiko for svangerskapskomplikasjoner sammenlignet med enkeltfødte kvinner (artikkel III).

Materiale og metoder: Hovedkilden til analysene var Medisinsk fødselsregister i Norge (1967-2020, artikkel I-III) med kobling til Dødsårsaksregisteret (artikkel I) og Nasjonal utdanningsdatabase ved Statistisk sentralbyrå (artikkel I-III). Personnummer ble brukt til å knytte sammen alle fødsler til en mor. Overlevelsesanalyser (artikkel I), lineære regresjonsmodeller (artikkel II) og generaliserte lineære modeller (artikkel III) ble brukt til å beregne hasard ratio (HR), gjennomsnittlig forskjell og relativ risiko (RR) med 95 % konfidensintervaller (KI) justert for mulige konfunderende faktorer. Alle statistiske analyser ble utført ved bruk av STATA (StataCorp LLC, College Station, Texas).

Resultater: Kvinner med kun ett svangerskap, tvilling eller enkeltfødt, har økt risiko for kardiovaskulær dødelighet (henholdsvis justert HR 1.72, 95% KI 1.21-2.43 og 1.92, 1.78-2.07) sammenlignet med kvinner med tre svangerskap med enkeltfødte. Kvinner med et første tvillingsvangerskap som fortsatte reproduksjonen har ikke økt risiko for kardiovaskulær dødelighet (justert HR 0.76, 0.48-1.19) sammenlignet med referansepopulasjonen som var kvinner med tre enkeltfødte. Kvinner med tvillingsvangerskap og kvinner med svangerskap med enkeltfødte får barn med sammenlignbar fødselsvekt i det påfølgende svangerskapet. Kvinner som selv var

født som tvilling har ikke økt risiko for svangerskapskomplikasjoner som svangerskapsforgiftning (justert RR 1.00, 0.93-1.09), prematur fødsel (justert RR 0.96, 0.90-1.02) og perinatalt tap (justert RR 1.00, 0.84-1.18) i egne svangerskap sammenlignet med kvinner som var enkeltfødt.

Konklusjoner: Til tross for at tvillingsvangerskap er klinisk mer komplisert og utfordrende, peker våre resultater mot at kvinner med tvillingsvangerskap ikke har økt dødelighet av kardiovaskulær sykdom, sammenlignet med kvinner med enkeltfødte. Videre finner vi at kvinner med et første tvillingsvangerskap får barn med sammenlignbar fødselsvekt som kvinner etter et svangerskap med enkeltfødte. Kvinner som selv var født som tvilling har ikke økt forekomst av svangerskapskomplikasjoner.

List of publications

I. Basnet Prativa, Skjærven Rolv, Sørbye Linn Marie, Morken Nils-Halvdan, Klungsøyr Kari, Singh Aditi, Mannseth Janne, Harmon Quaker E., Kvalvik Liv G. Long-term cardiovascular mortality in women with twin pregnancies by lifetime reproductive history, Paediatric Perinatal Epidemiology. 2023;37:19-27. https://doi.org/10.1111%2Fppe.12928

II. Basnet Prativa, Skjærven Rolv, Harmon Quaker E., Wilcox Allen J., Klungsøyr Kari, Sørbye Linn Marie, Morken Nils-Halvdan, Kvalvik Liv G. Birthweight of the subsequent singleton pregnancy following a first twin or singleton pregnancy. Acta Obstet Gynecol Scand. 2023;102:1674-1681. doi:10.1111/aogs.14644

III. Basnet Prativa, Skjærven Rolv, Harmon Quaker E., Sørbye Linn Marie, Morken Nils-Halvdan, Singh Aditi, Klungsøyr Kari, Kvalvik Liv G. Risk of adverse pregnancy outcomes in twin- and singleton-born women: an inter-generational cohort study. BJOG. 2023. doi:10.1111/1471-0528.17690

Contents

1. Definitions and abbreviations

Lifetime reproductive history	In this thesis, in *paper I*, women's reproductive history ascertained at the end of reproduction or 2020 (end of the study period), consisting of six mutually exclusive categories: 1) Women with only one twin pregnancy, 2) Women with only one singleton pregnancy, 3) Women with only two singleton pregnancies, 4) Women with a first twin pregnancy and continued reproduction, 5) Women with a first singleton pregnancy and twins in later reproduction and 6) Women with three singleton pregnancies.
Cardiovascular diseases mortality	In this thesis defined as Atherosclerotic Cardiovascular Disease (ASCVD) mortality for the deaths due to ischemic heart disease or cerebrovascular disease or peripheral arterial disease in women before 70 years of age.
MBRN	The Medical Birth Registry of Norway
Gestational age	Gestational age estimates were based on reported last menstrual period. Ultrasound based estimates have been recorded in the MBRN from 1999, and were used, when available, for women with missing information on last menstrual period or with a difference between ultrasound-based estimate and last menstrual period estimates of more than 10 days.
Birthweight	Offspring birthweight measured at delivery and recorded in grams.
Inter-pregnancy interval	Interval between the date of the subsequent delivery minus the date of the first delivery minus the gestational age of the subsequent pregnancy

Preeclampsia	An increased blood pressure to at least 140 systolic or 90 mmHg diastolic combined with proteinuria (protein excretion of ≥0.3 g/24 h or ≥1+ on dip-stick) after 20 weeks of gestation.
Perinatal loss	Any fetal loss registered in the MBRN after 16 gestational weeks and neonatal deaths during the first week after birth (one or both infants in case of twin pregnancies)
ART	Assisted Reproductive Technology. ART refers to methods used to treat infertility. In vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) are two types of ART.
SGA	Small for Gestational Age (usually defined as birthweight $<10^{th}$ percentile of gestational age)
FSH	Follicle Stimulating Hormone
BMI	Body Mass Index
PPV	Positive Predictive Value

Statistical abbreviations

CI	Confidence interval
OR	Odds ratio
SD	Standard deviation
SE	Standard error
HR	Hazard ratio
RR	Relative risk

2. Introduction and background

Twin pregnancies are common in the modern world, with 1.6 million twin pairs born each year.(1) An early scientific account of twins appeared in the mid-1800s, when Mackenzie published the paper "Statistics of multiple births" in the Lancet.(2) Some years later Mattew Duncan, a Scottish obstetrician, provided maternal and perinatal characteristics of twin pregnancies in the Edinburgh Medical Journal.(3) Later in 1875, Francis Galton recognized the value of studying twins to disentangle nature (heredity) and nurture (environment) by examining twins from infancy through adulthood.(4) Twins have inspired and challenged medical professionals and researchers since then, and they continue to do so in modern obstetric care and fetal medicine.

It is well established that twin pregnancies constitute significant risk of adverse outcomes to both mother and fetuses compared to singleton pregnancies.(5-7) Women with a history of pregnancy complications generally have higher cardiovascular disease mortality.(8-10) Women with twin pregnancies are more often exposed to pregnancy complications. Most previous studies on twin pregnancies have focused on obstetric and perinatal outcomes, while very few studies have investigated the long-term morbidity and mortality of women with twin pregnancies.(11, 12) There is also limited research on reproductive outcomes for women born as twins.

2.1 The twin phenomenon

Although Galton highlighted the significance of twin research already in the 19th century, the various sub-types of twins were not identified until the early 20th century.(13) According to their fertilization process, twins can either be dizygotic (commonly known as non-identical or fraternal) or monozygotic (commonly known as identical) (Figure 1).(14) Dizygotic twins occur from two ova that are fertilized by separate spermatozoids. They have different chromosomes; and may or may not be of the same sex. Each embryo has its own individual amniotic sac and placenta. Monozygotic twins occur when a single ovum is fertilized by a single spermatozoid

and the egg divides, thereafter, establishing two embryos. The embryos have identical chromosomes, and the same sex. The monozygotic twins may develop in three different types of uterine environments; two placentas and two amniotic sacs (dichorionic-diamniotic), one placenta and two amniotic sacs (monochorionic-diamniotic), or one placenta and one amniotic sac (monochorionic-monoamniotic).(15-17) Monozygotic conjoined twins occur due to delayed separation of the zygote and is a very rare twin sub-type (Figure 1).

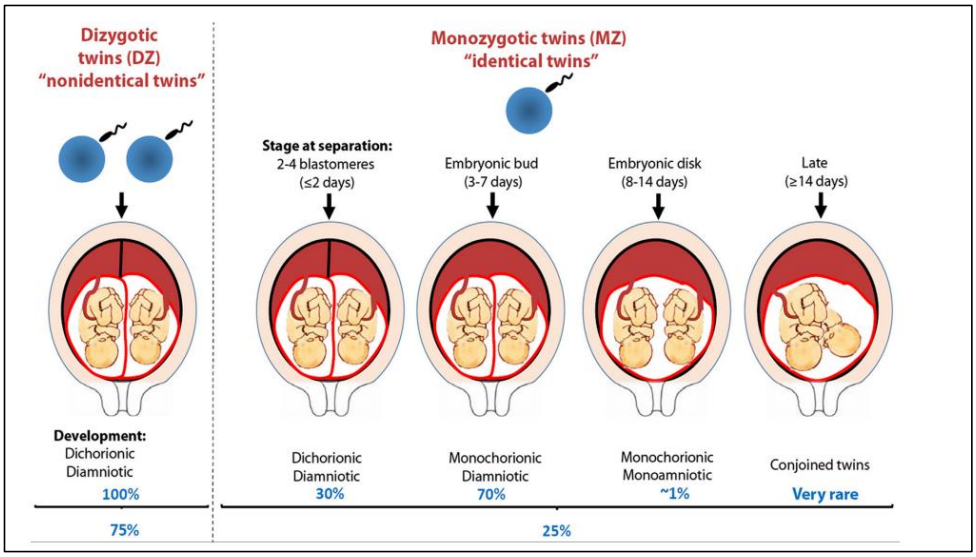


Figure 1: Twin pregnancy development and distribution by zygosity and chorionicity (Figure illustrator Louise Sudour, published with permission from the author and the publisher).(14)

In general, 75% of twin pregnancies are dizygotic, while 25% are monozygotic (Figure 1).(14) While women with dizygotic twins have been found to have an increased concentration of follicle-stimulating hormone (FSH), the cause of monozygotic twinning remains unclear.(16, 18, 19) Depending on biological factors such as zygosity, chorionicity and location of umbilical cord insertion in the placenta, significant variations in pregnancy outcomes have been observed between sub-types of twins.(20, 21) For example: Monozygotic twins seem to have higher rates of

perinatal mortality, stillbirths, neonatal mortality and lower birthweight compared to dizygotic twins.(22, 23) In this thesis, the focus is more on the general twin phenomenon, as data on zygosity and chorionicity were unavailable. Thus, the term *twin* in this thesis refers to any of its sub-types.

2.2 Prevalence and trends in twin births internationally and nationally

The occurrence of natural twinning varies greatly around the globe and within populations. A study published in 2021 by Monden and colleagues showed that the global incidence of twinning have increased markedly from 9 per 1000 to 12 per 1000 deliveries between 1980-1985 to 2010-2015.(1) According to this study, African countries have the highest twinning rates in the world and account for 42% of the world's twin deliveries during 2010-2015.(1) The study also demonstrated that twinning rate reached more than 15 per 1000 deliveries in Canada, the United States, Israel, South Korea, Taiwan and in several countries in Europe.(1) A study from 2016 found a wider variation in twinning rates across the European countries.(24) The lowest rate was found in Romania (9 per 1000) and the highest rate was found in Cyprus (25 per 1000), with a median twinning rate of 16.8 twin births per 1000 women having live or stillbirths across European countries in 2010.(24)

In Norway, a report by Fellman (25) showed that the twinning rates peaked during the 1910s and 1920s, after which there was a decline until the 1970ies.(25) Figure 2 shows the percentage of twin pregnancies among all pregnancies in Norway registered in the Medical Birth Registry of Norway (MBRN), 1967-2020, both natural conceived and those conceived by assisted reproductive technology (ART). There was a sharp increase in twin pregnancies during the 1990ties until reaching a peak after the millennium. After 2002, there has been a declining trend of twin pregnancies.

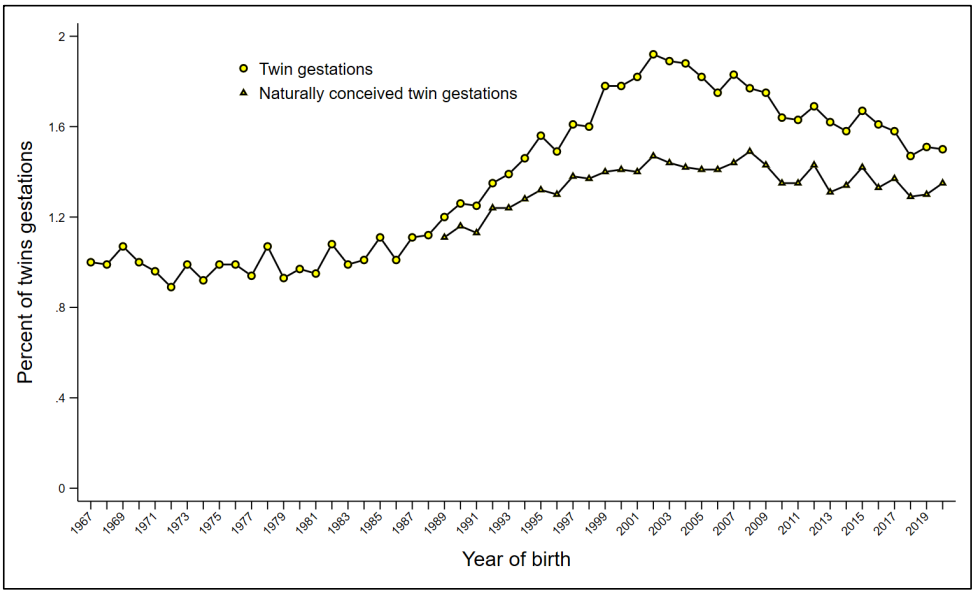


Figure 2. Percent of twin among all pregnancies by year of birth in Norway, 1967-2020 (MBRN), above 16 gestational weeks.

2.3 Causes of twinning and possible explanations for changes in twin proportions

The cause of monozygotic twin pregnancy is essentially a random event among spontaneously conceived twins.(16) The occurrence of dizygotic twin pregnancy is influenced by several biological, environmental and genetical factors.(26) One of the important drivers of twin pregnancies in many countries is the delayed age at conception.(19, 27-32) Studies have shown a link between advanced maternal age and twin pregnancy.(30, 33) In Norway, Tandberg et al. reported a 2.5-fold (95% Confidence Interval (CI) 2.2-2.8) increased risk for natural conceived twin pregnancy for women older than 38 years compared to women below 20 years of age.(34) Additionally, women in higher parities are more likely to have twin pregnancies independent of their age.(35) The correlation between maternal age and spontaneous

twin pregnancy is believed to be caused by age related increase of natural multiple follicular growth, which is linked to elevated FSH levels at later age.(26, 36)

Another important driver for increasing twinning rates globally is the availability of various types of ART. Twin pregnancy rates are higher for women receiving ART because of the need to stimulate surplus follicles and transfer excess embryos to achieve the intended pregnancy.(18) In Norway, the use of ART appears to have contributed to an increase in twin pregnancies during 1990ties, which however has declined in the recent years. In 2004, Thurin and colleagues published a randomized study that demonstrated effectiveness of single-embryo transfers to achieve live births and reduction of multiple births.(37) A change in clinical ART practice, may possibly explain some of the decline in total twin proportions in Norway in the new millennium (Figure 2).(38) When studying the frequency of twins excluding those conceived by ART, there was an increasing trend towards the millennium which leveled off towards the recent years. Several studies demonstrated increased twinning rates with the availability of ART.(6, 39, 40) A study using data from the Danish National Birth Cohort with births between 1998 and 2001 found that 15.5% of women with ART had twins, while only 1.3% of women who did not report ART had twins.(40) Further, this study also showed that women's body mass index (BMI) ≥ 30 or more was positively associated with spontaneous twin pregnancy.(40) A study from Norway also found that women with BMI ≥30 or height ≥ 173 cm had a higher chance of having twins, when adjusted for potential confounding factors such as age, parity and smoking.(41) A study in the United States also demonstrated an association between maternal weight and height and twinning.(42) Another factor influencing twin pregnancy is family history. Several studies from various parts of the world have reported familial association of twinning (26, 43, 44), particularly if the mother was twin.(45)

2.4 Twin pregnancies and health outcomes

In comparison to singleton pregnancies, twin pregnancies constitute increased obstetric and perinatal risk to both the mother and her offspring. Previous findings on long- and short-term outcomes in twin mothers and in her offspring are summarized in the chapter below.

2.4.1 Pregnancy complications in women delivering twins

The physiological changes during pregnancy exhibit more pronounced burden to the maternal organ systems in twin pregnancy compared to singleton pregnancy.(5) Women with twin pregnancies have larger placentas,(46) higher cardiac output,(47) greater nutritional demand,(48-50) evidence of systolic and diastolic dysfunction (51) and altered circulating angiogenic factors.(52) It has been well established that women with twin pregnancy have an increased risk of hypertensive disorders of pregnancy such as preeclampsia, although the underlying mechanism remains unclear.(53) A study from Norway by Laine and colleagues reported that independent of confounders such as maternal age, parity, educational level, smoking, comorbidities and use of ART, risk of preeclampsia in women with twin pregnancies was 4-fold higher compared to women with singleton pregnancies (OR 4.07, 3.65-4.54).(53) More than 50% of women with twin pregnancies deliver preterm (<37 gestational weeks). (54) In the U.S population, women with twin pregnancies were at almost 6-fold greater risk of delivering preterm (<37 gestational weeks) and 8-fold increased risk of delivering before 32 gestational weeks compared to women delivering singletons.(55) A study from European countries showed a 9-fold increased risk of preterm delivery (<37 gestational weeks) and 12-fold increased risk of very preterm delivery (<32 gestational weeks), compared to singleton pregnancies.(24) In a recent systematic review of twelve cohort studies, Wu and colleagues showed that hypertensive disorders in pregnancy increased the risk of preterm delivery for women with twins (OR 1.86; 1.36-2.55).(56) Several other adverse outcomes such as gestational diabetes, caesarean section delivery, postpartum

hemorrhage, post-partum depression and maternal mortality are increased among women with twin pregnancies.(24, 53, 57-70) These pregnancy complications have a link to women's long-term health and will be described below.

2.4.2 Short- and long-term health outcomes for twins

Twin offspring have a higher risk of fetal and infant morbidity and mortality compared to singleton offspring. As discussed in the previous paragraph more than half of the twin pregnancies are preterm (<37 completed gestational weeks).(54) Tingleff and colleagues showed that 54.7% of twin and 6.1% of singleton pregnancies were preterm in nulliparous women in Norway.(54) An earlier study demonstrated that mean gestational age for women with naturally conceived twin pregnancies was 36 weeks.(34) A larger proportion of twin pregnancies results in stillbirths or mortality during the neonatal period compared to singleton pregnancies. Scher and colleagues described a 5-fold increased risk of stillbirth and a 7-fold increased risk of neonatal death in twin pregnancies compared with singleton pregnancies in the United States and Australia.(71) A study from Europe showed that median fetal mortality rate at or after 28 gestational weeks was 7.0 per 1000 total births among multiple pregnancies, while the rate was 2.8 per 1000 among singleton pregnancies.(24) It is believed that the larger placental size in twin pregnancies may worsen placental perfusion leading to more complications in twin offspring.(72, 73) Correspondingly, prior studies have extensively demonstrated increased risk of cardiovascular defects, cerebral palsy, low birth weight, small for gestational age, and perinatal and infant mortality in twin offspring.(6, 28, 54, 66, 71, 74-79) Further, studies have shown that pregnancies conceived by ART, both singletons and twins, have more adverse perinatal outcomes compared to naturally conceived pregnancies (80-82) and that multiple pregnancies are especially high risk.(81, 83) One of these studies, a meta-analysis of 39 cohort studies demonstrated that multiple pregnancies by ART were at higher risk of adverse pregnancy outcomes: preterm birth (<37 completed gestational weeks) (RR 1.08, 1.03-1.14), very preterm birth (<32 completed gestational weeks) (RR 1.18, 1.04-1.34), low birthweight (<2500 grams)

(RR 1.04, 1.01-1.07), and very low birth weight (<1500 grams) (RR 1.18, 1.04-1.34).(81)

The long-term health outcomes of twin offspring have been evaluated quite extensively. Stern et al. reported that twin-born were more likely to have adverse long term health effects if they were born before 28 gestational weeks.(84) A recent retrospective cohort study from Israel followed twin-born babies up to 18 years of age and found increased proportions of morbidity in twin offspring compared to singleton offspring: cardiac (1.9% versus 1.5%), respiratory (8.4% versus 7.1%), neurological (7.7% versus 7.4%), infectious (26.0% versus 24.1%) and malignancies (0.7% versus 0.4%).(85) The increased occurrence of morbidities was mostly linked to preterm born twins. The reproductive outcomes of twin-born have also been briefly studied, which will be discussed in a later chapter.

2.5 Reproductive history and maternal long-term health

Pregnancy is associated with significant physiologic adaptations in the maternal system while nurturing and accommodating the growing foetus.(86) The changes in the maternal system during pregnancy may exert numerous mechanisms for short and long-term impact on woman's health. Below is a summary of studies on exploring the link between reproductive history and long-term maternal health.

2.5.1 Parity and maternal long-term health

Studies have shown an association between parity (number of children) and long-term maternal health and mortality. There is a J-shaped relationship between parity and risk of long-term all-cause mortality, with the lowest risk among women with two pregnancies.(87-90) Several studies have explored the association between parity and later life cardiovascular disease. While an earlier study by Colditz et al. reported no significant association between reproductive events and the risk of long-term cardiovascular disease,(91) subsequent studies have shown an association between

parity and cardiovascular disease in later life.(92-94) When accounted for cardiovascular risk factors such as smoking, type 2 diabetes and BMI in these studies the association remained the same.(92-94) A Swedish study showed a J-shaped association between parity and later life cardiovascular disease with the lowest risk in women with two births, also when accounting for potential confounders such as pregnancy-related complications and socioeconomic factors.(95) A review of ten cohorts studies found a relative risk (RR) of cardiovascular disease of 1.14 (1.09-1.18) among parous versus nulliparous women. The authors also commented on a J-shaped curve between parity and cardiovascular disease.(96) An earlier meta-analysis of ten prospective studies also suggested a potential J-shaped association between parity and cardiovascular disease mortality.(97) However, Halland et. al reported an association between parity and cardiovascular death only among women with low education.(98) The underlying biological mechanism behind these associations is not fully understood. It is possible that pregnancy leads to numerous cardiometabolic changes such as changes in the circulatory system, endothelial function, abdominal fat, pro-atherogenic lipid levels and systemic inflammation,(99-101) which may have a long-term impact on the cardiovascular system, increasing woman's risk of cardiovascular disease in later life. There may be other unknown factors contributing to these associations. Additionally, pregnancy complications have been linked to long-term maternal cardiovascular disease.(102)

2.5.2 Pregnancy complications and maternal long-term health

The association between pregnancy complications and long-term cardiovascular disease morbidity and mortality has been widely studied.(8-10, 103-117) Studies have consistently demonstrated that women with a history of pregnancy complications such as preeclampsia, preterm birth, gestational diabetes, gestational hypertension are more likely to develop cardiovascular disease in later life compared to woman without any history of these complications.(103, 104, 118-123) In Norway, Irgens et al.(124) found that women who delivered before 37 gestational weeks with preeclampsia had an 8-fold increased risk of cardiovascular disease mortality (HR

8.12, 4.31-15.33) compared to women who delivered after 37 gestational weeks without preeclampsia, when women were followed until 13 years after preeclampsia. Even without preeclampsia, women who delivered before 37 weeks had almost 3-fold increased risk of cardiovascular disease mortality (HR 2.95, 2.12-4.11).(124) Studies have demonstrated a range of risks between preterm delivery and maternal cardiovascular disease morbidity and mortality using different gestational age cut-offs.(9, 105, 125-128) Skjaerven et al. (8) showed that parity as well as complications are critical predictors of long-term maternal mortality (Figure 3). They found almost 2-fold (1.9-2.0) increased risk of long-term cardiovascular disease mortality in women with one lifetime pregnancy compared to women with two or more births. When accounting for pregnancy complications in adjusted analyses (maternal education, age at first birth and year of first pregnancy), women with one lifetime pregnancy and preterm preeclampsia had an almost 9-fold increased risk of future cardiovascular deaths (HR 9.4, 6.5-13.7) compared to women with two or more children without preeclampsia.(8) Lifetime number of pregnancies and associated complications seem to be important risk factors for future cardiovascular disease mortality.

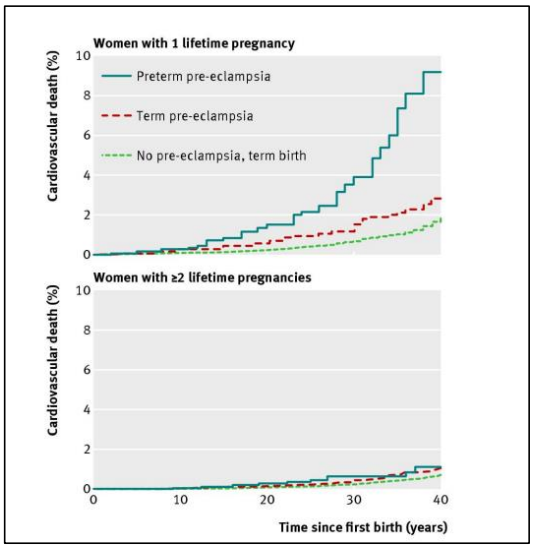


Figure 3. Cumulative risk of cardiovascular death for women according to preeclampsia status at first pregnancy and number of subsequent lifetime pregnancies (Skjærven et. al 2012, reused with permission from the BMJ publishing group).(8)

Pregnancy can be viewed as a "stress test", that provides a window of opportunity to identify women at high-risk for chronic diseases.(129, 130) This information could potentially be used for prevention of the chronic conditions. The vast majority of prior literature on reproductive history and maternal cardiovascular disease mortality are mainly focused on women with singleton pregnancies. Also, research on the association between pregnancy complications and subsequent maternal health have been based on complications in the first pregnancy. Identifying woman's total reproductive history, including twin pregnancies and associated complications may reveal heterogeneity in risk of future cardiovascular disease mortality. Analyses limited to the first pregnancy outcomes cannot capture this.(88)

2.5.3 Twin pregnancies and maternal long-term health

The long-term impact of twin pregnancies on maternal morbidity and mortality of women has received increasing attention in the recent years.(11, 12, 131, 132) A

study from Israel published in 2016 showed that women who ever had a twin pregnancy did not have increased risk of cardiovascular hospitalization compared to women without twins (OR 1.0, 0.8-1.1).(11) These results were adjusted for age, diabetes, parity, obesity (BMI>30kg/$m^{2)}$, preeclampsia and fertility treatment. In 2020, Bergman and colleagues showed that in Sweden, women with a first twin pregnancy with and without preeclampsia did not have an increased risk of long-term cardiovascular disease compared to women who had a singleton pregnancy without preeclampsia: aHR 1.25, 0.83-1.86 and aHR 0.94, 0.79-1.10, respectively.(12) When adjusting for maternal age, chronic hypertension before birth, education and time period of birth, results were not altered.(12) A recent study from Canada showed that hypertensive disorder in twin pregnancies were less likely to be associated with future cardiovascular disease compared to singleton pregnancies with hypertensive disorders in pregnancy.(131) Consistent to these findings, a new study from the Netherlands also demonstrated that women with twin pregnancy with hypertensive disorder of pregnancy did not have increased risk of cardiovascular mortality compared to singleton women with hypertensive disorder of pregnancy.(132) We have not been able to identify earlier studies that have evaluated how twin pregnancy and woman's full reproductive history are associated with long-term cardiovascular disease mortality.

2.5.4 Cardiovascular disease in women

Cardiovascular diseases comprise of a set of heart and blood vessels disorders: coronary heart disease, cerebrovascular disease, rheumatic heart disease and other related conditions.(133) Cardiovascular diseases are the major cause of death worldwide in both men and women.(133) In 2019, 6.2 million deaths occurred due to cardiovascular disease at age 30 to 70 years worldwide.(134) In Europe, cardiovascular diseases account for 45% of all deaths in women, with central and eastern European countries having the largest burden of cardiovascular diseases globally.(135, 136) Since the 1980s, there has been a declining trend in age-standardized cardiovascular disease mortality risk in most European countries.(137,

138) In the Nordic countries, cardiovascular diseases are among the major diseases contributing to the disease burden.(139) In Norway, cardiovascular disease mortality has declined after 2009,(140) but remains as one of the most common causes of death in the recent years.(141)

There are multiple risk factors contributing to the cardiovascular disease. One of the common risk factor of cardiovascular diseases include high blood pressure.(142) Another marker of cardiovascular disease is high cholesterol level (defined as ≥5.0 mmol/L). Obesity is another contributing risk factor for cardiovascular disease.(143) Smoking and alcohol continue to be a major health concerns in the Nordic region. In 2017, smoking alone was found to be responsible for 16% of the cardiovascular disease burden in the Nordic countries.(139) In Norway, a declining smoking trend has been observed among both men and women since 1973.(144) Further, studies have found that genetic factors significantly affect the risk factors of cardiovascular disease.(145-147)

2.6 Factors affecting birthweight and the role of parity

Several maternal and fetal factors are predictive of birthweight. Gestational age is the most important determinant of birthweight.(148) Birthweight increases with increasing gestational age.(149) Smoking habits and maternal BMI also affect birthweight and gestational age.(150, 151) Also, long and short inter-pregnancy interval have been linked to low birthweight in singletons,(152) however, to our knowledge, this has not been studied for twin pregnancies.

There is a tendency for gestational age and birthweight to be repeated in the successive singleton births due to underlying pregnancy factors, that are not explained by prior adverse pregnancy outcomes or by factors that contribute to adverse outcomes in subsequent pregnancies.(153) Studies have found a parity effect on birthweight, that successive singleton babies are about 80-170 grams larger

compared to the birthweight of a first singleton baby. This has been shown in several populations.(154-157) However, the causes of this parity effect on birthweight remain unclear. Based on the studies in singleton pregnancies, the biological explanation for higher birthweight in subsequent pregnancy could be that structural changes in spiral and uterine arteries in subsequent pregnancy seem to provide better uterine capacity.(158-160) Pregnancy related changes in the cardiovascular system such as increased ventricular volume and cardiac output and decreased systemic vascular resistance may be incompletely reversed postpartum, which may result in a more favorable uterine environment in a subsequent singleton pregnancy.(161) Parous uteri have greater placental blood flow, which may allow more efficient oxygen and nutrient delivery to the fetus.(162, 163)

In Norway, the mean birthweight for a newborn is about 3650 grams.(164) The mean birthweight of a twin fetus is about 2600 grams,(165) but the total birthweight of twins is greater as compared to a singleton birthweight. Exploring if this difference is linked to higher birthweight in the next singleton pregnancy could offer insight into the parity effect of birthweight.

2.7 Inter-generational studies

According to Debbie Lawlor, Sam Leary, and George David Smith, inter-generational studies "are studies in which the relationship between characteristics obtained from family members from at least two different generations (e.g parents and offspring) are explored".(20) Below is a brief summary of papers exploring adverse pregnancy outcomes across generations.

2.7.1 Inter-generational studies in singletons

Several studies have investigated familial patterns of recurrence of preeclampsia, preterm delivery, small for gestational age (SGA), breech delivery and intrauterine growth to determine the effect of maternal and fetal factors or genes, a shared

environment, or a combination of these factors on the risk of these adverse outcomes across generations.(166-172) In a Norwegian population, Lie and colleagues found that fetal genes from the father contributed to increased preeclampsia risk in the offspring.(166) Another study from the MBRN demonstrated that preeclampsia can be passed down through generations due to heritable traits carried by the maternal as well as the fetal genes.(173) Consistent to these results, a study from Sweden showed that preeclampsia is linked to family history on both the paternal and maternal side.(174) This study showed dominance of maternal genes, with variance of heritability estimated as 35% maternal genes, 20% fetal genes, 13% to the couple effect and remaining 32% to other effects.(174) Another Swedish study found that complete full sisters and mother-daughters shared a genetic component responsible for the development of preeclampsia and gestational hypertension, which was not found in half-sisters to both parents.(175) In Iceland, the prevalence of eclampsia and preeclampsia were increased for daughters born to eclamptic or preeclamptic mothers compared to daughters-in-law who were not exposed to preeclampsia.(176) In the United States, a study by Espin and colleagues demonstrated that men and women exposed in utero to preeclampsia had a 2-3-fold increased risk of developing preeclampsia in their later or partner's later pregnancies.(177) Another inter-generational study from the United States also demonstrated the role of fetal genes in triggering preeclampsia in offspring.(178)

Early inter-generational registry studies on preterm birth observed no significant recurrence across generations.(167, 179, 180) However, later studies revealed that preterm delivery recurs across generations. Wilcox et al. found that preterm delivery across generation seems to be transmitted through the mothers.(170) It has also been found that increasing paternal birthweight seem to be linked to an increased risk of preterm birth when the mother herself was born small.(181) Another study also confirmed that preterm-born women but not men were at increased risk of having preterm offsprings.(182) Moreover, women born SGA were at higher risk of placental abruption, preeclampsia and preterm birth in Sweden.(183) Another inter-

generational study found a higher risk of perinatal death in offspring born to very preterm mother or mothers with birthweight below 2000 grams in Norway.(184) In the United Kingdom, women who were born spontaneously preterm or had siblings who were preterm were likely to have spontaneous preterm delivery.(185) In Norway, other complications have also been explored in the inter-generational context such as stillbirths being more frequent in offspring born to diabetic mothers (186) and longer pregnancy duration at own birth was associated with having offspring with pregnancies of long duration.(187, 188) These inter-generational studies have been valuable in understanding whether the underlying etiology of adverse pregnancy outcomes are transmitted across generations.

2.7.2 Inter-generational studies in twins

Twin-born offspring or women giving birth to twins have received little attention in the context of inter-generational research. In 1992, Emauel and colleagues published an inter-generational study involving twins. This study from the United Kingdom showed that twin-born women had offspring of about 700 grams lighter compared to birthweight of offspring's to singleton-born women.(189) Another study from the Swedish twin registry also showed that twin-born women with higher birthweights gave birth to larger singletons in their later pregnancies.(190) Another study from Sweden using a large sample size found that the recurrence of preterm across generation was stronger for preterm singleton-born women compared to preterm twin-born women (aOR 1.39, 1.29-1.50 versus aOR 1.06, 0.79-1.44).(191) An earlier inter-generational study from Norway described that twin offspring were at increased risk of perinatal mortality if the mother was born preterm or growth restricted.(192) These studies suggest less recurrence of adverse outcomes across generations in twin-born women. Twins are more exposed to preeclampsia in utero, however, to our knowledge, the recurrence of these outcomes in later reproduction have not been studied among twin-born women.

Literature review was completed September 2023.

3. Aims of the study

The overall aim was to expand the understanding on how twin pregnancy was associated with different health outcomes for the women and for the offspring. Specifically, we investigated if women who deliver twins have different short-term (birthweight in the next singleton) and long-term health (cardiovascular disease mortality) outcomes compared to women who give birth to singletons. We also aimed to reveal if women born as twin have more adverse reproductive outcomes than women born as singletons.

The specific aims were:

Paper I. To estimate risk of long-term cardiovascular disease mortality in women with naturally conceived twins compared to women with singleton pregnancies, accounting for lifetime number of pregnancies and pregnancy complications.

Paper II. To compare birthweight in singleton pregnancies following a first twin relative to a first singleton pregnancy to get a better understanding of the general parity effect on birthweight.

Paper III. To compare the risk of adverse pregnancy outcomes between twin-born and singleton-born women. We also evaluated whether in utero exposure to preeclampsia or preterm delivery affected adverse pregnancy outcomes (preeclampsia, preterm delivery and perinatal loss) in the next generation.

4. Materials and methods

4.1 Study design

All three studies were based on data from the population-based national registries of Norway: MBRN and in *paper I* we also used data from the Norwegian Cause of Death registry. In *paper I and II*, we used sibling-linked data, with mothers as the unit of analysis. In *paper III*, we used an inter-generational design, with women born as either twin or singleton as the unit of analysis. *Papers I-III* are registry-based cohort studies, with prospectively recorded pregnancy data from 1967-2020.

4.2 Data sources

4.2.1 Medical Birth Registry of Norway

The MBRN is a national population-based registry established in 1967. The primary objective of the registry was to monitor birth abnormalities and perinatal health problems for early prevention, as well as to provide data for research on causes and consequences of pregnancy and birth.(193, 194) The register records all live births, stillbirths, and pregnancy losses from 16 gestational weeks onwards by mandatory notification regulated by Norwegian law.(195) The vast majority of births in Norway takes place in public hospitals and the proportion of live births captured by the MBRN is close to 100%. A standardized notification form is used by the attending midwife or obstetrician to prospectively record information on women's health before and during pregnancy, the delivery and the immediate postpartum period, including demographic information, complications and interventions during delivery and infant outcomes. Data were recorded as free text until 1999; or by predefined variables or check boxes in addition to free text after 1999. Since 2006, a gradual transition to electronic birth notification took place (complete in 2014), and the notifications are now based on pre-specified extractions from the medical records at the delivery units.

Every live-born infant in Norway, as well as all immigrants who become Norwegian inhabitants, are provided with a unique national identification number by the National Population Register, enabling data record linkage across national registries. The MBRN is routinely matched with the National Population Register and receives all national identification numbers and all dates of death through this linkage.

4.2.2 Norwegian Cause of Death Registry

The Norwegian Cause of Death Registry collects death certificates verified by doctors. The causes of death are coded by the International Classification of Diseases (ICD) coding system. Since its establishment in 1925 until 2014, Statistics Norway had the responsibility for the statistics on causes of death. From 2014, the Norwegian Institute of Public Health became solely responsible for operating the registry. In *paper I*, we used data from the Norwegian Cause of Death Registry.

4.2.3 Statistics Norway

Statistics Norway (SSB) governs the official national statistics in the country. Education level of the study population was derived from the National Education database located at Statistics Norway. Information on highest educational attainment of women was used in *papers I-III*.

4.2.4 The National Population Register

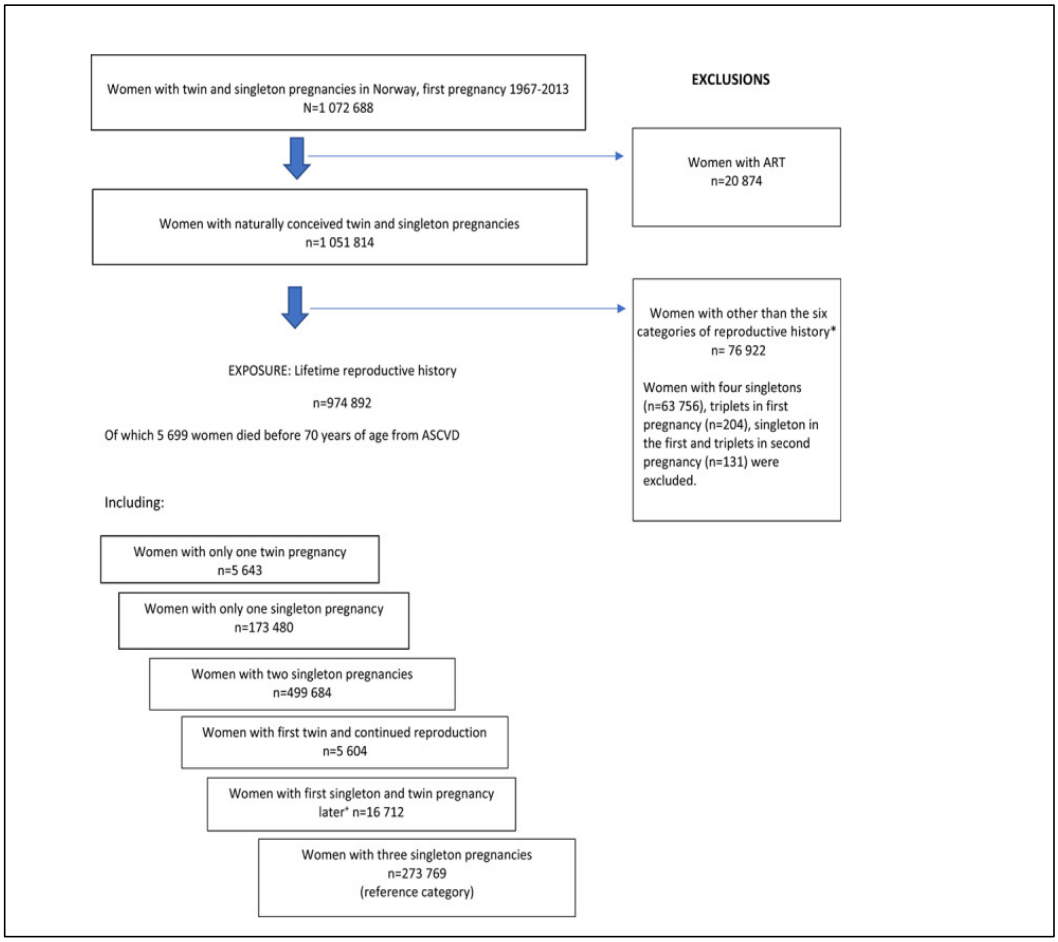
The Norwegian Directorate of Taxes governs the National Population Register. For each newborn, except stillbirths, a unique 11-digit national identification number (ID) is generated. These 11 digits serves several purposes: the first 6 digits represents the person's date of birth (DDMMYY), the next 3 digits contains a unique serial number of the newborn while the last 2 digits hold a control number for the previous 9 digits. Finally, to ensure the quality of the control; boys are provided an odd number while girls get an even number. The reporting of the birth information of the newborn to the MBRN includes the new ID number of the child in addition to the parents' numbers.

Women's country of birth used in *Paper II* are also available from the National Population Register.

4.3 Study populations

Paper I

The study population in *Paper I* consisted of 974 892 women with their first pregnancy registered in the MBRN and cause of death registered in the Norwegian Cause of Death Registry. We studied the reproductive patterns of the women with first pregnancy registered between 1967 and 2013, followed to 2020. Our study population included women with i. only one twin pregnancy (n=5643); ii. only one singleton pregnancy (n=173 480); iii. only two singleton pregnancies (n=499 684); iv. a first twin pregnancy and continued reproduction (n=5604); v. a first singleton pregnancy and twins in later reproduction (n=16 712) and vi. three singleton pregnancies (n=273 769). Inclusions and exclusions are presented in the flowchart below (Figure 4). Our focus in this paper were reproductive history including twins and comparable reproductive history of women with singletons. Other reproductive histories than these were not included in the study population.

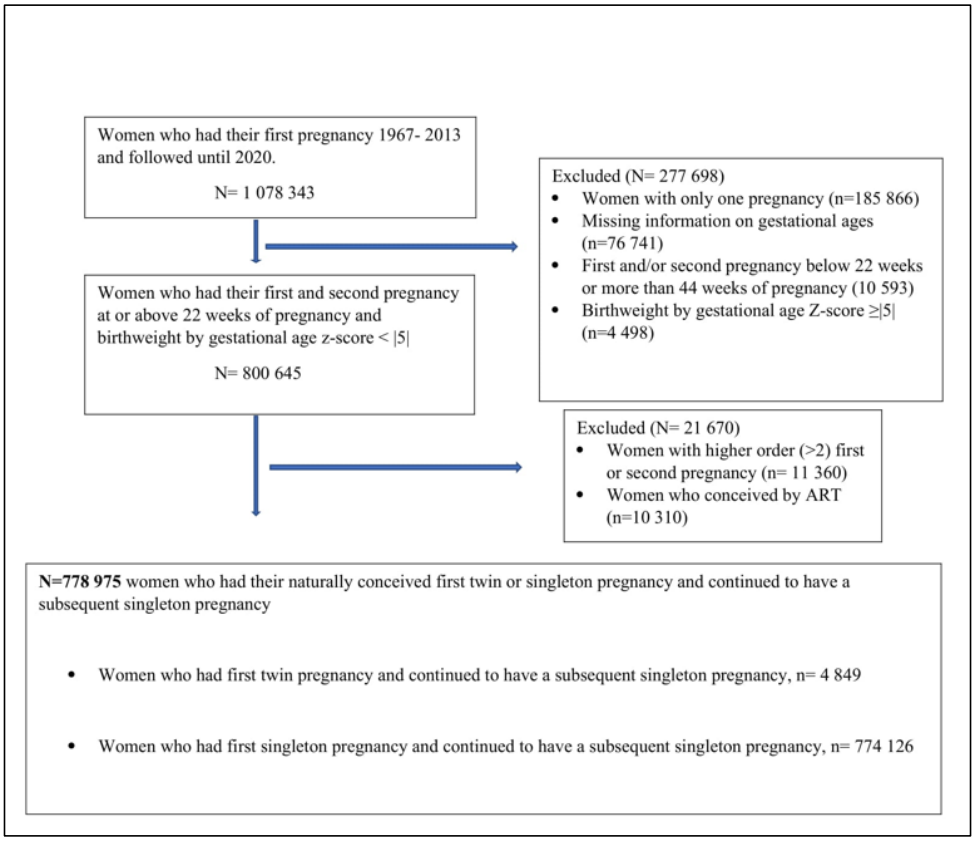


ART: Assisted Reproductive Technology

Figure 4. Flowchart of the study population (*Paper I*).

Paper II

In *paper II* we used data from women with their first and second pregnancies registered in the MBRN. The final study population consisted of 778 975 women with a subsequent singleton pregnancy of which 4849 had a first twin pregnancy and 774 126 had a first singleton pregnancy (See flowchart in Figure 5).



ART: Assisted Reproductive Technology

Figure 5. Flowchart of the study population (*Paper II*).

Paper III

The study population of *paper III* consisted of 502 078 twin- or singleton-born women with their reproduction registered in the MBRN. It is based on 9184 twin-born women and 492 894 singleton-born women during 1967-2005, with their later singleton pregnancies during 1981-2020. Inclusions and exclusions are presented in the flowchart below (See Figure 6).

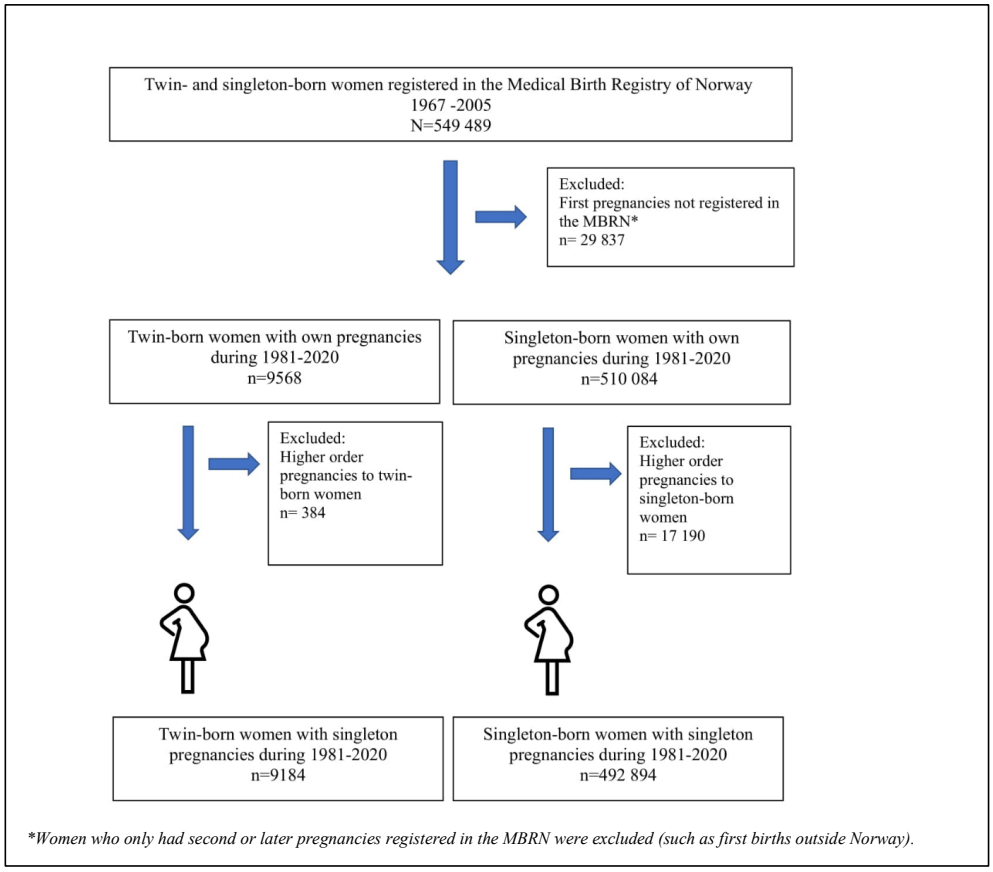


Figure 6. Flowchart of the study population (*Paper III*)

4.4 Variables and methods

4.4.1 Exposures

Lifetime reproductive history

Lifetime reproductive history of women was the exposure variable in *Paper I*. As described above in the study population, we constructed a composite variable for lifetime reproductive history ascertained at the end of reproduction or 2020, with six mutually exclusive categories. The composite variable accounted for women's total number of pregnancies, plurality status of the pregnancy and the sequence of pregnancy as recorded in the MBRN.

Table 1. Categories of reproductive history of women with a first twin or singleton pregnancy used in *Paper I.*

	The exposure variable showing various categories of women's reproductive history						
Source exposure variable		i. Women with only one twin pregnancy	ii. Women with only one singleton pregnancy	iii. Women with two singleton pregnancies	iv. Women with first twin pregnancy and continued reproduction	v. Women with first singleton pregnancy and twins in later[b] reproduction	vi. Women with three singleton pregnancies
Number of pregnancies	1	√	√				
	2			√			
	≥2				√	√	√
Plurality	Twins	√			√	√	
	Singleton		√	√			√
Sequence of Plurality	First twin	√			√		
	First singleton		√			√	√

[b]*Women with a twin pregnancy either in second, third or fourth pregnancy.*

We further stratified the six exposure categories by occurrence of any of the adverse pregnancy outcomes: preeclampsia, preterm delivery or perinatal loss giving us 12 exclusive exposure categories.

Preeclampsia

Preeclampsia is based on the clinical criteria applied by the Norwegian Society of Gynecology and Obstetrics,(196) aligned with the criteria recommended by the American College of Obstetricians and Gynecologists.(197) The definition of preeclampsia in the MBRN has changed somewhat over time in accordance with the update of the clinical criteria by the Norwegian Society of Gynecology and Obstetrics.(196, 198) The core criteria, throughout the study period has though been increased blood pressure to at least 140 systolic or 90 mmHg diastolic combined with proteinuria (protein excretion of ≥0.3 g/24 h or ≥1+ on dip-stick) after 20 weeks of gestation. In our analyses, preeclampsia included preeclampsia, HELLP syndrome (hemolysis, elevated liver enzymes and low platelet levels), eclampsia, as well as chronic hypertension with superimposed preeclampsia. Preeclampsia cases have been registered in the standardized notification form either as free text or, since 1999, by check box in the MBRN.

Perinatal loss

We defined perinatal loss as any fetal loss registered in the MBRN after 16 gestational weeks and neonatal deaths during the first week after birth (one or both infants in case of twin pregnancies).

Plurality of the first pregnancy

In the MBRN, number of children born to woman in each pregnancy is indicated. In *papers I* and *II*, the plurality of the first pregnancy was determined by the type of pregnancy, twin or singleton at birth (excluding higher order births such as triplets etc.).

Birthweight

Offspring birthweight has been measured at the time of delivery and recorded in grams in the MBRN. Distribution of birthweights in first and subsequent singleton pregnancies were plotted using categories of absolute grams (ranging from 500-7249 grams). In first-born twin pregnancies we used combined birthweights of twin pairs

and for singleton we used individual infant weights to describe birthweight distributions.

Gestational age

Gestational age estimates were based on reported last menstrual period. Ultrasound based estimates have been recorded in the MBRN from 1999, and were used, for women with missing information on last menstrual period or with a difference between ultrasound-based estimate and last menstrual period estimates of more than 10 days.

Preterm delivery

Preterm delivery was defined as births before 37 completed weeks of gestation. We used the same definition of preterm delivery in all three papers.

Z-score

Z-scores for birthweight by gestational age were based on a Norwegian standards.(149)

Inter-pregnancy interval

Inter-pregnancy interval was calculated as the date of the subsequent delivery minus the date of the first delivery minus the pregnancy length of the subsequent pregnancy. Inter-pregnancy interval was expressed in years showing birthweight by 1 year increments up to 3.9 years and the longer inter-pregnancy intervals (> 3.9 years) were combined as 4-5.9, 6-7.9, 8-9.9 and 10-11.9 years.

Plurality at birth

The plurality status was based on twin or singleton status of the women at her own birth. Based on this plurality status at birth, women were followed for their later reproduction.

In-utero exposure to adverse pregnancy outcomes

We also explored possible modification by in utero exposure to adverse pregnancy outcomes: preeclampsia or preterm delivery among twin-born versus singleton-born women in *paper III*. The definition of preeclampsia and preterm delivery was similar as already defined above.

4.4.2 Outcomes

Paper I

The main outcome of interest was Atherosclerotic Cardiovascular Disease (ASCVD) mortality defined as death from ischaemic heart disease or cerebrovascular disease or peripheral arterial disease (PAD) in women before 70 years of age. We used codes from the International Statistical Classification of Diseases and Related Health Problems (ICD) to define ASCVD. The codes from ICD 8th, 9th and 10th revisions were: a. Ischaemic heart disease: I20-I25 (ICD-10), 410-414 (ICD 8 and 9), b. Cerebrovascular disease: I60-I69 (ICD-10), 430-438 (ICD 8 and 9) and c. Peripheral arterial disease: I70-I72, I74 (ICD-10), 440-444 (ICD 8 and 9). In addition, in a sensitivity analyses we used more expansive definition of CVD. This extended CVD definition included in addition to ASCVD, hypertensive heart disease: I10-I15 (ICD-10), 400-405 (ICD 8 and 9) and cardiomyopathy: I42 (ICD-10), 425 (ICD 8 and 9).

Paper II

The main outcome of interest was birthweight (grams) in the subsequent singleton pregnancy after a first twin or singleton pregnancy.

Paper III

The main outcomes of interest were preeclampsia, preterm delivery and perinatal loss in any pregnancy to twin-born compared with singleton-born women.

4.4.3 Potential confounding factors

In *paper I*, the covariates were obtained from the MBRN except information on women's education. The covariates used were year of first delivery, mother's age at first birth (in years), and chronic medical conditions available in the MBRN (type 1 or type 2 diabetes mellitus, hypertension, kidney disease and rheumatoid arthritis). We tried to account for the confounding cohort effect on the exposure, (both twin or singleton pregnancy and parity), and on the outcome (cardiovascular disease mortality) by controlling for age and the year of first delivery. Also, chronic medical conditions are likely to influence the number of pregnancies and the chance of dying early due to cardiovascular disease in later life. Educational attainment was another potential confounder controlled as a categorical variable in our data. Education was used as a proxy for socioeconomic position. In Norway, education is shown as an indicator of both the family size and later life risk of cardiovascular disease mortality.(98)

In *paper II*, we adjusted for possible confounding variables available in our data that could affect plurality in the first pregnancy and birthweight in the subsequent: year of first delivery (categorized: 1967-1976; 1977-1986; 1987-1996; 1997-2006 and 2007-2020) and mother's age at first delivery (in years: ≤19; 20-25; 26-30; 31-35 and >35). Mother's BMI could potentially confound our results. Information on BMI was not available for the full study period; however, it is related to maternal education,(199) and we also adjusted for highest level of maternal education (<11 years, 11-13 years and >=14 years). It is known that twin pregnancies and consequently birthweight varies depending on geographical location.(30, 200, 201) To account for this variation, we controlled for women's country of birth as a potential confounder (categorized as Nordic: women born in Norway, Finland, Sweden, Denmark and Iceland; non-Nordic: women born outside the Nordic countries).

In *Paper III,* estimates were adjusted for the decade of the twin-born or singleton-born women's birth (categorized:1967-1969, 1970-1979, 1980-1989, 1990-1999 and 2000-2005), and their mother's educational attainment through 2020 (categorized as <11, 11-13 and ≥14 years) which are likely to affect the exposure and the outcome. In a sensitivity analyses, we further accounted for total number of pregnancies to women (categorized as 1, 2, 3+ registered in the MBRN through 2020), and her own educational attainment through 2020 (categorized as <11, 11-13 and ≥14 years). Women's total number of pregnancies was included to capture the "opportunity" to have adverse outcomes which increases with increasing number of pregnancies. Education was used as a surrogate for behavioural factors (smoking, BMI etc) which may "transmit" through generations but were only recently added to the MBRN.

4.4.4 Exclusions

Pregnancies conceived by ART were excluded from the main analyses in *papers I* and *II*. Infertility/subfertility are associated with reproductive patterns and could be associated with underlying factors predisposing to cardiovascular disease.(202, 203) Also pregnancies conceived by ART are more likely to have twins. Additionally, there is time-dependent missing data for ART. ART started in Norway in 1982 but systematic reporting of ART to the MBRN only started in 1988. In our study population, we have included mothers who have given birth since 1967. In *paper I* we also performed sensitivity analysis including women with ART, which however did not change our main results. We also excluded women with any higher order multifetal pregnancies (≥3 fetuses), as these pregnancies are both fewer in number and may be associated with specific obstetric challenges.

Further, in *paper I*, we excluded women with other reproductive patterns than the six defined as our exposure variable. Such as women with four singleton pregnancies (n=63 756). In *paper II*, we excluded women who gave birth before gestational week 22 or after 44 weeks or had implausible birthweight by gestational age z-score <-5 and >5. In *paper III*, we excluded women born in higher order pregnancies (>2

fetuses) and women who only had second or later pregnancies registered in the MBRN (such as first births outside Norway or who started their reproduction before 1967). We only included singleton pregnancies to twin-born and singleton-born women.

4.5 Statistical analysis

All statistical analyses were performed using STATA version 17 (*Paper I*) and version 18 (*Papers II* and *III*), StataCorp LLC, College Station, Texas.

Paper I

Descriptive characteristics of women's first pregnancy were presented as frequencies, proportions and percentages. We used Cox proportional hazard regression models to estimate hazard ratios with 95% CIs for ASCVD mortality by six reproductive history categories. We used maternal age as the underlying time variable in the cox models. Women were considered at risk of death from the age of their last pregnancy and censored at death, age 70, or when follow-up ended in 2020, whichever came first. Models were adjusted for: age at first birth, year of first birth, maternal education, and chronic medical conditions.

Paper II

Descriptive baseline characteristics of women were presented as means with standard deviations (SD) for continuous variables and as numbers (n) and percentage (%) for categorical variables. We evaluated the association between twin or singleton status of the first pregnancy and birthweight for subsequent singleton pregnancies as a continuous outcome by linear regression models. We also adjusted for the possible confounders: maternal age at first delivery, year of first delivery, maternal education and country of birth. Plots were used to visualize the distribution of birthweight in the subsequent singleton pregnancy after a first twin or singleton pregnancy. Differences in length of inter-pregnancy intervals and birthweight at different intervals were also

explored visually using plots. Inter-pregnancy interval was expressed in each year increments initially but for graphical presentation of birthweight by inter-pregnancy interval, the longer inter-pregnancy intervals (> 3.9 years) were combined as 4-5.9, 6-7.9, 8-9.9 and 10-11.9 years due to small numbers.

Paper III

The pregnancy characteristics of twin-born or singleton-born women were presented as frequencies, proportions and percentages. We used generalized linear models with log link binomial distribution to estimate RRs with 95% CIs for associations between twin-born women and later adverse pregnancy outcomes relative to singleton-born women adjusted for potential confounders: decade of women's birth and maternal education. We used separate models for each outcome and each model used clustered standard errors to account for correlations between siblings. We also obtained stratified results based on in utero exposure to preeclampsia or preterm delivery and adverse outcomes in later pregnancies: preeclampsia, preterm delivery and perinatal loss as the main outcome. These results were presented using Knol and VanderWeelee's recommended (204) methods for presenting RR. Further, E-values (205) were obtained for estimates with CIs outside 1 to assess the influence of unmeasured confounding.

4.6 Missing information

In general, missing data on the exposure and outcome variables were rare. In *paper I*, information on maternal age and year of birth of first child was complete in our study. Less than 1% of educational attainment was missing. In *papers I* and *II*, about 4% of the women's gestational ages were missing in the first pregnancy. In *paper II*, also about 4% of the gestational age were missing in second singleton pregnancy. Less than 0.1% of the women did not have information on the country of birth.

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An overview of material and methods is outlined in Table 2, below:

Table 2. Materials and methods

	Paper I	***Paper II***	***Paper III***
Aims	To estimate risk of long-term cardiovascular disease mortality in women with naturally conceived twins compared to singleton pregnancies, accounting for life-time number of pregnancies and pregnancy complications.	To compare birthweight in subsequent singleton pregnancy after a first twin pregnancy relative to a first singleton pregnancy.	To compare risk of adverse pregnancy outcomes between twin-born and singleton-born women.
Design	Nationwide, population-based cohort from Norway		
Data source	MBRN, Cause of Death Registry of Norway and Statistics Norway	MBRN and Statistics Norway	MBRN and Statistics Norway
Study population	974 892 women with first pregnancies registered in the MBRN during 1967-2013.	778 975 women with a first twin or singleton pregnancy and a subsequent singleton pregnancy during 1967-2020.	9184 twin-born and 492 894 singleton-born women during 1967-2005, with their pregnancies registered during 1981-2020.

Main exposure	Reproductive history of women: i. Only one twin pregnancy, ii. Only one singleton pregnancy, iii. Two singleton pregnancies, iv. Women with a first twin pregnancy and continued reproduction, v. A first singleton pregnancy and twins in later reproduction and vi. Women with three singleton pregnancies as the referent group	Twin or singleton status of first pregnancy	Twin-born or singleton-born status at birth
Outcome	Cardiovascular disease mortality before 70 years	Birthweight in subsequent singleton pregnancy	Preeclampsia, preterm delivery or perinatal loss
Covariates	Maternal age at first pregnancy, year of first birth, maternal education and chronic medical conditions	Maternal age at first pregnancy, year of first birth, maternal education and country of birth	Decade of women's birth and her mother's educational attainment.
Measure of association	Hazard ratios, 95% CIs	Mean difference, 95% CIs	Relative risks, 95% CIs

MBRN: Medical Birth Registry of Norway; CI: Confidence Interval

4.7 Ethical considerations

All the three papers included in this thesis are in accordance with the guidelines of the Declaration of Helsinki,(206) and comply with the Vancouver Recommendations.(207) All papers were based on de-identified, routine compulsory data and therefore individual consent was not necessary. The studies were approved by the Regional Committee for Medical Ethics Western Norway REC WEST 13818 on July 1st 2020.

We acknowledge our findings may be alarming for women concerned about their long-term health (*Paper I*). We are cautious about the language we use to explain our findings. While we do identify a population of women with higher risk of cardiovascular mortality before 70 years of age, we believe there is potential for preventive measure to reduce the risk for these women.

5. Summary of main results

5.1 Paper I

Women with a first twin pregnancy more often delivered preterm compared to women with a first singleton pregnancy (48% versus 6%). Preeclampsia (14% versus 4%) and perinatal loss (6% versus 1%) were more frequent in women with a first twin pregnancy. A total of 42 182 women died before the age of 70 years during 1967-2020, of which 5 699 (13.5%) died of cardiovascular related causes. Cardiovascular deaths (ASCVD) among women with twins in any pregnancy accounted for 2.8% of all cardiovascular deaths.

Women with only one lifetime pregnancy, twin or singleton, had increased risk of ASCVD death (adjusted estimates aHR 1.72, 1.21-2.43 and aHR 1.92, 1.78-2.07, respectively), compared to women with three lifetime singleton pregnancies (reference group). Women with a first twin pregnancy and continued reproduction did not have increased risk of ASCVD death (aHR 0.76, 0.48-1.19) compared to the reference population. The aHRs for women with two lifetime singleton pregnancies and women with first singleton pregnancy and later twin pregnancies were 1.08 (1.01-1.15) and 1.49 (1.22-1.81), respectively. The unadjusted estimates were slightly higher than the adjusted estimate, which was mostly driven by maternal age and education.

When accounting for the presence of one or more pregnancy complications, women with only one lifetime pregnancy (twin and singleton) had substantially increased risk of dying from ASCVD (twin: aHR 2.36, 1.49-3.71 and singleton: aHR 3.56, 3.12-4.06). Women with one lifetime pregnancy without complications also had an elevated risk of ASCVD death if the pregnancy was a singleton (aHR 1.99, 1.82-2.17). The estimated risk of dying from ASCVD for women with one lifetime twin pregnancy without complications was aHR 1.57 (0.92-2.66). Women with a first twin

pregnancy and continued reproduction had a lower risk of future ASCVD with and without any complications (aHR 0.95, 0.55-1.64 versus 0.70, 0.31-1.56).

In several sensitivity analyses, our results remained similar. To evaluate the robustness of our estimates, we restricted to women who had reached 40 years of age, we included women who conceived using ART, we restricted to women with pregnancies on or above 22 weeks as well as using an extended definition of CVD as outcome.

5.2 Paper II

The total mean birthweight for a twin pair was 4628 grams and 3444 grams for a singleton. For women whose first two births were singletons, mean birthweight increased by an average of 151 grams from first to second birth. The occurrence of preterm delivery and preeclampsia in the subsequent singleton pregnancies was similar for women with a first twin pregnancy or a first singleton pregnancy (4.5% versus 4.2% and 2.0% versus 2.0%, respectively).

The mean birthweight in singleton offspring after a first twin pregnancy was 3621 grams whereas singletons after a first singleton pregnancy was 3595 grams, resulting in a crude mean difference of 26 grams. The adjusted difference in mean birthweight in subsequent singletons among women with a first twin pregnancy compared to offspring of women with a first singleton was 21 grams (5.2-36.7). Further. there was no difference in the mean gestational age in the subsequent singleton pregnancy after a first twin or first singleton pregnancy (39.6 weeks versus and 39.7 weeks).

We found a distinct pattern of offspring birthweights between a first twin pregnancy and a first singleton pregnancy. However, in the subsequent singleton pregnancy, the offspring birthweights distributions were almost similar.

5.3 Paper III

When estimating the risk of adverse outcomes in twin- or singleton-born women's own pregnancies, we found no increased risk for twin-born women: preeclampsia aRR 1.00 (0.93-1.09), preterm delivery aRR 0.96 (0.90-1.02) or perinatal loss aRR 1.00 (0.84-1.18) compared with singleton-born women.

Twin-born women delivered from a non-preeclamptic pregnancy had no increased risk of preeclampsia (aRR 0.98, 0.90-1.07), preterm delivery (aRR 0.97, 0.91-1.04) and perinatal loss (aRR 1.04, 0.87-1.24) compared with singleton-born women from non-preeclamptic pregnancies. The occurrence of preeclampsia was, however, more frequent among both twin-born and singleton-born women who themselves had been exposed to preeclampsia compared to unexposed twin-born and singleton-born women (Twin-born: 9.8% versus 6.2%. Singleton-born: 13.4% versus 6.4%). The estimated risk of preeclampsia and preterm delivery in their own pregnancies was lower in twins exposed to preeclampsia aRR 0.73 (0.58-0.91) and preterm delivery aRR 0.71 (0.56-0.90) compared to singletons exposed to preeclampsia.

Women who were term twins had slightly decreased risk of preeclampsia (aRR 0.90, 0.82-1.00) and preterm delivery (aRR 0.91, 0.84-0.99) in their own pregnancies compared to women who were term singletons, with no association with perinatal loss (aRR 0.94, 0.76-1.17). Women born as a preterm twin had no increased risk of preeclampsia (aRR 1.05, 0.92-1.21), perinatal loss (aRR 0.99, 0.71-1.37), and reduced risk of preterm delivery (aRR 0.83, 0.74-0.94) compared to singletons born preterm. The occurrence of preeclampsia was slightly higher among preterm twin-born and singleton-born compared to term twin-born or singleton-born women (Twin-born: 8.0% versus 5.9%; Singleton-born: 7.7% versus 6.5%).

In a sensitivity analysis, we also adjusted our main models for other potentially confounding factors such as woman's educational status and total number of pregnancies. The estimates remained stable when adjusted for these factors.

6. Discussion

6.1 Discussion of methods

6.1.1 Study design

Papers I, II and III were all based on population-based registry data derived from prospectively collected database on women's complete reproductive history. The population-based registry data included every woman with a pregnancy above 16 gestational weeks, providing all twin pregnancies in Norway within the study period. Our study population had a fair sample size of twin pregnancies followed up until death or the end of study period (2020). The population in Norway has been fairly stable (208) with low emigration among those born in Norway.(209) However, immigration has increased in the recent decades under study.(209)

Paper I was based on a cohort of women characterized by their lifetime reproductive history and followed for cardiovascular disease mortality before 70 years of age. To ensure the complete reproductive history of women, we restricted our study group to women with their first pregnancy registered in the MBRN. Also, to allow for a second pregnancy within the study period, we limited inclusion of women with the first pregnancy before 2013. We evaluated the lifetime reproductive history for women with a twin pregnancy compared to women with a singleton pregnancy. As two or three pregnancies are a common family size in Norway,(210, 211) we also estimated the risk of cardiovascular disease mortality for women with this reproductive history. In our analysis, women with three singletons had lower cardiovascular disease mortality compared to women with two singleton pregnancies (4.82 versus 5.22 per thousand). Therefore, we selected women with three singletons as our reference group. We considered three pregnancies as a plausible stopping point for both twin and singleton first births, with two pregnancies for those who start with twins or three pregnancies for those who start with a singleton. A previous study in

the same population showed that cardiovascular disease mortality for women with two or three lifetime pregnancies was similar.(98)

Paper II was based on a cohort of women with two consecutive pregnancies. Woman with either a first twin or singleton pregnancy and a subsequent singleton pregnancy contributed to the study. We focused on the parity effect on birthweight from first to second birth and not birthweight in later pregnancies because this sequence is when the increase in birthweight is largest. Also, we only included second singleton pregnancies for a homogeneous comparison in the outcome. We included women who gave birth within gestational age 22 and 44 weeks and birthweight by gestational age z-scores > -5 and < 5 to exclude implausible gestational age records.

Paper III was based on a generation-linked data file. Twin- or singleton-born women during 1967-2005 contributed with their pregnancies registered in the MBRN during 1981-2020. Using inter-generational data has a unique potential to investigate aetiology of pregnancy outcomes from one generation to the next. We accounted for the dependency between siblings by using clustered standard errors. CIs changed only by a few decimal values, when accounting for sibling correlations. The study design limited the study population to include only twin- or singleton-born women whose births were registered in the MBRN since 1967 and who themselves reproduced and had their own pregnancies registered in the MBRN. Women who themselves were not born in Norway were not part of the study population, leading to a more homogeneous study population. Though this will not be representative of the more ethnically diverse population of Norway today,(212) it has been argued that "statistical representativeness leads to particular statement about the world, not general statement about nature."(213) Also, we restricted to singleton offspring to twin-born and singleton-born women to have a comparable outcome, which we believe have further strengthened the study design.

The benefits of registry data for research include their low cost, easy access, reliability, as well as the fact that they contain data collected over several decades that enable research on maternal long-term health as well as across generations.(193, 214) The data containing mandatory notification of births are vital resources to study rare exposures and outcomes.(215, 216) Likewise, the linkage of the personal identification number between registries enable to design studies with long follow-up time to have a life course perspective.(217) On the other hand, the difficulty in drawing causal conclusions is an issue in observational studies.(215) For example, registry data may not cover all relevant variables and confounder information will often be incomplete.(218)

6.1.2 Precision

In an observational study, two types of error can lead to inaccuracy of results; random error (affecting statistical precision) and systematic error (affecting the internal validity of the study).(215) Techniques such as increasing the sample size or modifying the study design can be used to improve the statistical precision of the reported associations and reduce random error. The plausible strength and the direction of the association can be better interpreted using CI values.

A major strength of this thesis is a large population-based dataset with mandatory notification of pregnancies in the registry. Since we have access to a large population-based material, statistical precision is usually not an issue. However, since our project has the main focus on twin pregnancies, and women with twin pregnancy constitute a smaller group compared to women with singleton pregnancies, it is important to interpret our results carefully. In *paper I,* the HR estimates of cardiovascular disease mortality of women with twin pregnancies and continued reproduction had wide CI compared to women with singleton pregnancies. Similarly in *paper III*, the RR estimates of twin-born women with a perinatal loss in later pregnancies had a wider CI compared to singleton-born women. These results should be interpreted with caution.

6.1.3 Validity

The validity of the study findings depends on both internal (i.e bias due to misclassification of study variables, selection bias and confounding) and external validity (i.e extent to which the study findings may be applicable to individuals outside the study population). This will be discussed in more detail below.

Internal validity

Internal validity refers to the extent of systematic error in a study.(215) The minimization of systematic error ensures the conclusion drawn are acceptable with regard to the source population. The three main sources of systematic error that can compromise internal validity are: Information bias (misclassification), selection bias and confounding.

Information/misclassification bias

Information or misclassification bias are a common source of bias in the estimates due to error in measuring of the exposure and outcome. This may occur during the recording/reporting of information in the source population.(215)

Misclassification bias of the exposure and outcome variables can lead to either differential or non-differential misclassification bias. Rothman described differential misclassification bias could occur "when the exposure is misclassified differentially according to a person's disease status or disease is misclassified differentially according to a person's exposure status".(219) This type of bias could lead to over- or underestimation of an association. In *papers I-III,* the exposures are registered before the outcome and should in that regard not be dependent on the outcome. For instance, in *paper I* reproductive patterns are registered before and in a different data source than registration of cause of death. Similarly, outcomes in our studies were registered independently of exposures. While differential misclassification may be more unlikely in our studies, non-differential misclassification may occur. According to

Rothman, non-differential misclassification error of a dichotomous exposure could lead to an underestimation of the true effect (bias towards the null value) provided that measurement error is not dependent on other variables.(215)

Misclassification of exposure

In *paper I*, women's reproductive patterns, including plurality status (twin or singleton status) were categorized according to women's pregnancies as registered in the MBRN. Plurality status was determined during the time of delivery. Plurality status was also used as an exposure in *papers II* and *III*. There are some challenges with the registration of twins. Pregnancies identified by twins in the first trimester may eventually not be delivered as twins.(220) "*Vanising twin"* defined as a spontaneous loss of a twin during the first trimester, has been found in about 15-35% of twin pregnancies.(221) In our study population, it may be likely that some of the twins from pregnancies with a "*vanishing twin*" may be captured as singletons. As our study population is based on a large sample, we do not expect this possible misclassification to be of significance, however it could potentially lead to an attenuation of our estimates when comparing outcomes of women delivering singletons and twins.

In *paper I,* we stratified the reproductive patterns on whether the women experienced preeclampsia, preterm delivery or perinatal loss. Also, in *paper III*, our exposure was twin- and singleton-born women with in utero exposure to preeclampsia or being born preterm. Especially in the early years of the registry preeclampsia might not have been captured completely. The data quality of preeclampsia cases registered in the MBRN have been validated over the years. Klungsøyr and colleagues showed that term preeclampsia cases increased after introduction of the new notification form in 1999.(222) Further, in a validation study of births 1967-2002, the proportion of pregnancies registered with preeclampsia in the MBRN that were verified using gold standards, registries and hospital records, was 88.3% (positive predictive value, PPV).(198) In another study of births 1999-2010, the PPV value of preeclampsia

registered in the MBRN among women participating in the Norwegian Mother Father and Child Cohort study (MoBa) was 83.9%.(223) However, the sensitivity was less than 50% in this study, meaning that less than half of cases with preeclampsia in the total population were registered. The preeclampsia cases that were missed in the registry were found to have less severe outcomes.(223)

In our data, misclassification of preterm delivery is possible. Since we use data from the beginning of the registry, when ultrasound measures were not used, we relied mainly on reported women's first day of last menstruation. Gestational age estimates based on women's last menstrual period may be imprecise for the preterm period.(224) However, a validation study of a selection of births between 1967-2012 found PPV above 90% for recording of preterm birth in the MBRN.(225) In the MBRN, information on gestational age was missing for approximately 4% of the study population. Missing data on gestational age occurred mainly before 1999. In *paper II*, we excluded about 76 741 women with missing data on gestational age in first and/or second pregnancy. When including women with missing gestational age in our analysis, we found a similar birthweight pattern. Also, as mentioned above, in *paper II* women with pregnancies with birthweight by gestational age z-scores less than -5 and above 5 were excluded to remove implausible gestational ages.

In our studies, preterm birth was defined as birth less than 37 completed gestational weeks for both twin and singleton pregnancies. This was done although the distribution of gestational length differs for singleton and twin pregnancies. However, a specific preterm definition for twin pregnancies is not established. In our study population, 6% of first-born singletons were delivered before 37 gestational weeks. If we defined "preterm" for twins at a similar cut-off, including 6% of those with the lowest gestational age, we would end up with a preterm cut-off at 28 gestational weeks for twins, that would surely be a very low cut-off. We want in future work to explore a twin specific cut-off for preterm birth applying a recent approach published by Wilcox et. al where they explore SGA cut-off points.(226)

In *paper I,* we also used perinatal loss as an outcome. Early neonatal deaths are registered both in the MBRN and through the Central Population Registry. In this way, two data sources ensure accuracy of the data. Stillbirths and early fetal losses are not given national identification number, and registration in the MBRN is the only source of this information. Underreporting of early stillbirths could be possible. Pregnancies with a "*vanishing twin"* have been described above and could lead to a twin being categorized as a singleton. This misclassification would most likely be non-differential and could potentially attenuate our results.

Misclassification of outcomes

In *paper I*, cause of death in the Norwegian Cause of Death Registry is ascertained by the medical professional using the death certificate.(227) The data quality in the Norwegian Cause of Death Registry has been ranked medium to high compared to other countries, with the reporting of "garbage codes" (codes that are not useful for public health analysis) continue to be a challenge.(227)

In *paper II*, birthweight was the main outcome, while z-scores of birthweight by gestational age were also reported. Birthweight recorded in the MBRN has been validated by a previous study finding low (<2500 grams) and high (>4500 grams) birthweights were accurately reported.(225) Also, birthweight for gestational age was based on a Norwegian standard.(149) With this standard we have excluded faulty birthweight by gestational age z-score (outside -5 and 5+).

In *paper III*, the outcomes were preeclampsia, preterm delivery and perinatal loss. The challenges with concerning registration of these outcomes have been described above.

Selection bias

Selection bias are errors due to the methods used to select the subjects and from factors that influence the study participation.(215) In this thesis, in *papers I-III*, the source population consisted of mandatorily registered birth records from the MBRN covering very close to 100% of live births in Norway. Since the complete population-based cohort data is the basis for inclusion in the *papers*, selection bias is less likely to affect the results.(216) However, there may be a possibility of potential biological selection into the different reproductive patterns in *papers I-III.*

In *paper I,* 1.2% (n=11 247) of women had twins in their first pregnancy, of which about 50% of these women continued to have another pregnancy. For women with a first singleton pregnancy, more than 80% of the women continued to have another pregnancy. We observed differences in maternal age and education among women with a first twin and singleton pregnancy. To account for these differences, when studying the association between reproductive history and long-term cardiovascular mortality, we adjusted for these variables in our models.

In *paper II,* we further investigated fertility following a first twin- and singleton pregnancy (Figure 7). A first preterm and early term delivery were associated with lower continuation of reproduction both for women with a first twin and women with a first singleton pregnancy compared to women term pregnancy. The difference in reproduction between women with a first twin or singleton pregnancy may indicate that women who had first twins and a subsequent singleton pregnancy represented a more selected group of women than women with a first singleton pregnancy and a subsequent pregnancy.

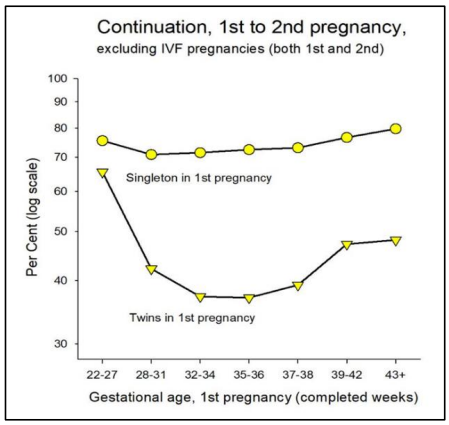

Figure 7: Panel A: Fertility after a first pregnancy, twins or singletons by gestational age in first pregnancy.

In the MBRN, women who stopped reproduction after a first twin pregnancy were older and had higher education than women who stopped after a first singleton pregnancy. However, no large difference in maternal age and education was observed for women who continued after a first twin or a singleton pregnancy (Table 3), which were the women who were the study population in *paper II*. In *paper I* these factors were adjusted for.

Table 3. Maternal age and education among women who continue or stop after a first twin or singleton pregnancy.

	Women who stopped after first pregnancy		**Women who continued after first pregnancy**	
	First twin pregnancy	First singleton pregnancy	First twin pregnancy	First singleton pregnancy
Mean age at first birth, (years) (95% CI)	29.56 (29.44-29.68)	27.59 (27.56-27.62)	25.51 (25.40-25.63)	24.60 (24.59-24.61)
Maternal highest educational attainment				
Low	14.17%	22.11%	16.62%	18.40%
Medium	34.74%	38.52%	35.91%	38.76%
High	50.02%	36.45%	47.04%	42.31%
Missing	1.07%	2.92%	0.43%	0.52%

Similarly, in *paper III*, we excluded individuals who did not reproduce. It is therefore possible that a biological selection of healthier twin-born women may be offsetting a slight increased risk of adverse outcomes resulting in the overall null association we see. However, as we have captured all births, a reduced adverse outcome in twin-born women is not biased in "statistical" sense, but rather a biological selection. We evaluated the probability of reproduction among twin-born women compared to singleton-born women among women born ≤1980 and survived until age 20. 77 % of twin-women reproduced compared to 84% of singleton-born women. Twin-born women had 8% lower reproduction than singleton-born women RR 0.92 (0.92-0.94). We looked further within strata of preeclampsia to assess reproduction within the subset of those women with an adverse in utero exposure. When restricting to women exposed to preeclampsia in utero, we found no difference in the probability of reproduction comparing twin-born and singleton-born women (RR 0.98, 0.93-1.02). Fertility in twin-born and singleton-born women likely depend on other factors, not necessarily captured by our study.

Confounding

Confounding is commonly a 'mixing' or 'blurring' of effects.(228) This is likely to occur when the relationship between exposure and outcome are mixed with a third factor, known as a confounding variable.(228) Stated otherwise, confounding is likely to occur when the link between exposure and outcome includes a non-causal component due to an uncontrolled shared cause. In the three papers presented in this thesis, we identified potential confounders based on our research hypothesis and prior literature. Controlling for potential confounders may improve the precision of our results. Further, the choice of confounders adjustment was limited by what was available in the MBRN and in Statistics Norway.

In *paper I*, for the association between lifetime reproductive history of women and long-term cardiovascular mortality, we used Cox proportional HR adjusted for maternal age at first birth, chronic diseases, maternal education and year of birth as

potential confounders (Figure 8. Conceptual framework for analysis, *Paper I*). There was slight reduction in the estimates upon adjusting for these confounders. With few covariates to choose from and sparse literature on twinning and reasons for stopping reproduction, we adjusted for age, and year of first birth to capture cohort effects over the 50 years of data. The only marker of socioeconomic status we had available was highest attained educational level. In Norway, education is strongly associated with both family size and cardiovascular mortality.(98) Women with low education (<11 years) had a higher risk of cardiovascular mortality, compared to women with a high education (11 or more years).(98) Chronic conditions could be associated with underlying factors predisposing to both the reproductive pattern of women and the long-term maternal health. The MBRN had information on some chronic health conditions including diabetes, hypertension, kidney disease and rheumatoid arthritis. Adjusting for these chronic conditions in our models did not substantially change the estimates.

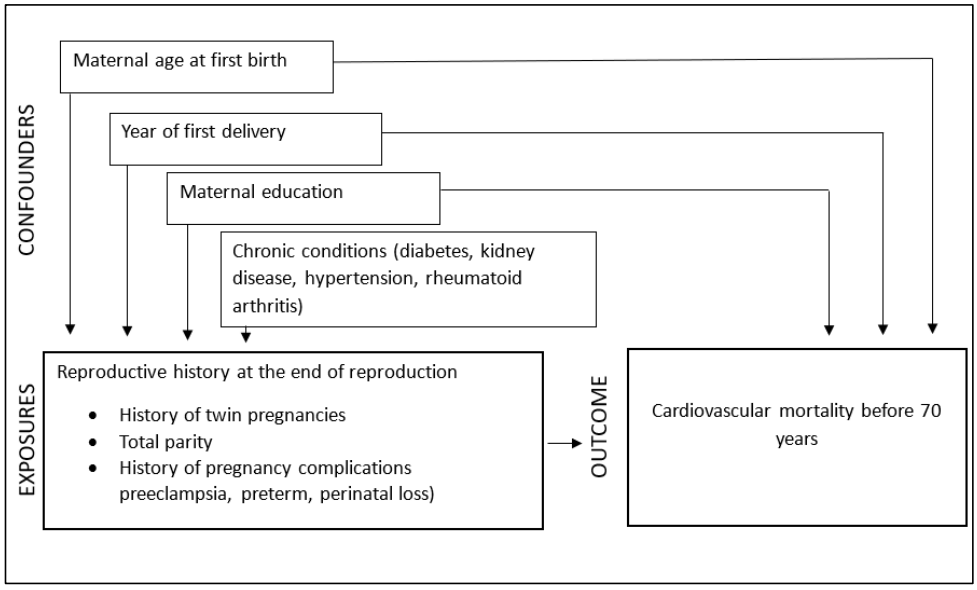

Figure 8. Conceptual framework for analysis, *Paper I*.

In *paper II*, for the mean difference in birthweight between the first and second pregnancy, we adjusted for maternal age at first birth, maternal education, year of

birth and country of birth as potential confounders as these factors could affect both plurality status of first pregnancy and birthweight in the second pregnancy. In the adjusted results, birthweight was only reduced by a few grams compared to the crude results.

In *paper III*, we adjusted for women's decade of birth and their mother's highest educational attainment as these may be associated with woman's own birth. However, adjustment did not alter our main conclusion. The reason for similarities in crude and adjusted results could be that the inter-generational impact was stronger than the relationships between confounding variables and the outcome. In a sensitivity analysis, we also accounted for woman's own educational attainment along with total number of pregnancies of a woman as a surrogate for the opportunity to experience adverse pregnancy outcomes. We saw very little change in the risk estimates with these adjustments.

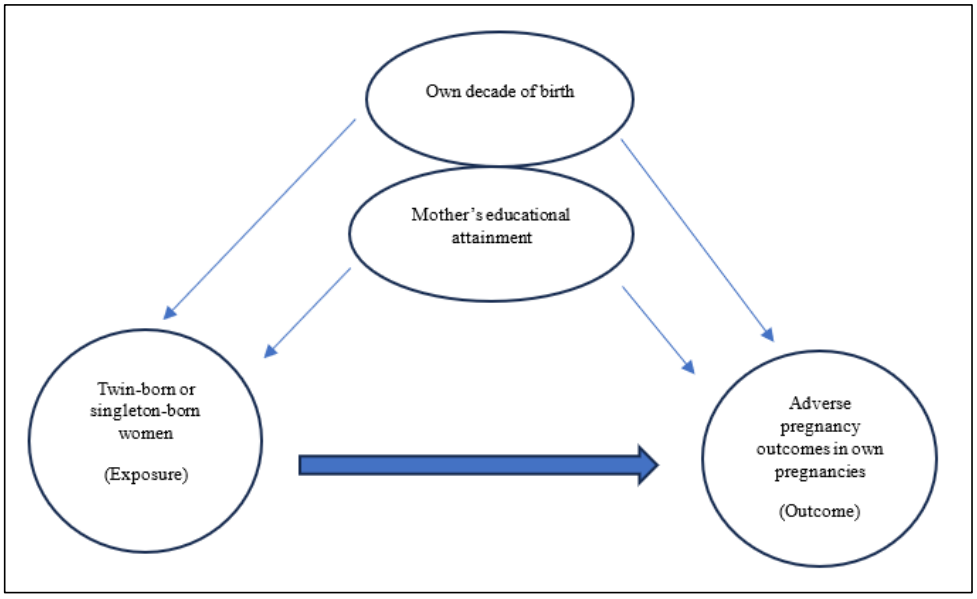

Figure 9. Conceptual framework for analysis (Plurality at birth and adverse pregnancy outcomes in later pregnancies)

Several other unmeasured confounding factors may have affected our results. We did not account for smoking, inter-pregnancy weight change and obesity in our analyses because these data were not completely available. Prior studies have shown the existence of a relationship between these factors and cardiovascular mortality, offspring birthweight and adverse outcomes in pregnancy.(229-231) As there is time-dependent missing data for these variables in the registry, we were not able to evaluate confounding effect of these factors in the overall cohort. Moreover, if we restricted our study population to the recent years, it would severely affect our sample size as the twin population is small compared to the singletons. In *paper III,* we obtained e-values for estimates with CIs excluding null. E-value has been defined as "the minimum strength of association, on the risk ratio scale, that an unmeasured confounder would need to have with both the treatment and outcome to fully explain away a specific treatment outcome association, conditional on the measured covariates."(205) The e-values for our estimates ranged from 1.4-2.2, suggesting that unmeasured confounding of such strength was required to move the estimate towards null.

External validity

External validity, also known as generalizability, refers to the validity of the findings and implications beyond the source population. Generalizability may depend on biological, social or genetical factors. In our study, the study participants were selected from the population-based national registry data which registered pregnancies from 16 gestational weeks.

Some factors in our study questions might increase the generalizability of our findings. Our findings are based on population-based data with close to 100% coverage of live births nationally. The research question in *paper I* explored differences between reproductive patterns including twins and singletons and later cardiovascular disease. Though cardiovascular disease morbidity and mortality may vary between countries, the increased physiological burden of a twin pregnancy

compared to a singleton pregnancy might be similar in contexts outside Norway, as it is representing a biological rather than an environmental/societal exposure. In *Paper II* the association between twin or singleton status in a first pregnancy and subsequent birthweight was studied. Although birthweight may vary between countries, the parity effect on birthweight has been found for several populations.(154-157) Also, as this research question focused on a difference in biological burden of twin and singleton mothers, the finding may be generalizable to other populations.

Other factors may limit the generalizability of our findings. In *Paper III* we looked at reproductive outcomes of being born twin or singleton. Several social and biological factors may influence reproduction both in the next generation, as well as subsequent reproduction after a first pregnancy, which may be different in other societies than Norway. Norway is a modern welfare state with generous maternal and paternal benefits related to childcare.(232) This could limit the generalizability of our results associated with reproduction across generations (in *paper III*) as well as with reproductive patterns after a first twin pregnancy (*paper I and II*). Our conclusions may be most applicable in a Nordic setting with similar welfare state benefits.

6.2 Discussion of main results

6.2.1 Paper I

In *paper I*, an increased risk of cardiovascular mortality before 70 years of age was found among women with one lifetime pregnancy, twin or singleton, compared to the reference of three lifetime singleton pregnancies. Compared with this reference population, women with a first twin pregnancy and continued reproduction did not have an increased risk of cardiovascular mortality while women with a first singleton pregnancy and with twin pregnancies in later reproduction had an increased risk of long-term cardiovascular mortality. Further, the risk of cardiovascular disease mortality for women with any pregnancy complications was more than 2-fold higher for women with one lifetime twin pregnancy and more than 3-fold higher for women with one lifetime singleton pregnancy compared to the women with three singleton pregnancies without any complications.

Over the past decades, the availability of health data from different sources (such as population-based registry data, hospital records or cohort data) has enabled researchers to study the association between pregnancy complications and long-term cardiovascular disease morbidity and mortality.(105-117, 123, 128, 233-240) These studies provide extensive evidence of an association between pregnancy complications and risk of cardiovascular morbidity and mortality in later life. The studies have used both nulliparous women and parous women in their study sample. However, most of the studies are based on singleton pregnancies and have estimated the overall risk of long-term cardiovascular morbidity and mortality without considering the lifetime reproductive pattern of a woman. The latter point is the most important novelty of our design, along with its main focus on women with twin pregnancies. In Norway, previous studies based on data from the MBRN highlighted different associations between various reproductive patterns and long-term cardiovascular health. As mentioned earlier, women with one lifetime singleton

pregnancy with preeclampsia had an increased risk of cardiovascular mortality in later life compared to women with more than one pregnancies.(8) Another study based on MBRN data also showed that risk of cardiovascular disease mortality was elevated for women with one perinatal loss and low education compared to women with higher education with a loss.(241) None of these studies accounted for twins in any pregnancies during a woman's reproductive career. As mentioned, in our study, we accounted for lifetime reproductive history of women with twin pregnancies, differentiating various pregnancy history of women, to describe women's overall risk of cardiovascular disease mortality before 70 years of age. The risk of cardiovascular mortality varied substantially in women by different lifetime reproductive history, which is an important finding.

There could be various mechanism leading to differences in cardiovascular disease mortality according to the reproductive history of women. In Norway, two children seem like a common norm for families.(232) As one twin gestation contributes to two children, women with one lifetime twin pregnancy may have achieved the desired family size after one twin pregnancy. Women who had first twins or singleton pregnancy may have different reasons for not having another pregnancy after one pregnancy. For women with one lifetime singleton pregnancy, stopping reproduction may be due to adverse pregnancy outcomes in the first pregnancy, underlying health issues, or subfertility. A woman's decision to become pregnant in the future may be influenced by her prior or preexisting adverse pregnancy outcomes.(241) Earlier studies that investigate cardiovascular disease risk in later life among women with twin or singleton pregnancies accounted for maternal age, underlying chronic conditions and fertility treatment.(11, 12, 131) In our study population, we restricted to women without ART and adjusted for both maternal age at first pregnancy and women's education. The risk of cardiovascular disease death may be driven by similar underpinnings among women with one lifetime twin or singleton pregnancy. However, the differences in risk estimates for women with twin pregnancy compared to women with singleton pregnancy may point to differences in underlying risk

profile among these two groups. Women who have twins after a first singleton birth may be further explored to understand their increased risk of cardiovascular disease mortality.

As shown in our data, women with twin pregnancies had higher occurrences of pregnancy complications such as preeclampsia, preterm delivery and perinatal loss. These complications in singleton pregnancies have been associated with increased risk of cardiovascular disease morbidity and mortality.(10, 102, 103, 105, 115, 124, 241-246) In our study population, the risk of long-term cardiovascular disease mortality differed among women with twin and singletons pregnancies with complications with pregnancy complications. This could imply several explanations. Pregnancy brings about a number of physiological changes that could clinically reveal a woman's underlying cardiometabolic risk.(130) Prior studies showed differences in the cardiovascular system, endothelial functions, haematological and metabolic adaptations between a singleton and twin gestation.(47, 247-249) Also, risk of preterm delivery differed between women with singletons and twins. This could mean that complications occurring in twin pregnancies might be less of a sign of cardiometabolic risk than in singleton pregnancies. Our conclusions did not alter when we controlled for maternal education and underlying chronic conditions during pregnancy. In line with previous studies, our findings show that risk of cardiovascular disease mortality is elevated more for women with singleton pregnancies compared to women with twin pregnancies. Consistent with other findings,(250, 251) our results overall add that twin pregnancy is less likely to exhibit health disadvantage for women with more than one pregnancy compared to women with only singleton pregnancies. Our results may have potential to prevent assumptions on women's long-term health based only on woman's first pregnancy. We believe our study contributes to the growing body of evidence on understanding the association between lifetime reproductive history of women with twin pregnancies and long-term cardiovascular disease mortality.

In our main analysis, we did not restrict our inclusion to any gestational ages. Our aim was to capture the full reproductive history of women including early losses as registered in the MBRN. However, as pregnancies ending before 22 gestational weeks could have more incomplete registrations, we did perform a sensitivity analysis restricted to women with gestational age above 22. This, however, did not alter our conclusions.

A major strength of our study was the population-based national registry data with longitudinal reproductive history of women, followed for median 24 years and linked to the Norwegian Cause of Death registry. Our limitation is that we were not able to adjust for possible predictors of cardiovascular disease, that were not recorded at the time of birth. Nevertheless, our study evaluated the risk of cardiovascular death based on the reproductive history of the women in Norway. Identification of risk profiles for cardiovascular disease has been one of the most important public health contributions of epidemiology, improving disease prevention, diagnosis and timely treatment.(215) Another important limitation in our study was that reasons for ending reproduction (our primary exposures) were not well characterized. However, our goal is to not suggest that having only one pregnancy (singleton or twin) is causally related to cardiovascular disease mortality, instead we intend to highlight that woman who complete reproduction with only one pregnancy (being either singleton or twin pregnancy) seem to have an increased risk of cardiovascular disease mortality before 70 years. This may help identify women who would benefit from earlier cardiovascular disease screening or tailored interventions.

6.2.2 Paper II

In *paper II*, we found offspring birthweight in second singleton pregnancy were comparable for women with a first twin and a first singleton pregnancy.

During pregnancy, a woman's fetus is influenced by the intrauterine environment which is largely affected by maternal diet, genes, underlying health, behaviors and

socioeconomic characteristics.(252) Birthweight is an important pregnancy outcome strongly associated with infant, child and later adult life health.(253, 254) As stated earlier, prior studies have shown subsequent singleton babies are about 80-140 grams larger than the first singleton, indicating an independent effect of parity on birthweight.(155-157, 255) Although we do not exactly know the reason why subsequent offspring's birthweight is generally larger than the first, there are some theories to it related to singleton pregnancies.(158-163) However, to our knowledge no previous study has examined the patterns in birthweight of singletons following a twin pregnancy.

Offspring's birthweights are larger for a twin pair than for a singleton foetus.(149, 165) We hypothesized that the enlarged uterine capacity due to two foetuses of a twin pair, along with amniotic fluid, and placental mass in a twin pregnancy, could accommodate a larger offspring in the subsequent pregnancy. On the other hand, as mentioned before, prior studies have shown independent parity effect on birthweight among singleton pregnancies. However, in our study the mean birthweight in subsequent singleton pregnancy was similar, whether the earlier birth was twin or singleton. After a twin pregnancy, the mean weight of a singleton birth was only 21 grams heavier than after a singleton pregnancy. Upon controlling for possible confounders such as age at first delivery, year of first delivery, maternal education and country of birth, the results were not affected. Thus, our study shows that the parity effect on birthweight reported by earlier studies (155-157, 255) (in the range of 80-140g) seems to be due to other mechanisms that is not yet clearly known.

Offspring birthweight has been suggested to be influenced by differences in maternal physiological factors that change in the first and subsequent pregnancy.(156) At the same time, growth of the fetus is also related to stable maternal factors, as women tend to have successive singleton pregnancies of similar size.(256, 257) As stated in earlier chapter, the perinatal outcomes differed for women with twin or singleton pregnancies such as gestational age and birthweight.(149, 165) However, successive

pregnancy outcomes after twin pregnancies have not been explored as much as singleton pregnancies due to the availability of data. We believe our study is the first study to report offspring's birthweight in a subsequent pregnancy after a first twin pregnancy. Twin pregnancies provided larger uterine distension due to multiple fetuses in addition to the amniotic fluid and the placentas. Therefore, we expected the differences in birthweight in subsequent singleton pregnancy to be greater than following a singleton pregnancy. However, additional weight and uterine expansion were associated with only a trivial increase of the birthweight in the subsequent singleton pregnancy. Prior studies have shown difference in birthweight by maternal education and geography.(201) Our results were adjusted for these factors. Our findings highlights that the mechanical burden exhibited by two fetuses in a twin pregnancy and the accompanying complications a woman with twin endures does not substantially influence the birthweight in the subsequent pregnancy in our population.

We also describe birthweight in the subsequent pregnancy after a twin pregnancy based on the inter-pregnancy interval between first and second pregnancy. Earlier studies using singleton populations have shown an increased risk of low birthweight after a long or short pregnancy interval,(152) however, to our knowledge birthweight in a subsequent singleton pregnancy following a twin pregnancy by inter-pregnancy interval has not been investigated before. In our study, the frequency distribution plots of inter-pregnancy interval showed that women who had singletons in the first pregnancy had a peak in frequency of a subsequent pregnancy at about 2-3 years (66%), while only 42% of women with a first twin pregnancy had a subsequent pregnancy within inter-pregnancy interval of 3 years. The birthweight patters of infants born within 3 years of a prior twin or singleton birth were similar. However, there were substantial difference in the offspring birthweight patterns for longer inter-pregnancy intervals. Interpretation of our results on the association between inter-pregnancy interval and birthweight after a first twin and singleton should be done with caution. Women with and long inter-pregnancy interval may be sub-fertile and may have underlying health concerns that may impact their offspring birthweight.

Women who have twins may have social reasons, such as caring of twin children, for having a long inter-pregnancy interval rather than underlying health or biological reasons. According to our findings, these women with longer inter-pregnancy intervals may be healthier as they had less reduction in offspring birthweight. Additionally, social factors like low education and change of partner may also be more frequent among singleton women with long inter-pregnancy interval compared to twin mothers with long inter-pregnancy intervals.

Further, preterm delivery and preeclampsia are more frequent in twin pregnancies compared to singleton pregnancies, and these complications are associated with reduced birthweight.(6) In our study, there was higher recurrence of preterm delivery and preeclampsia in a subsequent singleton pregnancy after a first singleton pregnancy compared to after a first twin pregnancy. We estimated aRR of recurrence of preterm delivery in the subsequent singleton pregnancy for women with a preterm twin pregnancy was aRR 1.99 (1.51-2.64) compared to women with a term first twin pregnancy. For women with a preterm singleton pregnancy the relative risk of recurrence was aRR 4.72 (4.61-4.84) compared to women with a term first singleton pregnancy. The recurrence risk of preeclampsia in the subsequent singleton pregnancy for women with a preeclamptic twin pregnancy was aRR 5.06 (3.39-7.56) compared to women without preeclampsia in a first twin pregnancy and for women with a preeclamptic singleton pregnancy was aRR 10.47 (10.14-10.82) compared to women without preeclampsia in their first singleton pregnancy. As shown by these results, while these complications tend to recur in subsequent pregnancies,(258) consistent with results from earlier studies, these complications do not recur as frequently following twin pregnancies as compared to singleton pregnancies.(59, 259) Although women with a twin pregnancy had higher pregnancy complication rates in the first pregnancy, the recurrence of pregnancy complications in the subsequent pregnancy was higher for women with a first singleton pregnancy. Thus, these pregnancy complications did not seem to affect the overall birthweight in the subsequent singleton offspring in our study population.

In our study, we chose to not stratify on gestational age. Stratifying by gestational age for twins and singletons in the first pregnancy may not yield interpretable results as the gestational age distribution of the twins and singletons are different. Stratification by gestational age may introduce paradoxical collider stratification bias, similar to what is seen when looking at gestational age of twins and infant mortality.(260) The peak growth of birthweight is earlier for twins than for the singletons.(165) The complex stratification structures may not provide for a fair comparison between twin and singleton pregnancies. Therefore, we decided to compare the mean birthweight in the subsequent singleton pregnancy after a first twin or singleton pregnancy using a standard preterm delivery definition.

The large population-based cohort data provided sufficient sample size to study the association in subsequent singleton pregnancy. The validation of measurement and reporting of birthweight which has been reported consistent overtime provides further assurance of our results.(225) Earlier studies have shown differences in the perinatal outcomes such as gestational age, birthweight in the monochorionic and dichorionic twins.(261-263) However, unfortunately we do not have information about the chorionicity of the twins to evaluate these perinatal differences.

6.2.3 Paper III

In *paper III*, we found no increased risk of adverse pregnancy outcomes such as preeclampsia, preterm delivery or perinatal loss among twin-born women compared with the singleton-born women. The increased risk of preeclampsia and preterm delivery in own pregnancies among women born with these complications have been established for singleton pregnancies.(170, 173) Our study provides novel information about the twin-born women who were in utero exposed to preeclampsia or preterm, had a reduced risk of preeclampsia or preterm delivery in their later pregnancies compared to the singleton women who were in utero exposed to preeclampsia or delivered preterm.

The in utero environment may influence later life health.(264, 265) Prior studies have found that infants born preterm or in pregnancies with preeclampsia are associated with adverse long-term effects such as cardiovascular disease in later life compared to infants born without these pregnancy complications.(266-268) Women with twin pregnancies are at increased risk of pregnancy complications such as preterm, preeclampsia and perinatal loss.(6, 7, 53) Both preterm delivery and preeclampsia are associated with increased risk of later maternal health consequences in singleton pregnancies,(104, 269) but there is little evidence about the risk of adverse pregnancy outcomes for twin-born women in their later reproduction.

The underlying reasons for the recurrence of pregnancy complications across generations have not been fully understood. Maternal genes and fetal genes from both the parents, are suggested to play a role in the development of preeclampsia in singleton-born women.(173, 177) Consistent to our findings, a study from Sweden showed less recurrence of preterm delivery in preterm twin-born women than preterm singleton-born women.(191) Taken together with prior literature, it seems that pregnancy complications in twin and singleton pregnancies have distinct origins. It may also seem that twin-born women are less likely to repeat the in utero complications in her own pregnancies. Maternal education and time trends did not seem to explain the reduced risk of these complications in twin-born women compared with singleton-born women. In general, there is a decreasing trend of preeclampsia in Norway, with a 37% decrease in preeclampsia prevalence during the last two decades.(270) A similar decline was also found among women with twin pregnancies. It has been suggested that changes in clinical handling such as aspirin use, and labor induction may partly explain this decline.(270) A decrease in prevalence might lead to lower recurrence across generations.

Studying pregnancy outcomes across generations was made possible by the prospectively collected data over 50 years. A relatively large study population

enabled a stratified analysis by in utero exposure to specific pregnancy complications among twin-born and singleton-born women. We did not account for factors which may predict specific adverse outcomes (smoking, BMI, inter-pregnancy interval) and may vary for each pregnancy over the whole reproductive course.

7. Conclusion

We found an increased risk of long-term cardiovascular disease mortality among women with one lifetime pregnancy, twins or singletons, compared to women with three singleton pregnancies. Women with twin pregnancies who continued reproduction had similar risk of cardiovascular disease mortality compared to the women with three singleton pregnancies. Thus, although women with twin pregnancies experience more pregnancy complications compared to women with singleton pregnancies, the long-term cardiovascular mortality risk does not appear to be higher. Our study findings provide novel information on the potential usefulness of incorporating full pregnancy history into the assessment of maternal long-term health of women with twin pregnancies.

We found that birthweights of the subsequent singleton offspring were similar for women with a first twin or a first singleton pregnancy. Twin pregnancies contribute to a greater combined total offspring birthweight including more extensive uterine expansion. However, this does not seem to explain the general parity effect seen in birthweight. Our findings indicate that a twin pregnancy does not contribute meaningfully to a parity effect of increased birthweight from first to second birth. Thus, physiologic reasons for the increased birthweight with parity remain to be established.

Additionally, although twin-born women are more often exposed to adverse pregnancy outcomes in-utero, the risk of preeclampsia, preterm delivery and perinatal loss in twin-born women are not increased in their own pregnancies compared with singleton-born women. Twin-born women exposed to preeclampsia in utero had a reduced risk of preeclampsia and preterm delivery in their own pregnancies compared with singleton-born women exposed to preeclampsia in utero. Preterm twin-born women had no increased risk of preeclampsia or perinatal loss in their own pregnancies and a reduced risk of preterm delivery compared with preterm singleton-

born women. These findings are relevant for the clinicians and families dealing with twins in assessing the immediate and long-term consequences of twin pregnancy.

Overall, our result indicates that, in comparison to singleton pregnancy, becoming a mother to twin offspring or being a twin-born women becoming a mother herself does not add any extra risk of later long-term cardiovascular disease mortality or adverse pregnancy outcomes in own pregnancies. Our study adds to the growing body of evidence on understanding the link of pregnancy complications and later cardiovascular disease mortality, and on twin pregnancies and their impact on long-term health.

8. Future implications

The evaluation of reproductive history including twins is complicated as the ideal cut-off for preterm delivery in twin pregnancies is not well-defined. Thus, future studies should focus on establishing a cut-off for preterm delivery in twin pregnancies, especially related to exploring associations of pregnancy complications and maternal long-term health. If we should use the similar preterm-term distribution that we find among first-born singletons (6% are born preterm), we would end up with a cut-off at 28 weeks. 6% of an already small group would mean that a large data source will be needed to study this.

In *paper I*, we highlight the remarkable high risk faced by women who stop reproduction after their first birth, and we include women with twin pregnancies who are often excluded. Future research should explore the reasons for this high risk and how they could benefit from follow-up. In our study, we were limited by our ability to consider specific complications in each pregnancy. Future studies may use similar approaches to study other non-communicable diseases to better understand the role of reproductive patterns related to twins and long-term maternal health. The findings in this thesis are based on pregnancies dating back to 1967. Future studies will be able to evaluate current obstetrical practices and their relation to maternal long-term health. Also, it would be interesting to replicate these analyses in countries with different economical support systems for maternal leave and childcare.

In *paper II*, we aimed to shed light on mechanisms related to the parity effect on birthweight. However, having a twin pregnancy with a greater combined total offspring birthweight did not lead to a substantial higher birthweight in the next singleton pregnancy. The physiological underpinnings of the parity effect on birthweight are still unclear and future studies are needed to gain more knowledge on this birthweight phenomenon. Also, in future studies twin related variables such as chorionicity and zygosity may be accounted for.

In *paper III*, we evaluated the own pregnancy outcomes of twin-born or singleton-born women. The study findings may be useful for clinicians to assess the recurrence of adverse pregnancy outcomes across generations for women born as twin. Future studies will be able to evaluate reproductive outcomes of twins surviving at lower gestational ages than earlier due to current obstetrical practices.

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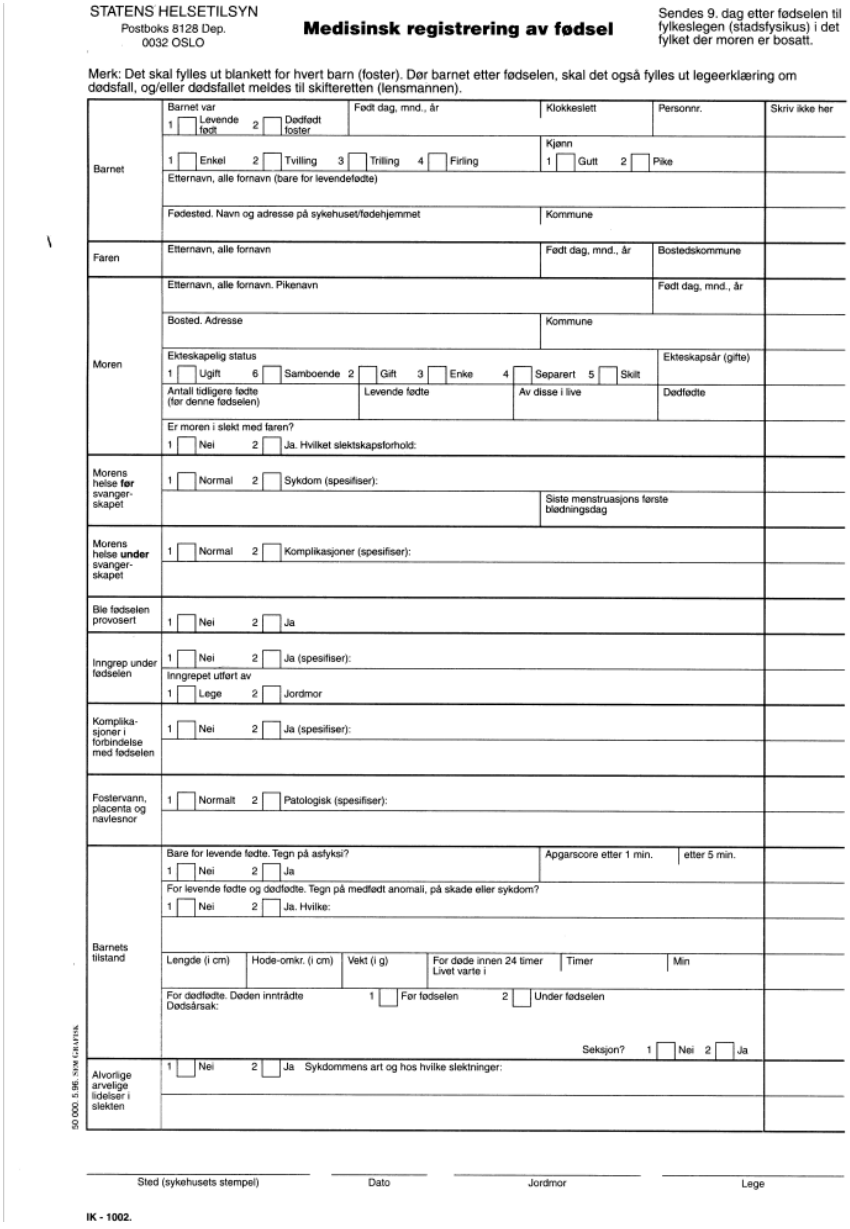
10. Appendix

The MBRN notification form until 1999



STATENS HELSETILSYN
Postboks 8128 Dep.
0032 OSLO

Medisinsk registrering av fødsel

Sendes 9. dag etter fødselen til fylkeslegen (stadsfysikus) i det fylket der moren er bosatt.

Merk: Det skal fylles ut blankett for hvert barn (foster). Dør barnet etter fødselen, skal det også fylles ut legeerklæring om dødsfall, og/eller dødsfallet meldes til skifteretten (lensmannen).

Barnet
Barnet var: 1 ☐ Levende født 2 ☐ Dødfødt foster
Født dag, mnd., år
Klokkeslett
Personnr.
Skriv ikke her
1 ☐ Enkel 2 ☐ Tvilling 3 ☐ Trilling 4 ☐ Firling
Kjønn: 1 ☐ Gutt 2 ☐ Pike
Etternavn, alle fornavn (bare for levendefødte)
Fødested. Navn og adresse på sykehuset/fødehjemmet
Kommune

Faren
Etternavn, alle fornavn
Født dag, mnd., år
Bostedskommune

Moren
Etternavn, alle fornavn. Pikenavn
Født dag, mnd., år
Bosted. Adresse
Kommune
Ekteskapelig status: 1 ☐ Ugift 6 ☐ Samboende 2 ☐ Gift 3 ☐ Enke 4 ☐ Separert 5 ☐ Skilt
Ekteskapsår (gifte)
Antall tidligere fødte (før denne fødselen)
Levende fødte
Av disse i live
Dødfødte
Er moren i slekt med faren? 1 ☐ Nei 2 ☐ Ja. Hvilket slektskapsforhold:

Morens helse før svangerskapet
1 ☐ Normal 2 ☐ Sykdom (spesifiser):
Siste menstruasjons første blødningsdag

Morens helse under svangerskapet
1 ☐ Normal 2 ☐ Komplikasjoner (spesifiser):

Ble fødselen provosert
1 ☐ Nei 2 ☐ Ja

Inngrep under fødselen
1 ☐ Nei 2 ☐ Ja (spesifiser):
Inngrepet utført av: 1 ☐ Lege 2 ☐ Jordmor

Komplikasjoner i forbindelse med fødselen
1 ☐ Nei 2 ☐ Ja (spesifiser):

Fostervann, placenta og navlesnor
1 ☐ Normalt 2 ☐ Patologisk (spesifiser):

Barnets tilstand
Bare for levende fødte. Tegn på asfyksi? 1 ☐ Nei 2 ☐ Ja
Apgarscore etter 1 min. | etter 5 min.
For levende fødte og dødfødte. Tegn på medfødt anomali, på skade eller sykdom? 1 ☐ Nei 2 ☐ Ja. Hvilke:
Lengde (i cm) | Hode-omkr. (i cm) | Vekt (i g) | For døde innen 24 timer Livet varte i | Timer | Min
For dødfødte. Døden inntrådte 1 ☐ Før fødselen 2 ☐ Under fødselen
Dødsårsak:
Seksjon? 1 ☐ Nei 2 ☐ Ja

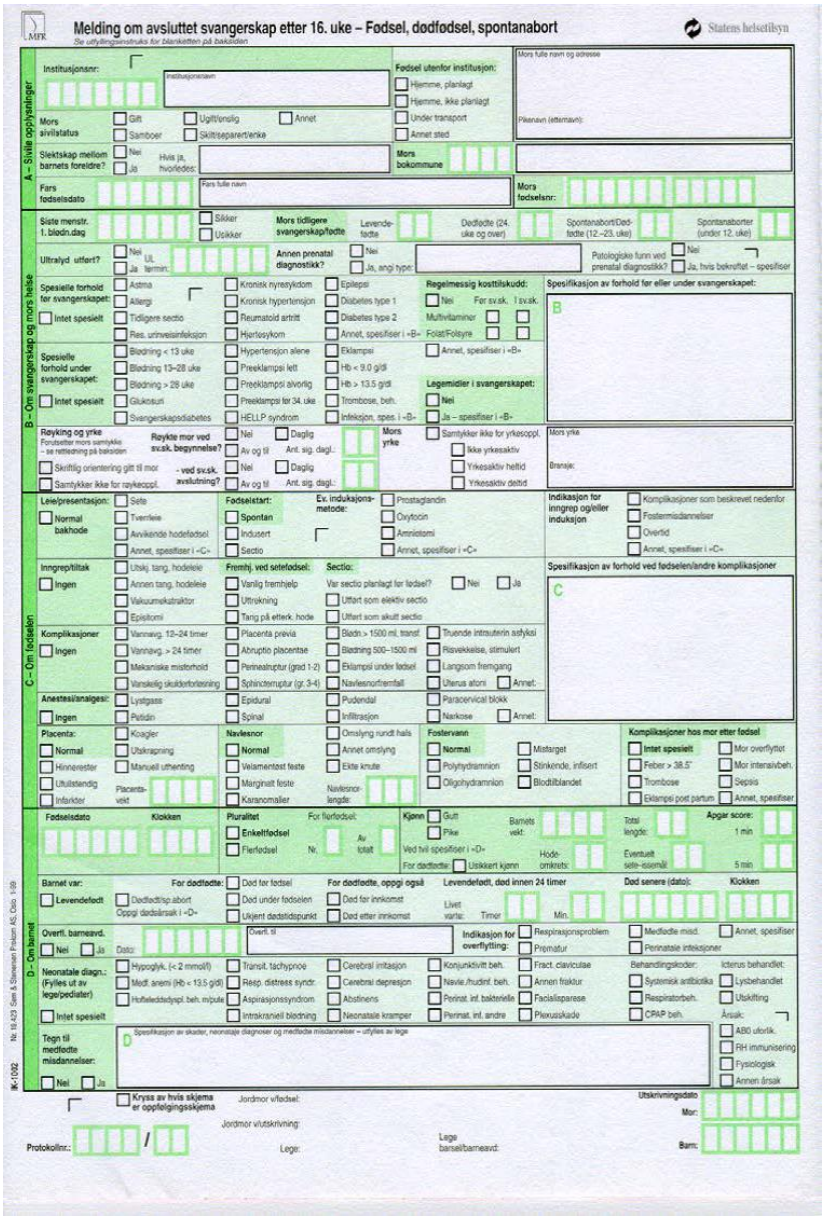
Alvorlige arvelige lidelser i slekten
1 ☐ Nei 2 ☐ Ja Sykdommens art og hos hvilke slektninger:

50 000. 5/86. SKM GRAFISK

Sted (sykehusets stempel) | Dato | Jordmor | Lege

IK - 1002.

The MBRN notification form from 1999



Melding om avsluttet svangerskap etter 16. uke – Fødsel, dødfødsel, spontanabort

Statens helsetilsyn

MBR

Se utfyllingsinstruks for blankettene på baksiden

A – Sivile opplysninger

Institusjonsnr: | Institusjonsnavn | Fødsel utenfor institusjon: Hjemme, planlagt; Hjemme, ikke planlagt; Under transport; Annet sted | Mors full navn og adresse | Pikenavn (ettemavn):

Mors sivilstatus: Gift; Samboer; Ugift/enslig; Skilt/separert/enke; Annet

Slektskap mellom barnets foreldre? Nei; Ja. Hvis ja, hvorledes: | Mors bokommune

Fars fødselsdato | Fars fulle navn | Mors fødselsnr:

B – Om svangerskap og mors helse

Siste menstr. 1. blødn.dag | Sikker; Usikker | Mors tidligere svangerskap/fødte: Levendefødte | Dødfødte (24. uke og over) | Spontanabort/Dødfødte (12.–23. uke) | Spontanaborter (under 12. uke)

Ultralyd utført? Nei; Ja. UL termin: | Annen prenatal diagnostikk? Nei; Ja, angi type: | Patologiske funn ved prenatal diagnostikk? Nei; Ja, hvis bekreftet – spesifiser

Spesielle forhold for svangerskapet: Intet spesielt; Astma; Allergi; Tidligere sectio; Res. urinveisinfeksjon; Kronisk nyresykdom; Kronisk hypertensjon; Reumatoid artritt; Hjertesykom; Annet, spesifiser i «B»; Epilepsi; Diabetes type 1; Diabetes type 2; Annet, spesifiser i «B»

Regelmessig kosttilskudd: Nei; For sv.sk.; I sv.sk.; Multivitaminer; Folat/Folsyre; Annet, spesifiser i «B»

Spesifikasjon av forhold før eller under svangerskapet: B

Spesielle forhold under svangerskapet: Intet spesielt; Blødning < 13 uke; Blødning 13–28 uke; Blødning > 28 uke; Glukosuri; Svangerskapsdiabetes; Hypertensjon alene; Preeklampsi lett; Preeklampsi alvorlig; Preeklampsi før 34. uke; HELLP syndrom; Eklampsi; Hb < 9.0 g/dl; Hb > 13.5 g/dl; Trombose, beh.; Infeksjon, spes. i «B»

Legemidler i svangerskapet: Nei; Ja – spesifiser i «B»

Røyking og yrke (Forutsetter mors samtykke – se retteanvisning på baksiden): Skriftlig orientering gitt til mor; Samtykker ikke for røykeoppl.

Røykte mor ved sv.sk. begynnelse? Nei; Daglig. Av og til. Ant. sig. dagl.:

– ved sv.sk. avslutning? Nei; Daglig. Av og til. Ant. sig. dagl.:

Mors yrke: Samtykker ikke for yrkesoppl.; Ikke yrkesaktiv; Yrkesaktiv heltid; Yrkesaktiv deltid | Mors yrke; Bransje:

C – Om fødselen

Leie/presentasjon: Normal bakhode; Sete; Tverrleie; Arvikende hodefødsel; Annet, spesifiser i «C»

Fødselstart: Spontan; Indusert; Sectio

Ev. induksjonsmetode: Prostaglandin; Oxytocin; Amniotomi; Annet, spesifiser i «C»

Indikasjon for inngrep og/eller induksjon: Komplikasjoner som beskrevet nedenfor; Fostermisdannelser; Overtid; Annet, spesifiser i «C»

Inngrep/tiltak: Ingen; Utskj. tang, hodeleie; Annen tang, hodeleie; Vakuumekstraktor; Episiotomi

Fremhj. ved setefødsel: Vanlig fremhjelp; Uttrekning; Tang på etterk. hode

Sectio: Var sectio planlagt før fødsel? Nei; Ja. Utført som elektiv sectio; Utført som akutt sectio

Spesifikasjon av forhold ved fødselen/andre komplikasjoner: C

Komplikasjoner: Ingen; Vannavg. 12–24 timer; Vannavg. > 24 timer; Mekaniske misforhold; Vanskelig skulderforløsning; Placenta prævia; Abruptio placentae; Perinealruptur (grad 1-2); Sphincterruptur (gr. 3-4); Blødn.> 1500 ml, transf.; Blødning 500–1500 ml; Eklampsi under fødsel; Navlesnorsfremfall; Truende intrauterin asfyksi; Risvekkelse, stimulert; Langsom fremgang; Uterus atoni; Annet:

Anestesi/analgesi: Ingen; Lystgass; Petidin; Epidural; Spinal; Pudendal; Infiltrasjon; Paracervical blokk; Narkose; Annet:

Placenta: Normal; Hinneretter; Utullstendig; Infarkter; Koagler; Utskrapning; Manuell uthenting; Placentavekt

Navlesnor: Normal; Velamentøst feste; Marginalt feste; Karanomalier; Omslyng rundt hals; Annet omslyng; Ekte knute; Navlesnorlengde:

Fostervann: Normal; Polyhydramnion; Oligohydramnion; Misfarget; Stinkende, infisert; Blodtiblandet

Komplikasjoner hos mor etter fødsel: Intet spesielt; Feber > 38.5°; Trombose; Eklampsi post partum; Mor overflyttet; Mor intensivbeh.; Sepsis; Annet, spesifiser

D – Om barnet

Fødselsdato | Klokken

Pluralitet: Enkeltfødsel; Flerfødsel. For flerfødsel: Nr.; Av totalt

Kjønn: Gutt; Pike; Ved tvil spesifiser i «D»; For dødfødte: Usikkert kjønn

Barnets vekt: | Hodeomkrets: | Total lengde: | Eventuelt sete-issmål

Apgar score: 1 min; 5 min

Barnet var: Levendefødt; Dødfødt/sp.abort. Oppgi dødsårsak i «D»

For dødfødte: Død før fødsel; Død under fødselen; Ukjent dødstidspunkt

For dødfødte, oppgi også: Død før innkomst; Død etter innkomst

Levendefødt, død innen 24 timer: Livet varte: Timer. Min.

Død senere (dato): | Klokken

Overfl. barneavd.: Nei; Ja. Dato:

Overfl. til

Indikasjon for overflytting: Respirasjonsproblem; Prematur; Medfødte misd.; Perinatale infeksjoner; Annet, spesifiser

Neonatale diagn.: (Fylles ut av lege/pediater) Hypoglyk. (< 2 mmol/l); Medf. anemi (Hb < 13.5 g/dl); Hofteleddsdyspl. beh. m/pute; Transit. tachypnoe; Resp. distress syndr.; Aspirasjonssyndrom; Intrakraniell blødning; Cerebral irritasjon; Cerebral depresjon; Abstinens; Neonatale kramper; Konjunktivitt beh.; Navle-/hudinf. beh.; Perinat. inf. bakterielle; Perinat. inf. andre; Fract. claviculae; Annen fraktur; Facialisparese; Plexusskade; Behandlingskoder: Systemisk antibiotika; Respiratorbeh.; CPAP beh.; Icterus behandlet: Lysbehandlet; Utskifting; Årsak: ABO uforlik.; Rh immunisering; Fysiologisk; Annen årsak

Intet spesielt

Tegn til medfødte misdannelser: Nei; Ja. Spesifikasjon av skader, neonatale diagnoser og medfødte misdannelser – utfylles av lege: D

Kryss av hvis skjema er oppfølgingsskjema

Jordmor v/fødsel:

Jordmor v/utskriving:

Lege:

Lege barsel/barneavd:

Utskrivningsdato Mor: Barn:

Protokollnr.:

Nr. 18.423 Sem & Stenersen Protom AS, Oslo 1/99

IK-1002

Papers I-III

I

Received: 7 April 2022 | Revised: 30 August 2022 | Accepted: 13 September 2022

DOI: 10.1111/ppe.12928

ORIGINAL ARTICLE

Paediatric and Perinatal Epidemiology WILEY

Long-term cardiovascular mortality in women with twin pregnancies by lifetime reproductive history

Prativa Basnet[1] | Rolv Skjærven[1,2] | Linn Marie Sørbye[1,3] | Nils-Halvdan Morken[1,4,5] | Kari Klungsøyr[1,6] | Aditi Singh[1] | Janne Mannseth[1] | Quaker E. Harmon[7] | Liv Grimstvedt Kvalvik[1]

[1]Department of Global Public Health and Primary Care, University of Bergen, Bergen, Norway

[2]Centre for Fertility and Health, Norwegian Institute of Public Health, Oslo, Norway

[3]Norwegian Research Centre for Women's Health, Oslo University Hospital, Rikshospitalet, Oslo, Norway

[4]Department of Clinical Science, University of Bergen, Bergen, Norway

[5]Department of Obstetrics and Gynecology, Haukeland University Hospital, Bergen, Norway

[6]Division for Mental and Physical Health, Norwegian Institute of Public Health, Bergen, Norway

[7]Epidemiology Branch, National Institute of Environmental Health Sciences, Durham, North Carolina, USA

Correspondence
Prativa Basnet, Department of Global Public Health and Primary Care, University of Bergen, Bergen, Norway.
Email: prativa.basnet@uib.no

Funding information
H2020 European Research Council, Grant/Award Number: 833076; The Intramural Research Program of the NIH National Institute of Environmental Health Sciences; Norwegian Research Centre for Women's Health at Oslo University Hospital

A commentary on this article appears on pages 28-30

Abstract

Background: Women with one lifetime singleton pregnancy have increased risk of cardiovascular disease (CVD) mortality compared with women who continue reproduction particularly if the pregnancy had complications. Women with twins have higher risk of pregnancy complications, but CVD mortality risk in women with twin pregnancies has not been fully described.

Objectives: We estimated risk of long-term CVD mortality in women with naturally conceived twins compared to women with singleton pregnancies, accounting for lifetime number of pregnancies and pregnancy complications.

Methods: Using linked data from the Medical Birth Registry of Norway and the Norwegian Cause of Death Registry, we identified 974,892 women with first pregnancy registered between 1967 and 2013, followed to 2020. Adjusted hazard ratios (aHR) with 95% confidence intervals (CI) for maternal CVD mortality were estimated by Cox regression for various reproductive history (exposure categories): (1) Only one twin pregnancy, (2) Only one singleton pregnancy, (3) Only two singleton pregnancies, (4) A first twin pregnancy and continued reproduction, (5) A first singleton pregnancy and twins in later reproduction and (6) Three singleton pregnancies (the referent group). Exposure categories were also stratified by pregnancy complications (pre-eclampsia, preterm delivery or perinatal loss).

Results: Women with one lifetime pregnancy, twin or singleton, had increased risk of CVD mortality (adjusted hazard [HR] 1.72, 95% confidence interval [CI] 1.21, 2.43 and aHR 1.92, 95% CI 1.78, 2.07, respectively), compared with the referent of three singleton pregnancies. The hazard ratios for CVD mortality among women with one lifetime pregnancy with any complication were 2.36 (95% CI 1.49, 3.71) and 3.56 (95% CI 3.12, 4.06) for twins and singletons, respectively.

Conclusions: Women with only one pregnancy, twin or singleton, had increased long-term CVD mortality, however highest in women with singletons. In addition, twin mothers who continued reproduction had similar CVD mortality compared to women with three singleton pregnancies.

KEYWORDS
CVD mortality, maternal survival, Norway, population-based study, twin pregnancy

1 | BACKGROUND

Cardiovascular disease (CVD) mortality risk is increased among women with one lifetime singleton births compared to women who continue reproduction.[1] Pregnancy complications including pre-eclampsia, preterm delivery and perinatal loss are also associated with elevated risk of CVD morbidity and mortality in singleton pregnancies.[2–8] Women with twin pregnancies have an increased risk of pregnancy complications[9–11] and may potentially stop reproduction after a first pregnancy with twins because two children are a common desired family size.[12,13] Twin pregnancies also have a greater biological demand on the mothers, which might impact their later health. However, the influence and interaction between lifetime number of pregnancies and pregnancy complications on maternal long-term CVD mortality have not been fully explored for twin pregnancies.

Due to the difficulty in linking pregnancies across a woman's reproductive lifetime, many previous studies have focused on associations between complications in the first pregnancy and later maternal health. However, analyses restricted to outcomes in first pregnancies do not account for possible heterogeneity in risk by the number of children.[14] To the best of our knowledge, no previous research has investigated long-term CVD mortality in women considering both plurality and complications in successive pregnancies across womens' reproductive period.

In Norway, a unique national identification number, provided to all residents, enables linkage of all pregnancies to a woman. With data on pregnancies since 1967, the Medical Birth Registry of Norway (MBRN) provides an opportunity to analyse women's complete reproductive history. Further linkage with the Norwegian Cause of Death Registry, allows an evaluation of the association between reproductive history and maternal cause-specific mortality. In linked pregnancy data (with the mother as the observational unit), we aimed to estimate long-term CVD mortality in women with twins by lifetime number of pregnancies compared to women with singleton pregnancies. We also assessed associations with long-term mortality by presence of pre-eclampsia, preterm delivery, perinatal loss as pregnancy complications are more common in twin pregnancies.[10,11] Findings may identify high-risk women for appropriate follow-up with interventions to lower their long-term risk of CVD related deaths.

Synopsis

Study question

Do women with twin pregnancies have increased risk of long-term cardiovascular disease (CVD) mortality?

What's already known

CVD mortality is increased among women with one lifetime singleton birth. Several complications in singleton pregnancies are associated with increased CVD mortality. Women with twin pregnancies have increased risk of pregnancy complications, such as pre-eclampsia, preterm delivery and perinatal loss, compared to singleton pregnancies.

What this study adds

In a population-based cohort study, women with only one pregnancy, twin or singleton, had increased risk of Atherosclerotic cardiovascular disease (ASCVD) mortality, compared to women with three singleton pregnancies. The increase was highest in women with singletons. Women with a first twin pregnancy and continued reproduction had similar ASCVD mortality compared to women with three singleton pregnancies.

2 | METHODS

2.1 | Data sources

The MBRN is a population-based registry, established in 1967, primarily to monitor birth defects and other maternal and perinatal health problems and to provide data for epidemiological research on causes and consequences of perinatal health problems.[15] The MBRN is based on mandatory notification of all live births, stillbirths and pregnancy losses from 16 weeks of gestation. The registry records prospectively collected information on women's health before and during pregnancy, the delivery and the immediate postpartum period, including demographic information, complications and interventions during delivery and infant outcomes. The attending midwife and obstetrician record data using a standardised notification form, either as free text or, since 1999, by predefined variables or check boxes in addition to free text. Since 2006, a gradual transition to electronic birth notification took place (complete in 2014), and the notifications are now based on pre-specified extractions from the medical records at the delivery units. Every live-born infant in Norway, as well as all immigrants who become Norwegian inhabitants, are provided with a unique national identification number by the National Population Register. The MBRN is routinely matched with the National Population Register and receives all national identification numbers and all dates of death and emigration through this linkage. The unique identification number was used to link all pregnancies to their mother in maternal pregnancy files, and linkage

with the Cause of Death Registry provided information on mother's causes of death. The Cause of Death Registry, established in 1954, contains information on the underlying and contributing causes of death, registered using ICD codes. The form is filled out by a medical doctor and is quality-assured using other national registries. Information on highest attained level of education by 2020 was obtained from the National Education Database at Statistics Norway.

We restricted our study population to women with their first pregnancy registered in the MBRN during 1967–2013 (Figure 1). This provided enough follow-up time for women to have a second pregnancy by 2020 as 95% of Norwegian women with two or more pregnancies have their second pregnancy within 7 years.[1] All women were followed until 2020 for deaths before 70 years of age.

There have been changes in the data quality of MBRN during the 50 years since its establishment, mainly due to the change of the notification form in 1999 from being based solely on free text to adding check boxes. These changes are unlikely to impact the reporting of singleton or multiple gestations over time. Reporting of some pregnancy complications including mild pre-eclampsia and late spontaneous abortions have improved over time. Registry-based research depends on valid information, and over the years, several MBRN variables have been validated with mostly acceptable results.[16] Pre-eclampsia was for example found to have a positive predictive value of 88.3% (births 1967–2002) in one study, using the diagnostic criteria at that time.[17] In a study of births 1999–2010, the positive predictive value of pre-eclampsia was 83.9%.[18]

2.2 | Lifetime successive pregnancies approach

By linking data on a woman's successive pregnancies through her lifetime to later health outcomes allows a more comprehensive study of possible associations between reproductive events and long-term health.[19] In this study, we linked consecutive pregnancies (as registered in the MBRN) to the women, to compare women with twin and singleton pregnancies accounting for their lifetime number of pregnancies.

2.3 | Exposure variables

Lifetime reproductive history, ascertained at the end of reproduction or 2020, consisting of six mutually exclusive categories were used as exposure: (1) Women with only one twin pregnancy, (2) Women with only one singleton pregnancy, (3) Women with only two singleton pregnancies, (4) Women with a first twin pregnancy and continued reproduction, (5) Women with a first singleton pregnancy and twins in later reproduction and (6) Women with three singleton pregnancies as the referent group (Figure 1). Given that two pregnancies are a common pregnancy pattern among singletons, we chose three pregnancies as the referent so that three children (two pregnancies for those that start with twins or three pregnancies for those who start with a singleton) were a possible stopping point for both twin and singleton first births.

Complications in each pregnancy were obtained from the MBRN. A diagnosis of pre-eclampsia is based on the definition provided by the Norwegian Gynaecological Association and aligned with the criteria recommended by the American College of Obstetricians and Gynaecologists (see further definition in Appendix S1). Preterm delivery was defined as births before 37 completed weeks of gestation. Perinatal loss included losses between 16 and 22 weeks, stillbirths and neonatal deaths during the first week after birth (one or both infants in case of twins). The six categories of reproductive history were further stratified by occurrence of pregnancy complications: pre-eclampsia, preterm delivery, perinatal loss in any pregnancy. This resulted in 12 exposure categories with women who had three singletons and no complication in any pregnancy as the referent.

2.4 | Outcome

The main outcome variable was Atherosclerotic Cardiovascular Disease (ASCVD) mortality defined as death from ischaemic heart disease or cerebrovascular disease or peripheral arterial disease in women before 70 years of age. We used codes from the International Statistical Classification of Diseases and Related Health Problems (ICD) to define our outcome as shown in Appendix S1. In addition, results using more expansive definition of CVD are presented in Appendix S1.

2.5 | Covariates

Estimates were adjusted for calendar year of first delivery, mother's age at first birth, maternal education: <9 years, 10–12 years and ≥13 years (reference) and chronic medical conditions available in the MBRN (Type 1 or Type 2 diabetes mellitus, hypertension, kidney disease and rheumatoid arthritis).

2.6 | Exclusions

Pregnancies conceived by assisted reproductive technologies (ART) were excluded from the main analyses as infertility/subfertility could be associated with underlying factors predisposing women for cardiovascular disease.[20,21] In addition, information on ART was not available for the whole study period in the MBRN. We also excluded women with any higher order multi-foetal pregnancies (≥triplets), as these pregnancies are rare and associated with specific obstetric challenges. Further, we excluded women with four singleton pregnancies (n = 63,756).

2.7 | Statistical methods

All data were analysed using STATA version 17. Descriptive statistics were presented as number and percentages. To estimate hazard ratios with 95% confidence intervals (CI) for ASCVD mortality by

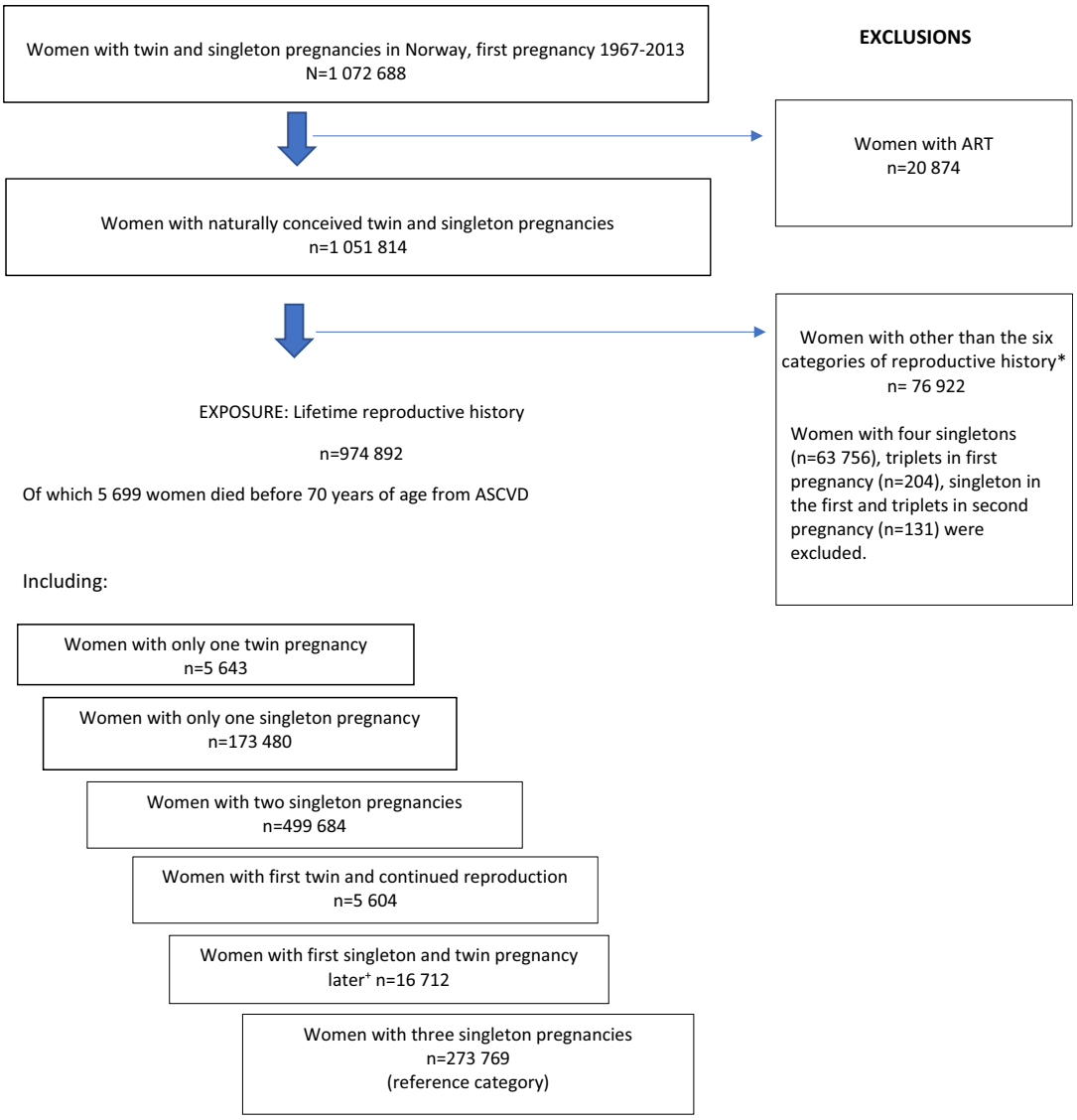


FIGURE 1 Flowchart of study population. ART, assisted reproductive technology; ASCVD, atherosclerotic cardiovascular disease. *Other reproductive history than the six categories presented above were not included in the analysis. For example, mothers with four singletons, triplets in first pregnancy or later etc. were excluded. +Women with twins either in second, third or fourth pregnancy.

the six categories of reproductive history in women, we used Cox proportional hazard regression models with women's age as the underlying time variable. We adjusted for age at first birth, year of first birth, education and chronic medical conditions as potential confounders. Women were considered at risk of death from the age at their last pregnancy. Women were censored at death, age 70 or when follow-up ended in 2020, whichever came first.

2.8 | Missing data

In our study population, missing data on the covariates were rare, we used complete case analysis. Less than 1% of the maternal education and 4.2% of the women's gestational ages were missing. Information on maternal age and year of birth of first child were complete.

2.9 | Sensitivity analysis

To evaluate the robustness of our findings, we conducted multiple sensitivity analyses. We assessed the risk of ASCVD mortality in women who had completed their reproduction (age 40). For this analysis, women who were not 40 years of age by the end of follow-up or women who died before 40 years of age were excluded. We also repeated the main analysis after including women who conceived using ART. We additionally performed the main analysis (ASCVD mortality in the six exposure groups) restricted to gestational age above 22 weeks to evaluate selection bias due to incomplete recording of pregnancies ending before 22 weeks. Finally, we evaluated whether associations by reproductive history changed when the outcome variable was extended to include hypertensive heart disease and cardiomyopathy.

3 | RESULTS

Maternal and pregnancy characteristics of 974,892 women by plurality of first pregnancy (singleton or twin) are shown in Table 1. In total 1.2% of first pregnancies were twins. Women with a first twin pregnancy were older, had higher frequency of university degrees, shorter gestations and more often delivered preterm (48% vs. 6%) compared to women with a first singleton pregnancy. Also, pre-eclampsia (14% vs. 4%) and perinatal loss (6% vs. 1%) were more frequent in women with a first twin pregnancy. In total 42,182 women died before the age of 70 years during 1967–2020, of which 5699 (13.5%) died of cardiovascular causes. ASCVD deaths among women with twins in any pregnancy accounted for 2.8% of all ASCVD deaths.

Table 2 shows the distribution of deaths across the six categories of reproductive history for ASCVD using women with three singleton pregnancies as the referent group. Women with only one lifetime pregnancy had increased risk of ASCVD death, both if their only pregnancy was with twins (adjusted HR (aHR) 1.72, 95% CI 1.21, 2.43) or a singleton (aHR 1.92, 95% CI 1.78, 2.07). The point estimate was slightly higher for women with one lifetime singleton pregnancy than women with one lifetime twin pregnancy. No increased risk was found for women with a first twin pregnancy and continued reproduction (aHR 0.76, 95% CI 0.48, 1.19). Women with a first singleton pregnancy and twins in later reproduction, however, had an increased risk of ASCVD death (aHR: 1.49, 95% CI 1.22, 1.81). A small increase was also found for women with two singletons (aHR 1.08, 95% CI 1.01, 1.15) compared to the referent.

Risk of long-term ASCVD mortality by one or more pregnancy complications (pre-eclampsia, preterm delivery, perinatal loss) is outlined in Table 3. Women with only one lifetime pregnancy had substantially increased risk of dying from ASCVD in the presence of one or more complications. This was true both for women with only one twin (aHR 2.36, 95% CI 1.49, 3.71) or women with only one singleton (aHR 3.56, 95% CI 3.12, 4.06). Women with one lifetime pregnancy without complications also had an elevated risk of ASCVD death if the pregnancy was a singleton (aHR 1.99, 95% CI 1.82, 2.17). The relative risk of dying from ASCVD for women with one lifetime twin pregnancy without complications was aHR 1.57 (95% CI 0.92, 2.66).

TABLE 1 Maternal and pregnancy characteristics of 974,892 women's first pregnancy registered in the Medical Birth Registry of Norway, 1967–2013

	Women with first twin pregnancy	Women with first singleton pregnancy
	N (%)	*N* (%)
Total	11,247	963,645
Maternal age at first birth		
≤19	768 (6.8)	108,321 (11.2)
20–24	3364 (29.9)	359,731 (37.3)
25–29	4006 (35.6)	320,707 (33.3)
30–34	2187 (19.5)	133,135 (13.8)
35–39	761 (6.8)	35,725 (3.7)
40–44	137 (1.2)	5754 (0.6)
≥45	24 (0.2)	272 (0.03)
Maternal education		
Primary school	1899 (16.9)	182,155 (18.9)
High school	4107 (36.5)	377,148 (39.2)
University	5150 (45.8)	395,289 (41.0)
Missing education	91 (0.8)	9053 (0.9)
Gestational age		
<28	578 (5.1)	4877 (0.5)
28–31	768 (6.8)	6353 (0.6)
32–33	987 (8.9)	7458 (0.8)
34–36	3059 (27.2)	38,017 (3.9)
37–38	3101 (27.6)	119,224 (12.4)
39+ weeks	2346 (20.8)	747,552 (77.6)
Missing	408 (3.6)	40,164 (4.2)
Perinatal loss	724 (6.4)	9758 (1.0)
Pre-eclampsia	1588 (14.1)	41,725 (4.3)
Preterm delivery	5392 (47.9)	56,705 (5.9)
Chronic conditions[a]	340 (3.0)	22,488 (2.3)

[a]Includes chronic medical conditions (diabetes, hypertension, kidney disease and rheumatoid arthritis).

When restricting analyses to deaths between 40 and 69 years of age and when including women with IVF, results were essentially the same (Tables S1 and S2). In addition, the results were substantially the same when restricted to gestational age above 22 weeks (Table S3). Additionally, using an extended definition of CVD also yielded similar results (Table S4).

4 | COMMENT

4.1 | Principal findings

Women with only one lifetime twin pregnancy and women with only one lifetime singleton pregnancy had similarly increased risk of ASCVD mortality compared to women with three singleton pregnancies. Although twin pregnancies are more likely to have pregnancy

TABLE 2 Hazard ratios (HRs) with 95% confidence intervals (CI) for atherosclerotic cardiovascular disease (ASCVD) mortality before 70 years of age by various categories of reproductive history in 974,892 women, first pregnancy 1967–2013 and follow-up until 2020. Medical Birth Registry of Norway and Cause of Death Registry

		ASCVD mortality				
Womens' reproductive history	Total women	No. of deaths	Deaths per 1000	Person-years	Unadjusted HR (95% CI)	aHR[a] (95% CI)
Only one twin pregnancy	5643	34	6.0	142,050	2.02 (1.44, 2.84)	1.72 (1.21, 2.43)
Only one singleton pregnancy	173,480	1611	9.3	4,841,214	2.38 (2.22, 2.57)	1.92 (1.78, 2.07)
Two singleton pregnancies	499,684	2607	5.2	12,792,455	1.16 (1.09, 1.24)	1.08 (1.01, 1.15)
First twin pregnancy and continued reproduction	5604	19	3.4	129,221	0.81 (0.51, 1.27)	0.76 (0.48, 1.19)
First singleton pregnancy and twins in later[b] reproduction	16,712	109	6.5	389,105	1.52 (1.25, 1.85)	1.49 (1.22, 1.81)
Three singleton pregnancies	273,769	1319	4.8	6,371,233	1.00 (Reference)	1.00 (Reference)

Abbreviations: aHR, adjusted hazard ratio; CI, confidence interval; HR, hazard ratio.

[a]Estimates were obtained using Cox regression and adjusted for year of first birth, maternal age at first birth, maternal education and chronic medical conditions (diabetes, hypertension, kidney disease and rheumatoid arthritis).

[b]Women with twins either in second, third or fourth pregnancy.

complications, these complications do not appear to further elevate the risk of ASCVD mortality once total parity is accounted for.

4.2 | Strengths of the study

A major strength of this study was the large population-based longitudinal dataset comprising of successive pregnancies with long follow-up and linked data from the Cause of Death Registry. This rich data source provided unique opportunities to study twin and singleton pregnancies accounting for pregnancy complications and evaluate long-term maternal ASCVD mortality, using lifetime successive pregnancies approach.

4.3 | Limitations of the data

Limitations included lack of information on several potential confounders, such as smoking and body mass index (BMI), that were not registered in the MBRN for most of the study period.

4.4 | Interpretation

Our findings are consistent with previous work in singletons showing that women with one lifetime pregnancy have increased long-term CVD mortality compared to women with more than one pregnancy[1]; however, the underlying mechanisms are uncertain. Several social and biological factors may contribute to the increased CVD mortality in women who stop their reproduction after one pregnancy. Previous research suggests that pregnancy influences endothelial function,[22-25] which may support the hypothesis that repeated pregnancies reduce the risk of CVD mortality.[26] On the other hand, women who stop reproducing may be a selected group of women with pre-existing medical conditions[27] or who suffered severe complications in pregnancy[1,20] or maybe due to changed relationship status. The underlying mechanism may also be related to subfertility issues,[28] which has been shown to be associated with later CVD mortality.[20] We were able to account for some important chronic medical conditions available in the MBRN.

We also examined pregnancy complications in women; pre-eclampsia, preterm delivery, perinatal loss, which are consistently reported to be associated with increased long-term CVD in women.[4–6,28–32] Most studies have focused on singletons and only analysed pregnancy complications in the first pregnancy without considering successive pregnancies and without specific evaluation of twin pregnancies. Twin pregnancies have an increased risk of pre-eclampsia.[33–35] In our study, we found that women with first twin pregnancies had more than three times higher risk of pre-eclampsia than women with singleton first pregnancies (14.1% vs. 4.3%). In our data, preterm delivery was also more common in first twin pregnancies compared to singletons (47.9% vs. 5.9%), as was perinatal loss (6.4% vs. 1.0%).

Although we found that pregnancy complications were more frequent in twin pregnancies, the complications may develop for different reasons[35] and may be viewed as less 'pathological'. Among those with only one pregnancy with complications, the increased relative risk of ASCVD mortality was higher for the

TABLE 3 Hazard ratios (HRs) with 95% confidence intervals (CI) for atherosclerotic cardiovascular disease (ASCVD) mortality before 70 years of age by various categories of reproductive history with and without pregnancy complications (pre-eclampsia, preterm delivery, perinatal loss) at least once in 974,892 women, first pregnancy 1967–2013 and follow-up until 2020. Medical Birth Registry of Norway and Cause of Death Registry

		ASCVD mortality			
Womens' reproductive history	Total women	No. of deaths	Deaths per 1000	Unadjusted HR (95% CI)	aHR[a] (95% CI)
Only one twin pregnancy with one or more complications	3116	19	6.1	2.73 (1.74, 4.31)	2.36 (1.49, 3.71)
Only one twin pregnancy without complication	2527	15	5.9	1.92 (1.15, 3.20)	1.57 (0.92, 2.66)
Only one singleton pregnancy with one or more complications	19,941	320	16.1	5.05 (4.44, 5.74)	3.56 (3.12, 4.06)
Only one singleton pregnancy without complication	153,539	1291	8.4	2.45 (2.25, 2.66)	1.99 (1.82, 2.17)
Two singleton pregnancies with one or more complications	66,143	523	7.9	2.13 (1.92, 2.38)	1.85 (1.66, 2.06)
Two singleton pregnancies without complication	433,541	2084	4.8	1.21 (1.12, 1.30)	1.12 (1.03, 1.21)
First twin pregnancy and continued reproduction with one or more complications	3503	13	3.7	1.04 (0.60, 1.80)	0.95 (0.55, 1.64)
First twin pregnancy and continued reproduction without complication	2101	6	2.9	0.74 (0.33, 1.65)	0.70 (0.31, 1.56)
First singleton pregnancy and twins later[b] with one or more complications	7969	52	6.5	1.87 (1.42, 2.48)	1.78 (1.35, 2.35)
First singleton pregnancy and twins later[b] without complication	8743	57	6.5	1.62 (1.24, 2.12)	1.58 (1.21, 2.07)
Three singleton pregnancies with one or more complications	51,350	384	7.5	1.73 (1.54, 1.95)	1.60 (1.42, 1.80)
Three singleton pregnancies without complication	222,419	935	4.2	1.00 (Reference)	1.00 (Reference)

Abbreviations: aHR, adjusted hazard ratio; CI, confidence interval; HR, hazard ratio.

[a]Estimates were obtained using Cox regression and adjusted for year of first birth, maternal age at first birth, maternal education and chronic medical conditions (diabetes, hypertension, kidney disease and rheumatoid arthritis).

[b]Women with twins either in second, third or fourth pregnancy.

women with a singleton aHR 3.56 (95% CI 3.12, 4.06) rather than a twin pregnancy aHR 2.36 (95% CI 1.49, 3.71) compared to the referent of three singletons with complications. This may support the hypothesis that pregnancy complications in twin pregnancies have important differences. Our finding is similar to an Israelian study that showed that even though women with twin pregnancy had more complications, twin pregnancy was not associated with increased risk of CVD hospitalisation.[34] Consistent to our finding, another recent study reported increased risk of CVD mortality among twin pregnancies complicated by hypertensive disorder compared to uncomplicated twin pregnancies.[36] However, a study from Sweden showed that women who had a multi-foetal pregnancy did not have increased CVD risk even if pre-eclampsia occurred, compared to women without pre-eclampsia in singleton pregnancy.[37] The Swedish study analysed women's first pregnancy only, and in contrast, our study incorporated both pregnancy complications and the number of pregnancies. We could, therefore, separate those with only one lifetime pregnancy, which was important for maternal long-term mortality.

While underlying CVD risk factors might predispose to both pre-eclampsia and later maternal CVD in singleton pregnancies, causes of pre-eclampsia in twin pregnancies may be less linked to long-term CVD.[37] A previous study that examined the association between complications in twin pregnancy and later life CVD, suggested different pathophysiological processes in twin and singleton pregnancies.[34] Likewise, studies have highlighted that there are differences in maternal adaptation during singleton and twin pregnancies; however, they have not found differences in indicators of maternal cardiovascular functions, such as blood pressure in later life.[38] We could not find any studies investigating long-term CVD mortality in mothers with twins who experienced preterm delivery or perinatal loss. As with pre-eclampsia, preterm delivery and perinatal loss, may have different association with maternal mortality in twin and singleton pregnancies.

The higher ASCVD mortality in women with one lifetime pregnancy could have more than one explanation. For women who start with one singleton, stopping reproduction may be due to underlying health concerns, severe pregnancy complications or subfertility,

which prevents further conception. Women with twins may stop reproduction for all the same reasons, however they may also stop due to having achieved their desired family size of two children. The elevated risk of ASCVD mortality in both groups, however, slightly higher for women with one lifetime singleton pregnancy than one lifetime twin pregnancy, suggests multiple pathways through which reproductive patterns can influence later health.

5 | CONCLUSIONS

Women with one pregnancy, twin or singleton, had increased risk of ASCVD mortality, compared to the referent of three singleton pregnancies. However, the relative increase in ASCVD mortality was slightly lower if this was a twin pregnancy. Women with a first twin pregnancy and continued reproduction had similar ASCVD mortality compared to women with three singleton pregnancies. Our findings do not suggest a greater long-term burden on ASCVD mortality in women with twin pregnancies. The heterogeneity in risk found between women with one lifetime pregnancy and women who continue reproduction should be explored in future research. Women who stop reproduction after their first pregnancy, twin or singleton, may benefit from timely follow-up and intervention to mitigate future risk of early deaths.

AUTHOR CONTRIBUTIONS

PB, RS and LGK conceived and designed the study. RS obtained access to the data. PB conducted the data analysis and drafted the initial version of the manuscript. RS is guarantor for data quality. All authors provided important insight during the data analysis and critically revised the manuscript.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Allen J. Wilcox at the National Institute of Environmental Health Sciences (NIEHS) for providing useful comments on earlier versions of this manuscript.

CONFLICT OF INTEREST

None declared.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from Norwegian Institute of Public Health. Restrictions apply to the availability of these data, which were used under license for this study. Data are available from https://www.fhi.no/en/hn/health-registries/medical-birth-registry-of-norway/medical-birth-registry-of-norway/ with the permission of Norwegian Institute of Public Health.

ORCID

Prativa Basnet https://orcid.org/0000-0001-9022-9957
Rolv Skjærven https://orcid.org/0000-0002-2983-1233
Linn Marie Sørbye https://orcid.org/0000-0002-3726-5198
Nils-Halvdan Morken https://orcid.org/0000-0002-8256-0778
Kari Klungsøyr https://orcid.org/0000-0003-2482-1690
Aditi Singh https://orcid.org/0000-0002-2302-1723
Janne Mannseth https://orcid.org/0000-0003-1386-5073
Quaker E. Harmon https://orcid.org/0000-0002-5866-848X
Liv Grimstvedt Kvalvik https://orcid.org/0000-0001-6520-9057

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Basnet P, Skjærven R, Sørbye LM, et al. Long-term cardiovascular mortality in women with twin pregnancies by lifetime reproductive history. *Paediatr Perinat Epidemiol*. 2023;37:19-27. doi: 10.1111/ppe.12928

Long-term Cardiovascular Mortality in Mothers with Twin Pregnancies by Lifetime Reproductive History

Prativa Basnet[1], Rolv Skjærven[1,2], Linn Marie Sørbye[1,3], Nils-Halvdan Morken[1,4,5], Kari Klungsøyr[1,6], Aditi Singh[1], Janne Mannseth[1], Quaker E Harmon[7], Liv Grimstvedt Kvalvik[1]

1) Department of Global Public Health and Primary Care, University of Bergen, Norway
2) Centre for Fertility and Health, Norwegian Institute of Public Health, Oslo, Norway
3) Norwegian Research Centre for Women's Health, Oslo University Hospital, Rikshospitalet, Oslo, Norway
4) Department of Clinical Science, University of Bergen, Norway
5) Department of Obstetrics and Gynecology, Haukeland University Hospital, Bergen, Norway
6) Division for Mental and Physical Health, Norwegian Institute of Public Health, Bergen, Norway
7) Epidemiology Branch, National Institute of Environmental Health Sciences, Durham, North Carolina, US

Definition of preeclampsia

The definition of preeclampsia has changed during the time span of our study and is today defined as two measurements of increased blood pressure after 20 weeks' gestation (defined as blood pressure ≥140 mm Hg; or diastolic blood pressure of ≥90 mm Hg), and proteinuria (≥0.3 g in 24 h urine specimen, or >1 point increase on urine dipstick).[1, 2] A validation of preeclampsia registration in the MBRN covering the years 1967-2005 showed that registered cases matched well to the medical records in the selected hospitals.[3]

Definition of outcomes:

The main outcome variable was Atherosclerotic cardiovascular disease (ASCVD) mortality defined as death from ischaemic heart disease, cerebrovascular disease or peripheral arterial disease in women before 70 years of age. The following codes from the International Statistical Classification of Diseases and Related Health Problems (ICD) 8th, 9th and 10th revisions were used:

- Ischaemic heart disease: I20-I25 (ICD-10), 410-414 (ICD 8 and 9)
- Cerebrovascular disease: I60-I69 (ICD-10), 430-438 (ICD 8 and 9)
- Peripheral arterial disease: I70-I72, I74 (ICD-10), 440-444 (ICD 8 and 9).

In addition, we also present results using more extended definition of CVD in the supplementary information. This extended CVD definition included ASCVD, hypertensive heart disease and cardiomyopathy. The following ICD codes were used for extended CVD definition in addition to ASCVD codes:

- Hypertensive heart disease: I10-I15 (ICD-10), 400-405 (ICD 8 and 9).
- Cardiomyopathy: I42 (ICD-10), 425 (ICD 8 and 9).

Table S1 Hazard ratios (HRs) with 95% confidence intervals (CIs) for maternal atherosclerotic cardiovascular disease (ASCVD) mortality between 40-69 years of age by various reproductive history in 969 940 women, first pregnancy 1967 to 2013, and follow-up until 2020. Medical Birth Registry of Norway and Cause of Death Registry.

		ASCVD Mortality			
Womens' reproductive history	**Total women**	**No. of deaths**	**Deaths per 1000**	**Unadjusted HR (95% CI)**	**aHR[a] (95% CI)**
Only one twin pregnancy	5 617	31	5.5	2.19 (1.53, 3.13)	1.71 (1.19, 2.46)
Only one singleton pregnancy	171 424	1420	8.3	2.42 (2.25, 2.62)	1.85 (1.70, 2.00)
Two singleton pregnancies	497 631	2395	4.8	1.17 (1.09, 1.25)	1.06 (0.99, 1.14)
First twin pregnancy and continued reproduction	5 591	16	2.9	0.74 (0.45, 1.21)	0.68 (0.41, 1.11)
First singleton pregnancy and twins in later reproduction[b]	16 649	101	6.1	1.52 (1.24, 1.87)	1.47 (1.20, 1.80)
Three singleton pregnancies	273 028	1236	4.5	Reference	Reference

[a]Estimates were obtained using Cox-regression and adjusted for year of first birth, maternal age at first birth, maternal education and chronic medical conditions (diabetes, hypertension, kidney disease and rheumatoid arthritis).
Abbreviations: HR, Hazard ratio; aHR, Adjusted hazard ratio; CI, Confidence interval.
[b]Women with twins either in second, third or fourth pregnancy.

Table S2 Hazard ratios (HRs) with 95% confidence intervals (CIs) for maternal atherosclerotic cardiovascular disease (ASCVD) mortality before 70 years of age by various reproductive history in 995 045 women without exclusion for Assisted Reproductive Technology (ART), first pregnancy 1967 to 2013, and follow-up until 2020. Medical Birth Registry of Norway and Cause of Death Registry.

		ASCVD mortality			
Womens' reproductive history	**Total women**	**No. of deaths**	**Deaths per 1000**	**Unadjusted HR (95% CI)**	**aHR[a] (95% CI)**
Only one twin pregnancy	7 641	35	4.6	1.87 (1.34, 2.62)	1.62 (1.15, 2.28)
Only one singleton pregnancy	177 954	1 619	9.1	2.39 (2.22, 2.57)	1.93 (1.78, 2.08)
Two singleton pregnancies	508 238	2 612	5.1	1.16 (1.09, 1.24)	1.08 (1.01, 1.15)
First twin pregnancy and continued reproduction	6 381	20	3.1	0.83 (0.53, 1.29)	0.78 (0.50, 1.22)
First singleton pregnancy and twins in later reproduction[b]	18 397	110	6.0	1.48 (1.22, 1.80)	1.46 (1.21, 1.78)
Three singleton pregnancies	276 434	1321	4.8	Reference	Reference

[a]Estimates were obtained using Cox-regression and adjusted for year of first birth, maternal age at first birth, maternal education and chronic medical conditions (diabetes, hypertension, kidney disease and rheumatoid arthritis).
Abbreviations: HR, Hazard ratio; aHR, Adjusted hazard ratio; CI, Confidence interval.
[b]Women with twins either in second, third or fourth pregnancy.

Table S3 Hazard ratios (HRs) with 95% confidence intervals (CIs) for maternal atherosclerotic cardiovascular disease (ASCVD) before 70 years of age by various reproductive history in 891 931 women restricted to pregnancies ending after 22 weeks, first pregnancy 1967 to 2013, and follow-up until 2020. Medical Birth Registry of Norway and Cause of Death Registry.

		ASCVD mortality			
Womens' reproductive history	**Total women**	**No. of deaths**	**Deaths per 1000**	**Unadjusted HR (95% CI)**	**aHR[a] (95% CI)**
Only one twin pregnancy	5 438	32	5.0	2.06 (1.45, 2.93)	1.74(1.21, 2.49)
Only one singleton pregnancy	164 931	1 490	9.0	2.42 (2.24, 2.62)	1.94 (1.79, 2.11)
Two singleton pregnancies	462 736	2 343	5.1	1.18 (1.10, 1.27)	1.10 (1.02, 1.18)
First twin pregnancy and continued reproduction	5 260	19	3.6	0.89 (0.57, 1.40)	0.83 (0.53, 1.31)
First singleton pregnancy and twins in later reproduction[b]	14 893	91	6.1	1.50 (1.21, 1.86)	1.47 (1.19, 1.82)
Three singleton pregnancies	238 673	1446	6.1	Reference	Reference

[a]Estimates were obtained using Cox-regression and adjusted for year of first birth, maternal age at first birth, maternal education and chronic medical conditions (diabetes, hypertension, kidney disease and rheumatoid arthritis).

Abbreviations: HR, Hazard ratio; aHR, Adjusted hazard ratio; CI, Confidence interval.

[b]Women with twins either in second, third or fourth pregnancy.

Table S4 Hazard ratios (HRs) with 95% confidence intervals (CIs) for extended cardiovascular disease (CVD[a]) mortality before 70 years of age by various reproductive history in 974 892 women, first pregnancy 1967 to 2013, and follow-up until 2020. Medical Birth Registry of Norway and Cause of Death Registry.

		Extended CVD mortality[a]			
Womens' reproductive history	**Total women**	**No. of deaths**	**Deaths per 1000**	**Unadjusted HR (95% CI)**	**aHR[b] (95% CI)**
Only one twin pregnancy	5 643	37	6.6	2.01 (1.45, 2.79)	1.70 (1.22, 2.37)
Only one singleton pregnancy	173 480	1773	10.2	2.40 (2.24, 2.58)	1.93 (1.79, 2.08)
Two singleton pregnancies	499 684	2862	5.7	1.16 (1.09, 1.24)	1.08 (1.01, 1.15)
First twin pregnancy and continued reproduction	5 604	20	3.6	0.78 (0.50, 1.21)	0.73 (0.47, 1.13)
First singleton pregnancy and twins in later reproduction[c]	16 712	119	7.1	1.52 (1.26, 1.83)	1.48 (1.23, 1.79)
Three singleton pregnancies	273 769	1446	5.3	Reference	Reference

[a] *includes Atherosclerotic cardiovascular disease (ASCVD), hypertensive heart disease and cardiomyopathy*

[b]Estimates were obtained using Cox-regression and adjusted for year of first birth, maternal age at first birth, maternal education and chronic medical conditions (diabetes, hypertension, kidney disease and rheumatoid arthritis).

Abbreviations: HR, Hazard ratio; aHR, Adjusted hazard ratio; CI, Confidence interval.

[c]Women with twins either in second, third or fourth pregnancy.

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Received: 9 January 2023 | Revised: 30 June 2023 | Accepted: 4 July 2023

DOI: 10.1111/aogs.14644

ORIGINAL RESEARCH ARTICLE

Birthweight of the subsequent singleton pregnancy following a first twin or singleton pregnancy

Prativa Basnet[1] | Rolv Skjærven[1,2] | Quaker E. Harmon[3] | Allen J. Wilcox[3] | Kari Klungsøyr[1,4] | Linn Marie Sørbye[5,6] | Nils-Halvdan Morken[7,8] | Liv G. Kvalvik[1]

[1]Department of Global Public Health and Primary Care, Faculty of Medicine, University of Bergen, Bergen, Norway

[2]Center for Fertility and Health, Norwegian Institute of Public Health, Oslo, Norway

[3]Epidemiology Branch, National Institute of Environmental Health Sciences, Durham, North Carolina, USA

[4]Division for Mental and Physical Health, Norwegian Institute of Public Health, Bergen, Norway

[5]Norwegian Research Center for Women's Health, Oslo University Hospital, Oslo, Norway

[6]Western Norway University of Applied Sciences, Faculty of Health and Social Sciences, Bergen, Norway

[7]Department of Clinical Science, University of Bergen, Bergen, Norway

[8]Department of Obstetrics and Gynecology, Haukeland University Hospital, Bergen, Norway

Correspondence
Prativa Basnet, Department of Global Public Health and Primary Care, Faculty of Medicine, University of Bergen, Alrek Helseklynge 17, Bergen 5020, Norway.
Email: prativa.basnet@uib.no

Funding information
H2020 European Research Council, Grant/Award Number: 833076; National Institute of Environmental Health Sciences, Grant/Award Number: ZIAES049003

Abstract

Introduction: Birthweight is an important pregnancy indicator strongly associated with infant, child, and later adult life health. Previous studies have found that second-born babies are, on average, heavier than first-born babies, indicating an independent effect of parity on birthweight. Existing data are mostly based on singleton pregnancies and do not consider higher order pregnancies. We aimed to compare birthweight in singleton pregnancies following a first twin pregnancy relative to a first singleton pregnancy.

Material and Methods: This was a prospective registry-based cohort study using maternally linked offspring with first and subsequent pregnancies registered in the Medical Birth Registry of Norway between 1967 and 2020. We studied offspring birthweights of 778 975 women, of which 4849 had twins and 774 126 had singletons in their first pregnancy. Associations between twin or singleton status of the first pregnancy and birthweight (grams) in subsequent singleton pregnancies were evaluated by linear regression adjusted for maternal age at first delivery, year of first pregnancy, maternal education, and country of birth. We used plots to visualize the distribution of birthweight in the first and subsequent pregnancies.

Results: Mean combined birthweight of first-born twins was more than 1000 g larger than mean birthweight of first-born singletons. When comparing mean birthweight of a subsequent singleton baby following first-born twins with those following first-born singletons, the adjusted difference was just 21 g (95% confidence interval 5–37 g).

Conclusions: Birthweights of the subsequent singleton baby were similar for women with a first twin or a first singleton pregnancy. Although first twin pregnancies contribute a greater combined total offspring birthweight including more extensive uterine expansion, this does not explain the general parity effect seen in birthweight. The physiological reasons for increased birthweight with parity remain to be established.

KEYWORDS
birthweight, parity effect, pregnancy, singleton pregnancy, twin pregnancy

Abbreviation: MBRN, Medical Birth Registry of Norway

1 | INTRODUCTION

Birthweight is an important pregnancy outcome strongly associated with infant, child, and later adult life health.[1,2] Previous research using both cross-sectional and longitudinal data indicates an independent effect of parity on birthweight, with subsequent singleton babies being 80–140 g larger than the first singleton.[3-6]

The reasons why birthweights of subsequent offspring are in general larger than the first are not fully understood. Possible mechanisms include functional and physiological adaptations during pregnancy, which likely impact the uterine function in subsequent pregnancy. For example, hemodynamic adaptations leading to increased uterine placental blood flow have been found in parous uteri, possibly allowing for more efficient oxygen and nutrient delivery to the fetus.[7,8] Structural changes in spiral arteries following a first pregnancy may improve vascular remodeling during the next pregnancy.[9] Also, pregnancy-related changes in the cardiovascular system, such as increased ventricular volume and cardiac output and decreased systemic vascular resistance, may be incompletely reversed postpartum, which may result in a more favorable uterine environment in a subsequent pregnancy.[10] Finally, uterine structural changes following the first pregnancy, including changes in connective tissue proteins, may provide a better uterine capacity in later pregnancies.[11,12] The current literature is, however, mostly based on successive singleton pregnancies and this association has not been studied for births following twins. Specifically, to our knowledge, no previous study has examined the patterns in birthweight of singletons following a twin pregnancy.

Women with twin pregnancies have larger placentas,[13] higher cardiac output,[14] evidence of systolic and diastolic dysfunction,[15] altered circulating angiogenic factors,[16] more pregnancy complications,[17,18] including shorter gestational age,[19] and greater fetal nutrition demand[20] than singleton pregnancies. In addition, twin pregnancies contribute a greater combined total offspring birthweight than singleton pregnancies. The resulting uterine capacity with twin pregnancy may be larger (combined birthweight, amniotic fluid, placental mass) and the uterine structural and functional changes may be greater than with singleton pregnancies. It remains unknown if these cardiovascular and uterine differences result in changes that impact birthweight in the subsequent pregnancy. Exploring birthweights of singletons following twin pregnancies may provide insight into the importance of factors resulting from pregnancy-related adaptation. In addition to physiological factors, other factors such as interpregnancy interval and pregnancy complications may differ based on the type of first pregnancy (twin or singleton). Women with singleton pregnancies with very short or long pregnancy interval are reported to be at increased risk of low birthweight[21] but, to our knowledge, no earlier studies have described the association among women with a first twin pregnancy.

We aimed to compare birthweight in singleton pregnancies following a first twin pregnancy relative to a first singleton pregnancy to get a better understanding of the general parity effect on birthweight. We also describe differences in interpregnancy interval and subsequent offspring's birthweight in women with first twin or singleton pregnancies. We hypothesized that the increased burden on a woman's physical capacity during a first twin pregnancy would be associated with a larger increase in a subsequent singleton's birthweights compared with a first singleton pregnancy. Our findings are relevant for clinicians who wonder whether a previous twin pregnancy could increase the risk of large subsequent singleton infant, suggesting a need for closer follow up towards term.

Key message

Although twin pregnancies contributed a greater combined offspring birthweight in the first pregnancy than a singleton pregnancy, birthweights of the subsequent singleton pregnancy were similar for offspring born after a twin pregnancy or after a singleton pregnancy.

2 | MATERIAL AND METHODS

2.1 | Study population

Data were obtained from the Medical Birth Registry of Norway (MBRN), a national population-based birth registry, established in 1967. Since then, the register has recorded all pregnancies lasting 16 or more gestational weeks (12th week since 2002) by mandatory notification. The unique national identification number provided to all residents in Norway allows women to be linked to all their pregnancies with women as the unit of observation. Our study population was restricted to women with a first pregnancy registered in the MBRN during 1967–2013 and followed for subsequent pregnancies until 2020. The main analyses consisted of a total of 4849 women who had a first twin pregnancy and a subsequent singleton pregnancy compared with 774 126 women with first and subsequent singleton pregnancies during the study period 1967–2020 (Figure 1). In our study, we excluded women who gave birth before gestational week 22 or after 44 weeks or had implausible *z*-score for birthweight by gestational age outside|5|. We further excluded triplet and quadruplet pregnancies because these pregnancies are both fewer in number than twin pregnancies and more complicated and might have different associations with birthweight and fetal growth in the subsequent pregnancy. Women who became pregnant through assisted reproductive technologies were more likely to have twins, and could have underlying conditions causing fertility problems that also affect birthweight. We therefore excluded the 0.4% of mothers who used assisted reproductive technologies in either their first or second pregnancies.

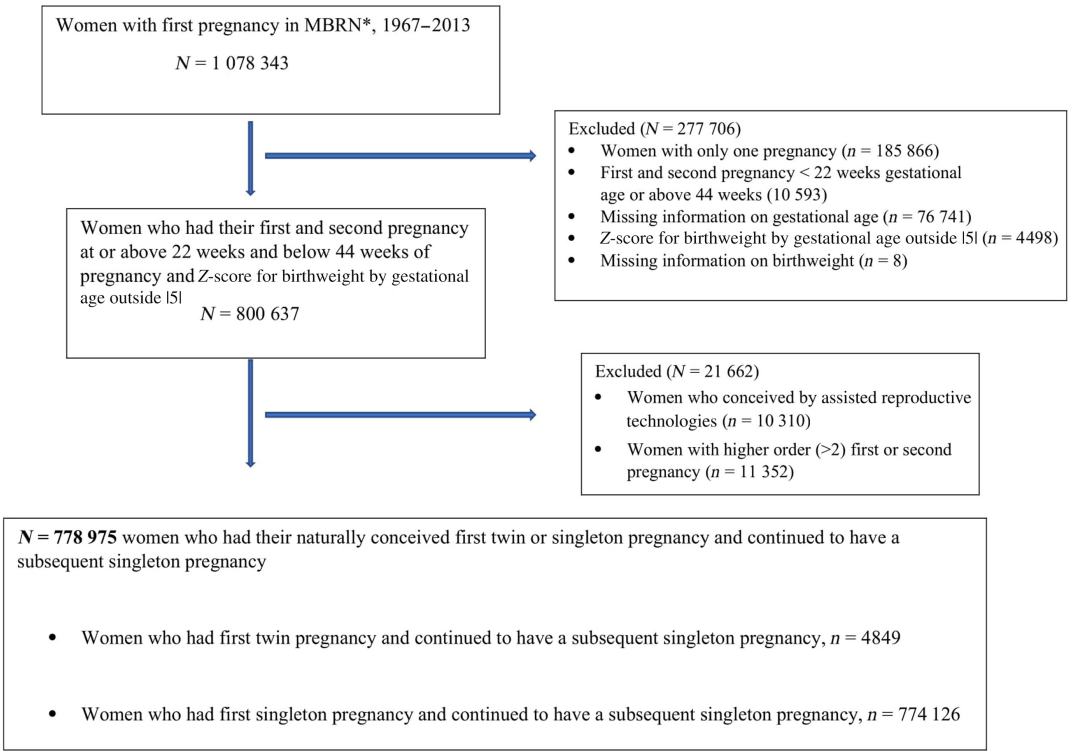

FIGURE 1 Flowchart of the study population.

2.2 | Exposure, outcome, and covariates

The exposure variable was twin or singleton status of the first pregnancy. Offspring birthweight was measured at delivery and recorded in grams (g) in the MBRN. Distribution of birthweights in first and subsequent singleton pregnancies was plotted using categories of absolute grams (ranging from 500 to 7000 g). In first-born twins we used both sum of birthweights in twin pairs and individual infant weights to describe birthweight distributions. Gestational age estimates were based on reported last menstrual period. Ultrasound-based estimates have been recorded in the MBRN from 1999, and were used, when available, for women with missing information on last menstrual period or with a difference between ultrasound-based estimate and last menstrual period estimates of more than 10 days. *Z*-scores for birthweight by gestational age were derived based on national birthweight and gestational age distributions.[22] Our main outcome was birthweight in the subsequent singleton pregnancy.

We adjusted for possible confounding variables available in our data that could affect plurality in the first pregnancy and birthweight in the subsequent: secular trends year of first delivery (1967–1976, 1977–1986, 1987–1996, 1997–2006, and 2007–2020) and mother's age at first delivery (in years: ≤19, 20–25, 26–30, 31–35, and >35). Other potential confounders could be mother's body mass index (BMI), which we did not have data on. However, BMI is related to maternal education, we therefore also adjusted for highest level of maternal education (<11 years, 11–13 years, and ≥14 years). There are also studies describing different rates of twinning[23,24] and general differences in birthweight across countries,[25] so mother's country of birth was also included as a potential confounder (Nordic: women born in Norway, Finland, Sweden, Denmark, and Iceland; non-Nordic: women born outside the Nordic countries). Information on highest attained level of maternal education was obtained from the National Education Database at Statistics Norway, 2020.

The frequency of pregnancy complications in the first and second pregnancy as well as the interpregnancy interval were calculated by twin or singleton status of the first pregnancy. Interpregnancy interval was calculated as the date of the subsequent delivery minus the date of the first delivery minus the pregnancy length of the subsequent pregnancy. Pregnancy complications were obtained from the MBRN. The definition of preeclampsia in the MBRN has changed somewhat over time in accordance with the clinical criteria applied by the Norwegian

Society of Gynecology and Obstetrics.[26] The core criteria have been an increased blood pressure to at least 140 mm Hg systolic or 90 mm Hg diastolic combined with proteinuria (protein excretion of ≥0.3 g/24 h or ≥1+ on dip-stick) after 20 weeks of gestation. Preterm delivery was defined as births before 37 completed weeks of gestation. Perinatal loss included pregnancy loss, stillbirths, and neonatal deaths during the first week after birth (one or both infants in the case of twins).

2.3 | Statistical analyses

All data were analyzed using STATA version 18 (StataCorp LLC, College Station, Texas). Descriptive statistics were presented as means with standard deviations (SD) for continuous variables (maternal age [years], gestational age [weeks], birthweight [grams] and interpregnancy interval [years]), and as numbers and percentage for categorical variables (maternal education, country of birth, initiation of delivery, pregnancy complications in the first and subsequent pregnancy). Association between twin and singleton status of the first pregnancy and birthweight for subsequent singleton pregnancies as a continuous factor was evaluated by linear regression adjusting for the confounders listed above. We also used plots to visualize the distribution of birthweight in the subsequent singleton pregnancy after a first twin or singleton pregnancy. Differences in length of interpregnancy intervals and birthweight at different interpregnancy intervals were explored visually using plots. Interpregnancy interval was expressed in 1-year increments initially but for graphical presentation of birthweight by interpregnancy interval, the longer interpregnancy intervals (>3.9 years) were combined as 4–5.9, 6–7.9, 8–9.9, and 10–11.9 years due to smaller numbers.

2.4 | Ethics statement

Norway by the Regional Committee for Medical Ethics Western Norway REC WEST 13818 on July 1, 2020.

3 | RESULTS

A flow chart of the study sample is presented in Figure 1. Missing values for the covariates (maternal education and country of birth) were rare (0.5% and <0.1%). These analyses are based on the 778 975 women with complete data.

3.1 | Maternal and pregnancy characteristics of study population

Baseline characteristics of the 778 975 women with a first twin ($n = 4849$) or singleton ($n = 774\,126$) birth and a subsequent singleton pregnancy are presented in Table 1. Mean maternal age at first delivery was similar in women with twin pregnancies (25.0 years) and women with singleton pregnancies (24.6 years). For women whose first two births were singletons, mean birthweight increased by an average of 151 g from first to second birth.

Mean gestational age was shorter for first twin pregnancies (252 days) than first singleton pregnancies (281 days). Combined

TABLE 1 Baseline characteristics of 778 975 women with a first twin ($n = 4849$) or singleton ($n = 774\,126$) pregnancy. Medical Birth Registry of Norway, 1967–2020.

	First twin pregnancy $N = 4849$ Mean ± SD or *n* (%)	First singleton pregnancy $N = 774\,126$ Mean ± SD or *n* (%)
Maternal age (years)	25.0 ± 4.3	24.6 ± 4.4
Gestational age (days)	252.1 ± 28.7	280.9 ± 14.9
Birthweight (g)[a]	4627.7 ± 1390.2	3443.6 ± 568.5
Maternal education		
Primary school	840 (17.3)	140 630 (18.2)
High school	1778 (36.7)	300 396 (38.8)
University	2208 (45.6)	329 047 (42.5)
Missing education	23 (0.5)	4053 (0.5)
Women's country of birth		
Nordic	4521 (93.2)	718 684 (92.84)
Non-Nordic	328 (6.8)	55 432 (7.2)
Missing	0	10
Preterm delivery	2388 (49.3)	44 166 (5.7)
Preeclampsia	634 (13.1)	32 039 (4.1)
Perinatal loss	428 (8.8)	8698 (1.1)
Initiation of delivery		
Spontaneous	3088 (63.6)	636 147 (82.2)
Induction	1171 (24.2)	119 305 (15.4)
Cesarean section	590 (12.2)	18 674 (2.4)
Interpregnancy interval (years)	4.2 ± 3.1	2.9 ± 2.4
<1	602 (12.4)	104 737 (13.5)
1–1.9	704 (14.5)	226 027 (29.2)
2–2.9	747 (15.4)	179 713 (23.2)
3–3.9	686 (14.1)	100 524 (13.0)
4–5.9	1047 (21.6)	89 707 (11.6)
6–7.9	548 (11.3)	37 418 (4.8)
8–9.9	272 (5.6)	17 942 (2.3)
10–11.9	132 (2.7)	9065 (1.9)
>12	58 (1.2)	4691 (0.6)
Missing	53 (1.1)	4302 (0.6)
Preterm in subsequent pregnancy	218 (4.5)	32 417 (4.2)
Preeclampsia in subsequent pregnancy	99 (2.0)	15 182 (2.0)

Abbreviation: SD, standard deviation.

[a]Combined mean birthweight of two fetuses for a twin pair.

mean birthweight was 4628 g for a twin pair and 3444 g for a singleton in the first pregnancy. More than 90% of the women were born in Nordic countries. As expected, first twin pregnancies had a much higher occurrence of preterm delivery (49%), preeclampsia (13%), and perinatal loss (9%) than first singleton pregnancies (6%, 4%, and 1%, respectively), and the initiation of delivery was more frequently by prelabor cesarean section (12%) and induction of labor (24%) than in first-born singletons (2% and 15%, respectively). The occurrence of preterm delivery and preeclampsia in the subsequent singleton pregnancies was similar for women with a first twin pregnancy or a first singleton pregnancy (4.5% vs. 4.2% and 2.0% vs. 2.0%, respectively).

3.2 | Birthweight and gestational age in the subsequent singleton pregnancy by first twin or singleton pregnancy

Mean birthweight was 3621 g in singleton pregnancies following a first twin pregnancy and 3595 g in singletons after a first singleton pregnancy (Table 2), resulting in a crude mean difference of 26 g. The mean gestational ages in the subsequent singleton pregnancy after a first twin or first singleton pregnancy were 39.6 and 39.7 weeks respectively. The *z*-scores for birthweight by gestational age in the subsequent singleton pregnancy were 0.15 and 0.08 after a first twin and first singleton pregnancy, respectively. The adjusted difference in mean birthweight in subsequent singletons among women with a first twin pregnancy compared with offspring of women with a first singleton was 21 g (95% confidence interval 5–37) after adjusting for maternal age at first delivery, year of first delivery, maternal education, and country of birth.

The distributions of birthweight in the first (Figure 2A) and subsequent (Figure 2B) pregnancies show that although the birthweights of twin and singleton infants are markedly different in the first birth (Figure 2A), the birthweight distributions are almost identical in the subsequent singleton birth (Figure 2B).

3.3 | Birthweight in the subsequent singleton pregnancy by interpregnancy interval

Women with a first twin pregnancy had a mean ± SD interpregnancy interval of 4.2 ± 3.1 years, whereas women with a first singleton pregnancy had a mean ± SD interpregnancy interval of 2.9 ± 2.4 years. The

TABLE 2 Mean birthweight, gestational age, and *z*-score of 778 975 infants born following a previous twin ($n = 4849$) or singleton ($n = 774\,126$) pregnancy, Medical Birth Registry of Norway, 1967–2020.

Subsequent singleton pregnancy						
	n	Birthweight (g), mean ± SD	Gestational age (wk), mean ± SD	*z*-score,[a] mean ± SD	Difference in birthweight (g) unadjusted[b] (95% CI)	Difference in birthweight (g): adjusted[c] (95% CI)
First twin pregnancy	4849	3621 (575)	39.6 (1.9)	0.15 (1.05)	26.07 (10.29–41.85)	20.92 (5.19–36.67)
First singleton pregnancy	774 126	3595 (559)	39.7 (1.9)	0.08 (1.01)	Reference	Reference

Abbreviations: CI, confidence interval; SD, standard deviation.

[a]Z-score for birthweight by gestational age.

[b]Linear regression.

[c]Adjusted for maternal age at first delivery, year of first delivery, maternal education, and country of birth.

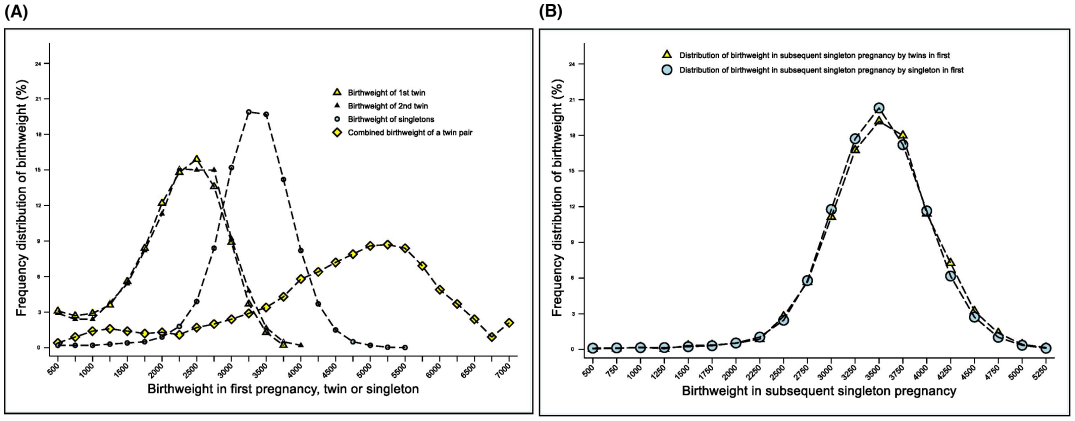

FIGURE 2 (A) Frequency distributions of mean birthweight in the first pregnancy, either twin or singleton. (B) Frequency distributions of mean birthweight in the subsequent singleton pregnancy by first twin or singleton pregnancy.

frequency distribution plots of interpregnancy interval showed that women who had singletons in the first pregnancy had a peak in frequency of a subsequent pregnancy at about 2 years (Figure 3A) and the majority (66%) of women with a first singleton pregnancy had interpregnancy interval of less than 3 years. In contrast the interpregnancy intervals in women with a first twin pregnancy were longer with a wider distribution and a less pronounced peak (Figure 3A). Following a first twin pregnancy only 42% of women had a subsequent pregnancy within 3 years. Although the birthweights of infants born within 3 years of a previous twin or singleton birth were similar (Figure 3B), there were differences in the birthweight patterns for longer interpregnancy intervals. Women with a first singleton pregnancy had an evident declining birthweight in the subsequent pregnancy with increasing interpregnancy intervals beyond 3 years, but a similar declining pattern was not observed among women with a first twin pregnancy.

4 | DISCUSSION

In this population-based cohort study using maternally linked sibship data in Norway, we found that although the combined birthweights of twins were on average more than a kilogram heavier than singleton pregnancies, the mean birthweight of singleton infants in the subsequent pregnancy were similar regardless of whether the earlier birth was twin or singleton. After a twin pregnancy, the adjusted mean weight of a singleton birth was only 21 g heavier than a singleton birth after a previous singleton pregnancy.

Earlier studies have suggested that birthweight is affected by differences in maternal physiological factors that change between the first and subsequent pregnancy.[3] These maternal physiological changes might impact the growth and size of the fetus. At the same time, growth of the fetus is also related to stable maternal factors, as women tend to have successive singleton babies of similar size.[27,28]

In our study, as expected, the mean total sum of birthweights in the first twin pregnancies was higher than the mean birthweight of first singletons. When amniotic fluid and placentas are also considered, it is likely that many women with twin pregnancies have a greater uterine distension than women with singleton pregnancies. This overdistension of the uterus in twin pregnancy has been hypothesized as a possible causal factor in the mechanisms leading to preterm delivery.[29,30] Increased birthweight in subsequent pregnancies might be the result of the improved uterine capacity and function

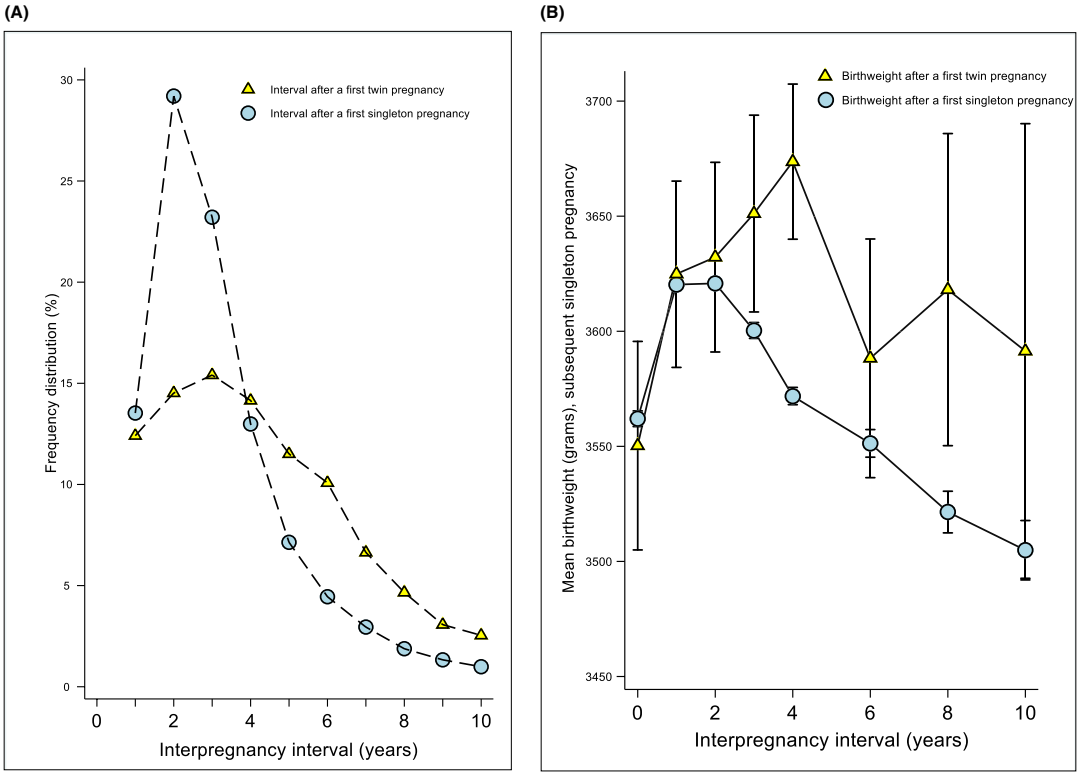

FIGURE 3 (A) Frequency distributions of interval between first and second pregnancy by plurality. (B) Mean birthweight (with 95% confidence interval) in the subsequent singleton pregnancy after a first twin or singleton pregnancy. In (B), interpregnancy interval in years is truncated as 0–0.9, 1–1.9, 2–2.9, 3–3.9, 4–5.9, 6–7.9, 8–9.9, and 10–11.9.

following a first pregnancy.[7,8,12] If this was the primary reason, we might expect that births following a first twin pregnancy would weigh substantially more than those following a first singleton pregnancy.

However, our data do not support this hypothesis. Although the combined birthweights of twin pregnancies were on average more than a kilogram heavier than singleton pregnancies, this additional weight and uterine expansion was associated with only a trivial increase in birthweight in the subsequent singleton pregnancy. The parity effect on birthweight (in the range of 80–140 g) therefore seems to be due to other mechanisms not yet understood.

The associations of birthweight with interpregnancy interval deserve special comment. We found that, after a singleton birth, the mean weight of the subsequent baby declined when the interpregnancy interval was longer than 3 years. This association probably reflects selection, in which the women who take longer to conceive after a singleton pregnancy are more likely to be subfertile and to have associated health problems that decrease birthweight. Such selection would not be as strong for mothers of twin babies, for whom having twins is itself a reason for a longer pregnancy interval (Figure 3A). After a twin birth, there are more healthy mothers with longer interpregnancy intervals, and less evidence of declining birthweights (Figure 3B). Further, adverse social factors like low education and change of partner may also be more frequent among singleton women with long interpregnancy interval compared with mothers of twins with long intervals. In this framework, pregnancy interval is not a confounder, and adjustment for pregnancy interval would not be justified. We can be further reassured that interpregnancy interval is not affecting our results by restricting to births within the first 2 years after delivery, during which time, selection should be less important. Within this time range, there is no evidence that a previous twin birth results in a heavier subsequent birth.

The main strength of our study is the maternally linked offspring design based on a population-based cohort with mandatory registration of mothers and offspring in Norway. This large cohort of births over 50 years provided sufficient sample size to study association in subsequent pregnancies. Another strength of the study is the valid measurement and reporting of birthweight, which has been consistent over time.[31]

We lacked information on some possible confounding factors such as smoking, gestational weight gain, and BMI resulting in possible residual confounding. Information on gestational diabetes was not available for the whole study period. Additionally, we did not have information on diet, weight change between pregnancies, and lifestyle factors that may have varied between first and subsequent pregnancy. Many of these factors are, however, related to maternal educational level, so by adjusting educational level we have likely reduced some of this residual confounding.

5 | CONCLUSION

Our study showed that women with a first twin pregnancy have singletons in the next pregnancy of similar birthweight to women with a first singleton pregnancy. Our findings indicate that the increased physiological and mechanical burden resulting from a twin pregnancy do not explain the general parity effect on the birthweight of first and second singleton births.

AUTHOR CONTRIBUTIONS

PB, RS, and LGK: Conceived and designed the study. PB: Performed statistical analysis and wrote the first draft of the manuscript. All authors contributed to analytical methods and discussion of results. RS: Is the guarantor of data quality. All authors took part in revision of the manuscript. All authors approved the manuscript.

ACKNOWLEDGMENTS

The Medical Birth Registry of Norway provided data for the analysis. We would also like to thank our colleague Aditi Singh for providing helpful comments on the manuscript.

FUNDING INFORMATION

Rolv Skjærven has received funding from the European Research Council advanced grant (ERC-ADG) under the European Union's Horizon 2020 research and innovation program (grant agreement no. 833076). This research was supported in part by the Intramural Research Program of the NIH, National Institute of Environmental Health Sciences (ZIAES049003).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the Norwegian Institute of Public Health. Restrictions apply to the availability of these data, which were used under license for this study. Data are available from https://www.fhi.no/en/hn/health-registries/medical-birth-registry-of-norway/medical-birth-registry-of-norway/ with the permission of the Norwegian Institute of Public Health.

ORCID

Prativa Basnet https://orcid.org/0000-0001-9022-9957
Rolv Skjærven https://orcid.org/0000-0002-2983-1233
Quaker E. Harmon https://orcid.org/0000-0002-5866-848X
Allen J. Wilcox https://orcid.org/0000-0002-3376-1311
Kari Klungsøyr https://orcid.org/0000-0003-2482-1690
Linn Marie Sørbye https://orcid.org/0000-0002-3726-5198
Nils-Halvdan Morken https://orcid.org/0000-0002-8256-0778
Liv G. Kvalvik https://orcid.org/0000-0001-6520-9057

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How to cite this article: Basnet P, Skjærven R, Harmon QE, et al. Birthweight of the subsequent singleton pregnancy following a first twin or singleton pregnancy. *Acta Obstet Gynecol Scand*. 2023;102:1674-1681. doi:10.1111/aogs.14644

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Accepted: 25 September 2023

DOI: 10.1111/1471-0528.17690

BJOG An International Journal of Obstetrics and Gynaecology

RESEARCH ARTICLE

Risk of adverse pregnancy outcomes in twin- and singleton-born women: An inter-generational cohort study

Prativa Basnet[1] | **Rolv Skjærven**[1,2] | **Quaker E. Harmon**[3] | **Linn Marie Sørbye**[4,5] | **Nils-Halvdan Morken**[6,7] | **Aditi Singh**[1] | **Kari Klungsøyr**[1,8] | **Liv Grimstvedt Kvalvik**[1]

[1]Department of Global Public Health and Primary Care, University of Bergen, Bergen, Norway

[2]Centre for Fertility and Health, Norwegian Institute of Public Health, Oslo, Norway

[3]Epidemiology Branch, National Institute of Environmental Health Sciences, Durham, North Carolina, USA

[4]Norwegian Research Centre for Women's Health, Oslo University Hospital, Rikshospitalet, Oslo, Norway

[5]Faculty of Health and Social Sciences, Western Norway University of Applied Sciences, Bergen, Norway

[6]Department of Clinical Science, University of Bergen, Bergen, Norway

[7]Department of Obstetrics and Gynaecology, Haukeland University Hospital, Bergen, Norway

[8]Division for Mental and Physical Health, Norwegian Institute of Public Health, Bergen, Norway

Correspondence
Prativa Basnet, Department of Global Public Health and Primary Care, University of Bergen, Alrek helseklynge, blokk D, Årstadveien 17 Postboks 7804 5020 Bergen, Norway.
Email: prativa.basnet@uib.no

Funding information
European Research Council- Advanced Grant; NIEHS Division of Intramural Research

Abstract

Objective: To compare the risk of adverse pregnancy outcomes between twin-born and singleton-born women. We also evaluated whether in utero exposure to pre-eclampsia or preterm delivery affected adverse pregnancy outcomes in women's own pregnancies.

Design: Population-based cohort study.

Setting: Medical Birth Registry of Norway 1967–2020.

Population: 9184 twin-born and 492 894 singleton-born women during 1967–2005, with their later pregnancies registered during 1981–2020.

Methods: Data from an individual's birth were linked to their later pregnancies. We used generalised linear models with log link binomial distribution to obtain exponentiated regression coefficients that estimated relative risks (RRs) with 95% confidence intervals (CIs) for associations between twin- or singleton-born women and later adverse pregnancy outcomes.

Main outcome measures: Pre-eclampsia, preterm delivery or perinatal loss in twin-born compared with singleton-born women.

Results: There was no increased risk for adverse outcomes in twin-born compared with singleton-born women: adjusted RRs for pre-eclampsia were 1.00 (95% CI 0.93–1.09), for preterm delivery 0.96 (95% CI 0.90–1.02) and for perinatal loss 1.00 (95% CI 0.84–1.18). Compared with singleton-born women exposed to pre-eclampsia in utero, twin-born women exposed to pre-eclampsia had lower risk of adverse outcomes in their own pregnancies; the aRR for pre-eclampsia was 0.73 (95% CI 0.58–0.91) and for preterm delivery was 0.71 (95% CI 0.56–0.90). Compared with preterm singleton-born women, preterm twin-born women did not differ in terms of risk of pre-eclampsia (aRR 1.05, 95% CI 0.92–1.21) or perinatal loss (aRR 0.99, 95% CI 0.71–1.37) and had reduced risk of preterm delivery (RR 0.83, 95% CI 0.74–0.94).

Conclusions: Twin-born women did not differ from singleton-born women in terms of risk of adverse pregnancy outcomes. Twin-born women exposed to pre-eclampsia in utero, had a lower risk of pre-eclampsia and preterm delivery compared with singleton-born women exposed to pre-eclampsia.

KEYWORDS
cohort study, epidemiology, inter-generational, perinatal loss, pre-eclampsia, preterm birth, twin pregnancy

1 | INTRODUCTION

Infants born preterm or in pregnancies with pre-eclampsia are disproportionately more likely to have long-term significant sequelae than are infants born without these adverse pregnancy complications.[1–3] There is accumulating evidence that exposure to complications in utero may influence later health.[4,5] Pre-eclampsia is a pregnancy-specific condition characterised by elevated blood pressure and proteinuria.[6] Both preterm delivery and pre-eclampsia are associated with increased risk of later maternal health consequences;[7,8] however, the exact cause of these complications is not fully understood.

Studies have investigated the inter-generational impact of adverse pregnancy outcomes, such as recurrence of pre-eclampsia or preterm delivery in daughters born preterm or those whose mothers had pre-eclampsia.[9,10] Twin gestations are associated with higher occurrence of adverse pregnancy outcomes such as pre-eclampsia, preterm delivery and perinatal loss.[11–13] However, little is known about the inter-generational recurrence of adverse pregnancy outcomes in twin-born women compared with singleton-born women.

Using a national population-based registry containing information on women's own birth and their later pregnancies, the objective in this study was to compare the risk of adverse pregnancy outcomes between twin-born and singleton-born women. We also evaluated whether in utero exposure to pre-eclampsia or preterm delivery were associated with adverse pregnancy outcomes in women's own pregnancies.

2 | METHODS

2.1 | Data source

This study used data from the nationwide population-based Medical Birth Registry of Norway (MBRN). Using unique national identification numbers, we linked birth record information of females born in 1967–2005 to the birth record information of their own offspring born in 1981–2020, providing information on pregnancies across two generations. Information on women's highest attained level of education by 2020 was obtained from the National Education Database at Statistics Norway.

The MBRN is based on mandatory notification of all live births, stillbirths and pregnancy losses from 16 weeks of gestation. The registry includes prospectively collected data on women's health before and during pregnancy, the delivery and the immediate postpartum period, including demographics, complications and treatments during delivery as well as infant outcomes.[14] The attending midwife and obstetrician record data using a standardised notification form, either as free text or, since 1999, by predefined variables or check boxes in addition to free text. Since 2006, the registry has undertaken a gradual transition to electronic birth notification (complete in 2014) and the notifications are now based on prespecified extractions from the medical records at the delivery units. Reporting of pregnancy complications including mild pre-eclampsia has improved over time.[15] The MBRN is routinely matched with the National Population Register and receives all national identification numbers through this linkage. Given that the registry has registered pregnancies for more than 50 years, this enabled us to study pregnancies to women who were themselves registered in the MBRN.

2.2 | Study population

To evaluate inter-generational associations, we studied women born 1967–2005 and registered in the MBRN, whose own singleton pregnancies were registered in the MBRN during 1981–2020. This enabled us to stratify the women by plurality at birth (twin-born or singleton-born) and retrieve information on their own intrauterine exposure to pregnancy complications (pre-eclampsia and/or preterm delivery).

2.3 | Exposure

The exposure variable was the plurality status (twin or singleton) of the women at their birth. We also explored possible modification by in utero exposure to pre-eclampsia or preterm delivery among twin-born versus singleton-born women. All exposures were obtained from the woman's birth record.

2.4 | Outcome

The main outcomes of interest were the risks of pre-eclampsia, preterm delivery or perinatal loss in any pregnancy of twin-born women compared with singleton-born women.

Pre-eclampsia was coded using the clinical definitions in place at the year of birth. The definition has been an increased blood pressure to at least 140 systolic or 90 mmHg diastolic combined with proteinuria (protein excretion of ≥0.3 g/24 hours or ≥1+ on dip-stick) after 20 weeks of gestation, the criteria corresponding to the Norwegian Society of Gynaecology and Obstetrics.[16] Preterm delivery was defined as pregnancies <37 completed weeks of gestations. Perinatal losses included miscarriages (16–21 weeks), stillbirths (≥22 weeks) and early neonatal deaths during the first week after delivery.

2.5 | Covariates

Estimates were adjusted for the decade of the twin-born or singleton-born women's birth (categorised as 1967–1969, 1970–1979, 1980–1989, 1990–1999 and 2000–2005) and the

women's mother's educational attainment through 2020 (categorised as <11, 11–13 and ≥14 years). In a sensitivity analysis, we also accounted for women's total number of pregnancies categorised as 1, 2, 3+ registered in the MBRN through 2020, and their own educational attainment through 2020 (categorised as <11, 11–13 and ≥14 years).

2.6 | Exclusion and inclusion

We excluded women born in higher order pregnancies (>2 fetuses) and women who only had second or later pregnancies registered in the MBRN (such as first births outside Norway). We only included singleton pregnancies to twin-born and singleton-born women for a homogeneous comparison.

2.7 | Statistical analysis

We used generalised linear models with log link binomial distribution to estimate relative risks (RRs) with 95% confidence intervals (CIs) for associations between twin-born women and later adverse pregnancy outcomes relative to singleton-born women. The estimates were adjusted for women's own decade of birth and their mother's educational attainment. We ran separate models for each outcome. Models accounted for correlations between siblings using clustered standard errors. We also ran stratified models based on in utero exposure to pre-eclampsia or preterm delivery with similar outcomes as the main analyses. We used Knol and VanderWeelee's recommended[17] methods for presenting RR for these strata. Further, we obtained E-values[18] for estimates with CIs that excluded the null to assess the suggested influence of unmeasured confounding. Statistical analyses were performed using STATA IC statistical software (version 17.0).

2.8 | Ethics approval

The study was approved in Norway by the Regional Ethics Committee REK VEST 13818 on 1 July 2020.

3 | RESULTS

The study population consisted of 9184 twin-born and 492 894 singleton-born women in 1967–2005, with their later births registered in the MBRN during 1981–2020 (Figure 1).

Table 1 shows the birth characteristics of twin-born and singleton-born women. About 40% of both twin-born women and singleton-born women were born during 1970s and 36–40% of twin-born or singleton-born women had their first pregnancies after 2009. Twin-born women were older at their first birth, but there was no difference in educational attainment for twin-born and singleton-born women. Almost 50% of both twin- and singleton-born women had two pregnancies. Twin-born women were more frequently exposed to in utero pre-eclampsia than were singleton-born women (8% versus 2%) and were more often born preterm (29% versus 4%).

Table 2 shows the risk of pre-eclampsia, preterm delivery and perinatal loss in women's own pregnancies among twin-born versus singleton-born women. There was no increased risk for adverse outcomes in twin-born women compared with singleton-born women: adjusted RR (aRR) for pre-eclampsia 1.00 (95% CI 0.93–1.09), for preterm delivery 0.96 (95% CI 0.90–1.02) and for perinatal loss 1.00 (95% CI 0.84–1.18). Analyses were adjusted for twin-born and singleton-born women's own decade of birth, and their mother's educational attainment.

We further investigated whether the risk of adverse outcomes differed by in utero exposure to pre-eclampsia (Table 3). Compared with singleton-born women with no in utero exposure to pre-eclampsia, singleton-born women exposed to pre-eclampsia had an increased risk of pre-eclampsia (aRR 2.17, 95% CI 2.07–2.28) and preterm delivery (aRR 1.23, 95% CI 1.17–1.30) in their own pregnancies. Twin-born women delivered from a non-pre-eclamptic pregnancy had no increased risk of any adverse pregnancy outcome compared with singleton-born women from non-pre-eclamptic pregnancies. Twin-born women exposed to pre-eclampsia in utero did have an increased risk of pre-eclampsia in their own pregnancies (aRR 1.57, 95% CI 1.26–1.97) compared with singleton-born women with no pre-eclampsia, but it was lower than that experienced by singletons exposed to pre-eclampsia (aRR 0.73, 95% CI 0.58–0.91; Table 3). Twins born with in utero exposure to pre-eclampsia had a possible decrease in the risk of perinatal loss in their own pregnancies compared with those singleton-born without pre-eclampsia (aRR 0.47, 95% CI 0.20-1.14) and with pre-eclampsia (aRR 0.45, 95% CI 0.19-1.10), but the estimates were imprecise due to small numbers. Analyses were adjusted for covariates as in the main analyses.

We also investigated whether the risk of adverse outcomes differed by preterm birth among twin-born versus singleton-born women (Table 4). Compared with singletons born term, singleton women born preterm had an increased risk of pre-eclampsia (aRR 1.18, 95% CI 1.12–1.24), preterm delivery (aRR 1.35, 95% CI 1.30–1.40) and perinatal loss (aRR 1.15, 95% CI 1.03–1.28) in their own pregnancies. Women who were term twins had a slightly decreased risk of pre-eclampsia (aRR 0.90, 95% CI 0.82–1.00) and preterm delivery (aRR 0.91, 95% CI 0.84–0.99) in their own pregnancy compared with women who were term singletons, with no association with perinatal loss (aRR 0.94, 95% CI 0.76–1.17). Women born as a preterm twin had an increased risk of pre-eclampsia in their own pregnancies (aRR 1.26, 95% CI 1.11–1.44) compared with women born as term singletons; however, this risk was not increased compared with singleton women born preterm (aRR 1.05, 95% CI 0.92–1.21). Preterm twin-born women had a slightly increased risk of preterm delivery compared with term singleton-born women (aRR 1.12, 95% CI 1.00-1.26); however, this risk was decreased (aRR 0.83, 95% CI 0.74-0.94) compared with singletons born preterm. Preterm twin-born women had no increased risk of

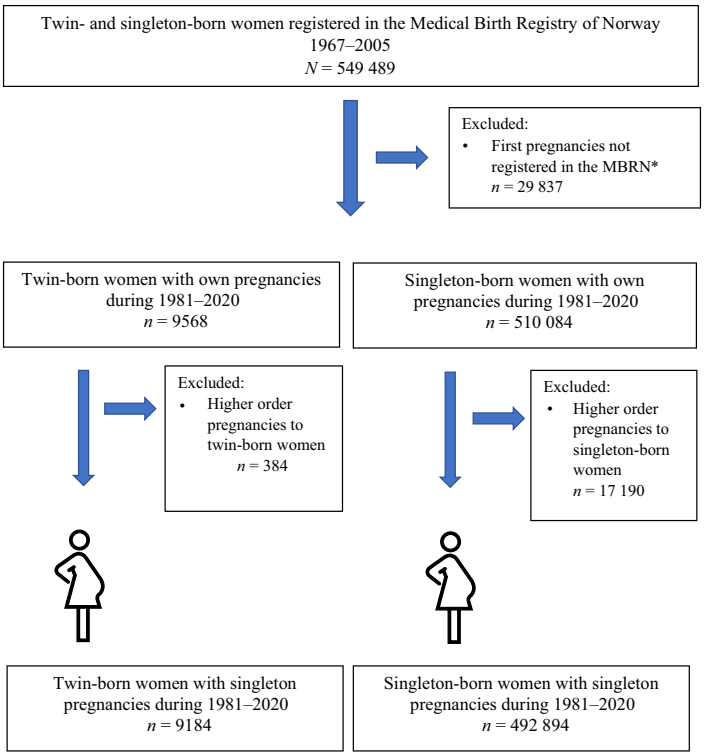

FIGURE 1 Flowchart of the study population. *Women who only had second or later pregnancies registered in the Medical Birth Registry of Norway (MBRN) were excluded (such as first births outside Norway).

perinatal loss in their own pregnancies compared with both term and preterm singleton-born women.

In a sensitivity analysis, we also adjusted our main models for other factors such as women's educational status and total number of pregnancies; the results were essentially the same (Table S1).

4 | DISCUSSION

4.1 | Main findings

We found that, on average, there was no difference in the risk of pre-eclampsia, preterm delivery or perinatal loss in the singleton pregnancies to twin-born women compared with singleton-born women, despite pre-eclampsia and preterm delivery being much more frequent in twin pregnancies. Twin-born women with in utero exposure to pre-eclampsia had a lower risk of pre-eclampsia and preterm delivery in their own pregnancies compared with singleton-born women with in utero exposure to pre-eclampsia. Further, preterm twin-born women did not differ in terms of their risk of pre-eclampsia or perinatal loss compared with preterm singleton-born women, and they had a reduced risk of preterm delivery.

4.2 | Interpretation

Earlier studies have evaluated several risk factors contributing to higher incidences of pregnancy complications in twin versus singleton pregnancies such as obstetric history, age and pre-existing hypertension.[19–21] In our study, we evaluated whether twin-born women have a higher risk of adverse pregnancy outcomes in their own pregnancies compared with singleton-born women. Our results therefore add to the existing literature by demonstrating that twin-born women, despite more frequently experiencing in utero exposure to pre-eclampsia or being born preterm, generally seem to have no increased risk of adverse outcomes in their own pregnancies compared with singleton-born women. This may be due to the different underlying causes of pregnancy complications in twin compared with singleton pregnancies. Some of the causes of pregnancy complications in twin pregnancies may be less likely to carry an increased inter-generational risk and be related more to the larger intrauterine volume of two growing fetuses.

Recurrence of pre-eclampsia across generations has been well documented in singleton-born women;[9,22] however, less is known for twin-born women. Although we see evidence for an increased inter-generational recurrence risk

TABLE 1 Pregnancy characteristics of twin-born ($n = 9184$) and singleton-born ($n = 492\,894$) women, from the Medical Birth Registry of Norway, 1967–2020.

	9184 twin-born women, *n* (%)	492 894 singleton-born women, *n* (%)
Decade of women's birth		
1967–1969	1367 (14.9)	74 571 (15.1)
1970–1979	3663 (39.9)	206 067 (41.8)
1980–1989	3073 (33.5)	161 187 (32.7)
1990–1999	1070 (117)	50 713 (10.3)
2000–2005	11 (0.1)	356 (0.1)
Characteristics of women's own pregnancy		
Decade of first pregnancy		
1981–1989	296 (3.2)	17 896 (3.6)
1990–1999	2222 (24.2)	131 326 (26.6)
2000–2009	2999 (32.7)	165 430 (33.6)
2010–2020	3667 (39.9)	178 242 (36.2)
Age at first pregnancy, years		
≤19	658 (7.2)	41 357 (8.4)
20–25	3375 (36.7)	189 074 (38.4)
26–30	3406 (37.1)	172 093 (34.9)
31–35	1339 (14.6)	71 402 (14.5)
>35	406 (4.4)	18 968 (3.8)
Years of attained education		
<11	1219 (13.3)	67 868 (13.8)
11–13	2891 (31.5)	149 096 (30.2)
≥14	5046 (54.9)	275 231 (55.8)
Missing	28 (0.3)	699 (0.1)
Number of pregnancies		
1	2456 (26.7)	120 535 (24.5)
2	4401 (47.9)	239 649 (48.6)
3	1908 (20.8)	107 002 (21.7)
4 or more	419 (4.6)	25 708 (5.2)
Characteristics of women's in utero exposures		
Women exposed to in utero pre-eclampsia		
Yes	755 (8.2)	11 507 (2.3)
No	8429 (91.8)	481 387 (97.7)
Women born preterm (<37 weeks)		
Yes	2647 (28.8)	18 527 (3.8)
No	6139 (66.8)	446 260 (90.5)
Missing	398 (4.3)	28 107 (5.7)

of pre-eclampsia in both singleton-born and twin-born women who themselves were exposed to pre-eclampsia compared with those who were not, twin-born women exposed to pre-eclampsia had a lower recurrence of pre-eclampsia compared with singleton-born women exposed to pre-eclampsia. One inter-generational study from Sweden has shown less recurrence of preterm delivery in preterm twin-born women than in preterm singleton-born women.[23]

TABLE 2 Relative risks (RRs) with 95% CIs for pre-eclampsia, preterm delivery (<37 weeks) and perinatal loss[a] in twin-born women compared with singleton-born women.

		Adverse outcomes in own pregnancy									
		Pre-eclampsia			Preterm delivery			Perinatal loss[a]			
Women born	*Total N*	*n* (%)	Crude RR (95% CI)	aRR[b] (95% CI)	*n* (%)	Crude RR (95% CI)	aRR[b] (95% CI)	*n* (%)	Crude RR (95% CI)	aRR[b] (95% CI)
Singleton	492 894	32 091 (6.5)	Reference	Reference	49 263 (10.0)	Reference	Reference	7290 (1.5)	Reference	Reference
Twin	9184	597 (6.5)	1.00 (0.92–1.08)	1.00 (0.93–1.09)	876 (9.5)	0.95 (0.89–1.02)	0.96 (0.90–1.02)	135 (1.5)	0.99 (0.84–1.18)	1.00 (0.84–1.18)

Abbreviations: CI, confidence interval; RR, relative risk.

[a]Miscarriages, stillbirths and early neonatal deaths <7 days of life.

[b]aRRs obtained by generalised linear models with log link binomial distribution. Analyses were adjusted for twin-born and singleton-born women's own decade of birth, and their mother's education.

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TABLE 3 Relative risks (RRs) with 95% CIs for pre-eclampsia, preterm delivery (<37 weeks) and perinatal loss[a] in twin-born women compared with singleton-born women, when the women were themselves exposed to pre-eclampsia.

	Adverse pregnancy outcomes in own pregnancy				
	Singleton-born women		Twin-born women		RR (95% CI) for twin- versus singleton-born women within strata of in utero exposure to pre-eclampsia
Outcome = Pre-eclampsia in own pregnancy	***n* with/without pre-eclampsia**	**aRR[b] (95% CI)**	***n* with/without pre-eclampsia**	**aRR[b] (95% CI)**	
In utero exposure					
Pre-eclampsia = 0	30 549/481 387 (6.3%)	Reference	523/8429 (6.2%)	0.98 (0.90–1.07)	0.98 (0.90–1.07)
Pre-eclampsia = 1	1542/11 507 (13.4%)	2.17 (2.07–2.28)	74/755 (9.8%)	1.57 (1.26–1.97)	0.73 (0.58–0.91)[c]
Outcome = Preterm delivery (<37 weeks) in own pregnancy	***n* with/without preterm delivery**		***n* with/without preterm delivery**		
In utero exposure					
Pre-eclampsia = 0	47 892/481 387 (9.9%)	Reference	812/8429 (9.6%)	0.97 (0.91–1.04)	0.97 (0.91–1.04)
Pre-eclampsia = 1	1371/11 507 (11.9%)	1.23 (1.17–1.30)	64/755 (8.5%)	0.88 (0.69–1.11)	0.71 (0.56–0.90)[c]
Outcome = Perinatal loss[a] in own pregnancy	***n* with/without perinatal loss[a]**		***n* with/without perinatal loss[a]**		
In utero exposure					
Pre-eclampsia = 0	7146/481 387 (1.5%)	Reference	130/8429 (1.5%)	1.04 (0.87–1.24)	1.04 (0.87–1.24)
Pre-eclampsia = 1	164/11 507 (1.4%)	1.03 (0.88–1.20)	5/755 (0.7%)	0.47 (0.20–1.14)	0.45 (0.19–1.10)

Abbreviations: CI, confidence interval; RR: relative risk.

[a]Miscarriages, stillbirths and early neonatal deaths <7 days of life.

[b]aRRs obtained by generalised linear models with log link binomial distribution. Analyses were adjusted for twin-born and singleton-born women's own decade of birth, and their mother's education.

[c]E-values for these estimates ranged from 2.1 to 2.2, suggesting that unmeasured confounding of such a strength would be needed to move this point estimate to null.

TABLE 4 Relative risks (RRs) with 95% CIs for pre-eclampsia, preterm delivery (<37 weeks) and perinatal loss[a] in twin-born women compared with singleton-born women, when the women themselves were born preterm.

	Adverse pregnancy outcomes in own pregnancy				
	Singleton-born women		Twin-born women		RR (95% CI) for twin- versus singleton-born women within strata of preterm delivery at birth
Outcome = Pre-eclampsia in own pregnancy	***n* with/without pre-eclampsia**	**aRR[b] (95% CI)**	***n* with/without pre-eclampsia**	**aRR[b] (95% CI)**	
In utero exposure					
Preterm delivery = 0	28 906/446 260 (6.5%)	Reference	360/6139 (5.9%)	0.90 (0.82–1.00)	0.90 (0.82–1.00)
Preterm delivery = 1	1420/18 527 (7.7%)	1.18 (1.12–1.24)	212/2647 (8.0%)	1.26 (1.11–1.44)	1.05 (0.92–1.21)
Outcome = Preterm delivery (<37 weeks) in own pregnancy	***n* with/without preterm delivery**		***n* with/without preterm delivery**		
In utero exposure					
Preterm delivery = 0	43 953/446 260 (9.9%)	Reference	548/6139 (8.9%)	0.91 (0.84–0.99)	0.91 (0.84–0.99)[c]
Preterm delivery = 1	2477/18 527 (13.4%)	1.35 (1.30–1.40)	289/2647 (10.9%)	1.12 (1.-00–1.26)	0.83 (0.74–0.94)[c]
Outcome = Perinatal loss in own pregnancy	***n* with/without perinatal loss[a]**		***n* with/without perinatal loss[a]**		
In utero exposure					
Preterm delivery = 0	6586/446 260 (1.5%)	Reference	86/6139 (1.4%)	0.94 (0.76–1.17)	0.94 (0.76–1.17)
Preterm delivery = 1	318/18 527 (1.7%)	1.15 (1.03–1.28)	43/2647 (1.6%)	1.12 (0.83–1.52)	0.99 (0.71–1.37)

Abbreviations: CI, confidence interval; RR: relative risk.

[a]Miscarriages, stillbirths and early neonatal deaths <7 days of life.

[b]aRRs obtained by generalised linear models with log link binomial distribution. Analyses were adjusted for twin-born and singleton-born women's own decade of birth, and their mother's education.

[c]E-values for these estimates ranged from 1.4 to 1.7, suggesting that unmeasured confounding of such a strength would be needed to move this point estimate to null.

We found similar patterns when looking at later preterm delivery among preterm twin- and singleton-born women. Together with prior literature, this supports the theory that pregnancy complications in twin pregnancies have both similar and distinct origins compared with singleton pregnancies. For instance, studies indicate that the placental size in twin pregnancies may worsen placental perfusion, leading to increased complications such as pre-eclampsia in twin pregnancies.[24,25] When the complications are due to the physical demands of the twin pregnancies, they may be less likely to be transferred to the next generation.

4.3 | Strengths and limitations

Prospectively collected data provided the opportunity to study pregnancy outcomes across generations. The large sample size and long follow-up allowed us to evaluate associations stratified by in utero exposure to specific pregnancy complications among twin-born and singleton-born women. Changes to the data recording system over the years are unlikely to impact the reporting of singleton or multiple gestations over time. Linked pregnancy complications across a woman's reproductive life are necessary for studies such as this one, but using linked birth registry data does have limitations. Data on covariates relevant to the mother's own birth (i.e. her mother's smoking or BMI, chorionicity of twins) were not collected. Given the analysis of a woman's whole reproductive course, we did not take into account intermediate factors (smoking, inter-pregnancy interval) that may predict specific adverse outcomes, but which will vary for each pregnancy. We did take into account factors that may be associated with the woman's own birth (mother's education, decade of birth). However, in a sensitivity analysis, we also accounted for woman's education along with their total number of pregnancies as a surrogate for the opportunity to experience adverse pregnancy outcomes. We saw very little change in the estimates with these adjustments. Further, there is no large difference in reproduction of twin- or singleton-born women. In our population, 77% of twin-born women had a recorded pregnancy as compared with 84% of singleton-born women. Finally, for the estimates which showed decreased risk of adverse outcomes for twins within strata of their own in utero exposure we have provided E-values. The E-values for these estimates ranged from 2.1 to 2.2 when women were stratified according to in utero exposure to pre-eclampsia and 1.4 to 1.7 when women were stratified according to preterm birth, suggesting that unmeasured confounding of such strength would be needed to move these point estimates to the null.

5 | CONCLUSION

Despite the fact that twin-born women are more often exposed to adverse pregnancy outcomes in utero, the risk of pre-eclampsia, preterm delivery and perinatal loss in twin-born women is not increased in their own pregnancies compared with singleton-born women. Twin-born women exposed to pre-eclampsia in utero had a reduced risk of pre-eclampsia, preterm delivery or perinatal loss in their own pregnancies compared with singleton-born women exposed to pre-eclampsia in utero. Preterm twin-born women had no increased risk of pre-eclampsia or perinatal loss in their own pregnancies and a reduced risk of preterm delivery compared with preterm singleton-born women.

AUTHOR CONTRIBUTIONS

PB, RS, LGK and QEH contributed to study design. PB performed the data analysis. RS, LGK, QH, LMS, N-HM, AS, KK contributed critical comments to the analysis. RS is guarantor for data quality. PB wrote the first draft of the paper. LGK, QEH, LMS, N-HM, KK, AS contributed to the revision. All authors agree with the final version of the paper.

ACKNOWLEDGEMENTS

The Medical Birth Registry of Norway provided data for the analysis.

FUNDING INFORMATION

This study was financially supported by The European Research Council under the European Union's Horizon 2020 Research and Innovation Program (ERC advanced grant 2018, agreement No 833076). The Norwegian Research Centre for Women's Health at Oslo University Hospital also contributed with financial support by adding human resources. This research was supported in part by the NIH, National Institute of Environmental Health Sciences.

CONFLICT OF INTEREST STATEMENT

None declared.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from Norwegian Institute of Public Health. Restrictions apply to the availability of these data, which were used under licence for this study. Data are available from https://www.fhi.no/en/hn/health-regis tries/medical-birth-registry-of-norway/medical-birth-registry-ofnorway/ with the permission of the Norwegian Institute of Public Health.

ORCID

Prativa Basnet https://orcid.org/0000-0001-9022-9957
Rolv Skjærven https://orcid.org/0000-0002-2983-1233
Quaker E. Harmon https://orcid.org/0000-0002-5866-848X
Linn Marie Sørbye https://orcid.org/0000-0002-3726-5198
Nils-Halvdan Morken https://orcid.org/0000-0002-8256-0778
Aditi Singh https://orcid.org/0000-0002-2302-1723
Kari Klungsøyr https://orcid.org/0000-0003-2482-1690
Liv Grimstvedt Kvalvik https://orcid.org/0000-0001-6520-9057

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Basnet P, Skjærven R, Harmon QE, Sørbye LM, Morken N-H, Singh A, et al. Risk of adverse pregnancy outcomes in twin- and singleton-born women: An inter-generational cohort study. BJOG. 2023;00:1–9. https://doi.org/10.1111/1471-0528.17690

Table S1. Adjusted relative risks (aRRs*) with 95% confidence intervals (CIs) for preeclampsia, preterm delivery (<37 weeks) and perinatal loss in twin-born women compared with singleton-born women.**

		Adverse outcomes in own pregnancy					
		Preeclampsia		Preterm delivery (<37 weeks)		Perinatal loss**	
Women born	N	n (%)	aRR* (95% CI[¤])	n (%)	aRR* (95% CI[¤])	n (%)	aRR* (95% CI[¤])
Singleton	492 894	32 091 (6.5)	1	49 263 (10.0)	1	7 290 (1.5)	1
Twin	9 184	597 (6.5)	1.01 (0.93-1.09)	876 (9.5)	0.97 (0.91-1.04)	135 (1.5)	1.05 (0.89-1.25)

[¤]*CI: Confidence Interval*

**aRRs obtained by generalized linear models with log link binomial distribution. Analyses were adjusted for twin-born and singleton-born women's own decade of birth, her mother's education, her total number of pregnancies and her own educational attainment.*

*** Miscarriages, stillbirth, and early neonatal deaths <7 days of life.*

Graphic design: Communication Division, UiB / Print: Skipnes Kommunikasjon AS

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ISBN: 9788230861165 (print)
9788230843581 (PDF)