Number of children: pre- and post-pregnancy lipids, effect of pregnancy outcome and modification by perinatal loss

Aleksandra Pirnat

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

This project has been carried out in the Research Group for Reproductive Epidemiology with a Lifecourse Perspective, at the Department of Global Public Health and Primary Care and the Research Group for Pregnancy, Fetal Development and Birth at the Department of Clinical Science, the Medical Faculty, University of Bergen.

The project has been funded by a three-year scholarship from the Norwegian Association for Public Health.

The main supervisor has been Professor Nils-Halvdan Morken at the Department of Clinical Science, University of Bergen and the Department of Obstetrics and Gynaecology, Haukeland University Hospital.

Co-supervisor has been Professor Rolv Skjærven at the Department of Global Public Health and Primary Care, University of Bergen.

During the course of my PhD education, I have been an active member of the National Research School in Population Based Epidemiology (EPINOR) and I have participated in local and national activities related to this affiliation. I was also an affiliated member of the Research School in Public Health and Primary Care, University of Bergen.

“Come forth into the light of things,

let Nature be your teacher”.

William Wordsworth

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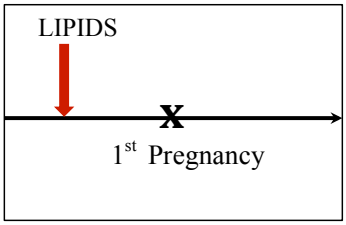
Abbreviations

ApoB	apolipoprotein B
BMI	body mass index
CI	confidence interval
CONOR	Cohort of Norway
CVD	cardiovascular disease
CS	cesarean section
HDL	high density lipoprotein
IVF	in vitro fertilization
LDL	low density lipoprotein
MBRN	Medical Birth Registry of Norway
OC	oral contraceptive
OR	odds ratio
PCOS	polycystic ovary syndrome
PE	preeclampsia
PTD	preterm delivery
RR	relative risk
SGA	small for gestational age
TG	triglyceride
TG/HDL	triglycerides to high density lipoprotein ratio
TFR	total fertility rate

Thesis at a glance



Paper I

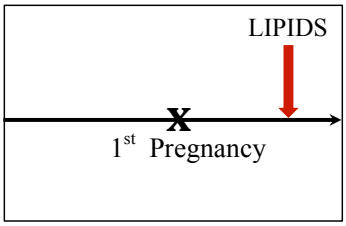
Question: Is there an association between pre-pregnant lipid levels and number of children?

Period: 1994-2003, followed for a 2nd birth until 2008.

Study population: 2 645 parous women and 1 677 nulliparous women participating in Cohort of Norway and with linked data from the Medical Birth Registry of Norway

Exposure: Pre-pregnant lipid levels

Outcome: Number of born children



Paper II

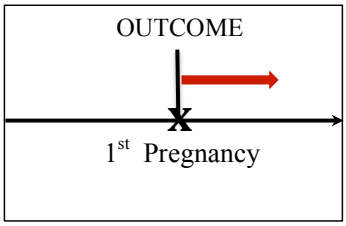
Question: What is the status of post-pregnancy lipid levels in one-child mothers compared to mothers with two or more children? Are women's post-pregnancy lipid levels associated with number of children?

Period: 1994-2003, followed for a 2nd birth until 2008.

Study population: 32 618 parous women examined after first childbirth in Cohort of Norway and with linked data from the Medical Birth Registry of Norway

Exposure: Post-pregnant lipid levels

Outcome: Number of born children



Paper III

Question: What is the risk of having one lifetime pregnancy by adverse pregnancy outcomes and how does perinatal loss modify this risk?

Period: 1967-2007, followed for a 2nd birth until 2014.

Study population: 882 803 mothers giving birth to their first singleton baby and followed until 2014 in the Medical Birth Registry of Norway

Exposure: Preterm delivery, preeclampsia, small for gestational age, cesarean section and perinatal loss

Outcome: One lifetime pregnancy

Abstract

Background: Cardiovascular disease (CVD) is an important public health problem and remains the number one cause of death in women. Substantial increase in CVD mortality has been found in women with only one child, and lipid disorders are suggested to play a role in both subfertility and later CVD development.

Objectives: To explore the extent to which pre-pregnant serum lipid levels of total, low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol, triglyceride (TG) and triglycerides to high density lipoprotein (TG/HDL) ratio are associated with having no and one lifetime pregnancy; to estimate post-pregnancy lipid levels in one-child mothers compared to mothers with two or more children and to assess these lipid's associations with number of children; to explore if preterm delivery (PTD), preeclampsia (PE), small for gestational age (SGA) and cesarean section (CS) at first birth are associated with having one lifetime pregnancy, and assess the modifying effect of perinatal loss.

Material and Methods: In paper I we analysed prepregnant lipid levels in 2 645 women giving birth to their first child during 1994 - 2003 and in 1 677 nulliparous women with linked data from Cohort of Norway (CONOR) and The Medical Birth Registry of Norway (MBRN). In paper II we used data on 32 618 parous women examined after first childbirth as part of CONOR (1994-2003) with linked data on reproduction and number of children from the MBRN (1967-2008). Paper III was a population-based study of 882 803 mothers giving birth to their first singleton infant (≥22 gestational weeks) during 1967 to 2007 who were followed for the occurrence of second birth in the MBRN until 2014. Logistic regression (Papers I and II) and generalized linear models (Paper III) were used to calculate odds ratios (ORs) and relative risks (RRs) with 95% confidence intervals (CIs) and to adjust for confounders.

Results: Assessed in quintiles, higher pre-pregnant TG and TG/HDL ratio levels were associated with increased risk of one lifetime pregnancy compared to having ≥ 2 children. Compared to the highest quintile, women in the lowest quintile of HDL cholesterol levels had an increased risk of one lifetime pregnancy (OR 1.7 95% CI 1.2-2.4), as were women with the highest LDL cholesterol, TG and TG/HDL ratio quintiles (compared to the lowest) (OR 1.2 95% CI 0.8-1.7; OR 2.2 95% CI 1.5-3.2; and OR 2.2 95% CI 1.5-3.2, respectively).

Similar effects were found in women with BMI ≥ 25 and the highest LDL and total cholesterol levels in risk of lifetime nulliparity. When examined after first childbirth in paper II, ORs for one lifetime pregnancy for the highest quintiles of LDL and total cholesterol (compared to lowest quintiles) were 1.30 (95%CI: 1.14-1.45) and 1.43 (95%CI: 1.27-1.61), respectively. In women with pregnancy complications where the infant survived the perinatal period, RRs for one lifetime pregnancy were increased (PTD: 1.21 [1.19-1.22], SGA: 1.13 [1.12-1.15], PE: 1.09 [1.07-1.11], CS: 1.24 [1.23-1.25]), but significantly reduced if the child was lost (PTD: 0.63 [0.59-0.68], SGA: 0.57 [0.51-0.63], PE: 0.69 [0.59-0.80], CS: 0.67 [0.56-0.79]), compared to women with no perinatal loss and no adverse outcome.

Conclusions and implications: Unfavorable pre-pregnant lipid levels were associated with having no and one lifetime pregnancy. The association with unfavourable lipids was also present for one child mothers with lipids measured more than a decade after childbirth. These findings provide a possible biological underpinning for a joint mechanistic pathway for reduced fertility and cardiovascular conditions. Associations between adverse outcomes of pregnancy and the risk of having one lifetime pregnancy were strongly modified by child survival in the perinatal period.

List of Publications

This thesis is based on the following original research papers, which will be referred to by their Roman numerals:

I) Pirnat A, DeRoo LA, Skjærven R, Morken NH. *Women's prepregnancy lipid levels and number of children: a Norwegian prospective population-based cohort study*. BMJ Open 2018;8:e021188.

II) Pirnat A, DeRoo LA, Skjærven R, Morken NH. *Lipid levels after childbirth and association with number of children: A population-based cohort study.* Manuscript (submitted).

III) Pirnat A, DeRoo LA, Skjærven R, Morken NH. *Risk of having one lifetime pregnancy and modification by outcome of pregnancy and perinatal loss.* Acta Obstet Gynecol Scan. 2019;98 (6):753-760.

Paper I was published as open access and no permission was needed for reprints. Paper III was reprinted with permission from Wiley and Sons.

1. Introduction

Cardiovascular disease (CVD) is an important public health problem and remains the number one cause of death in women (1). Although recent Norwegian reports suggest a decrease in CVD incidence for men, the decline has been less pronounced for women (2). Several Norwegian studies have observed higher CVD mortality among women with only one child (3, 4). Relative to mothers of two children, childless women and those with one child had higher mortality risks for nearly all causes (5, 6).

Efforts to elucidate this association are many, but inconclusive, ranging from lifestyle risk factors associated with childrearing (7), sex hormone fluctuations (8) and protective effect of future pregnancies (8). Considering the increased CVD mortality among women with low parity (3, 4, 5, 6, 8), the information about possible underlying biological factors adds critical value from a public health perspective. Given the lipid's role in both CVD and female fertility (9, 10, 11) and the lack of lipid-fertility studies in humans, increased knowledge of the association between women's lipid profile and the number of children may provide valuable insights for the observed inverse parity-CVD mortality association. Apart from specific biologic links, other pathways may also play a role in reproductive patterns. As previous pregnancy experience may shape women's decision towards future pregnancies (1, 12), insights into the adverse pregnancy effects on women's future reproduction could add valuable knowledge about reproductive patterns.

In this thesis, we sought to explore possible biological underpinning for the association between low parity and increased CVD mortality as well as to investigate the effect of adverse pregnancy outcomes on future reproduction.

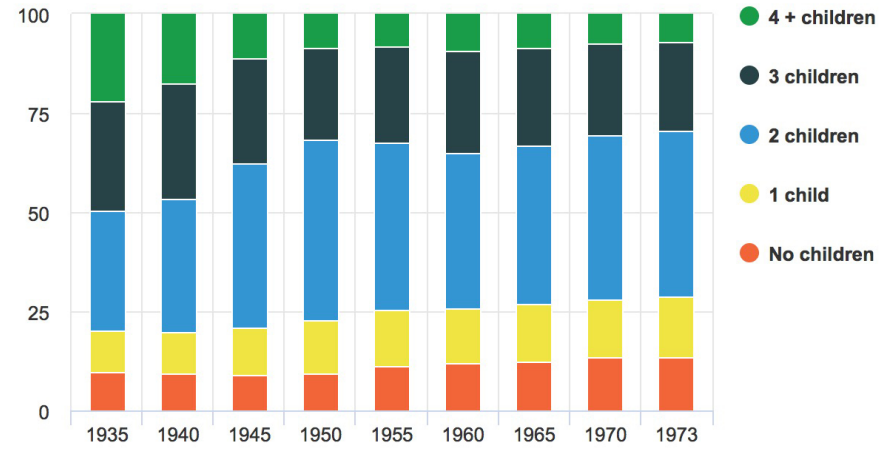
1.1 Background

1.1.1 Fertility trends in Norway, Scandinavia and Europe

In Norway, the total fertility rate (TFR) has over the last 25 years been higher than the Nordic average, with only Iceland having a consistently higher TFR than Norway (13). In Europe, only France, Ireland and the United Kingdom had higher TFR than Norway in 2013 (13). Recent data show an overall decline in TFR in Scandinavia, with Iceland, Norway and Finland showing record-low TFR, while other European countries have followed the same declining trend (14).

Historically, the decline in fertility in Norway from 2.5 (in women born during the 1930s) to the current level has largely been attributed to an increased proportion of women who stopped their childbearing *after reaching two births* (13). A decline in higher order births started during the 1960s and lasted for approximately a decade, leading to final stabilization (with the exception of a smaller decline in 2000-2002) (13). In Norway, we currently have a rather stabile second birth rate, while the major contributors to the fertility decline are suggested to be advanced age at first birth and lower third birth rates (15) (Figure 1).

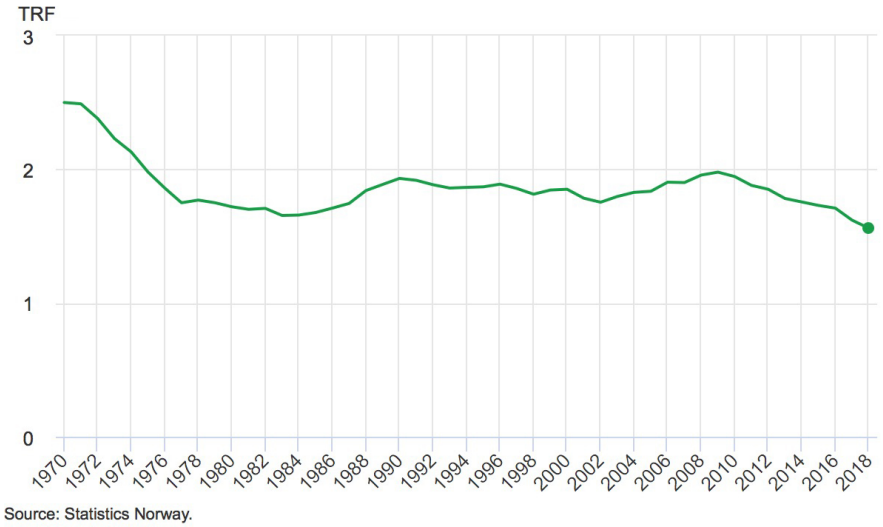
Figure 1. Women in selected cohorts of births, by number of children at age 45 (x-axis: birth cohorts; y-axis: %)



Source: Statistics Norway.

Fertility rates for European countries show a similar decline in TFR from the mid-1960s until the turn of the century (14). At the beginning of the 2000s, fertility in most European countries displayed signs of rising until 2010. After that, we have had a more or less stabile European decline, with more advanced age at first childbirth, similar to the Norwegian trend (Figure 2).

Figure 2. Total fertility rate (TRF) for women, Norway 1970-2018



Source: Statistics Norway.

Across Europe, higher order births are less common today with a decrease in the frequency of large families (14). Most of the fertility declines are reflected in a drop in the proportion of third and higher order births, and an increase in the share of first order births. At the European level, almost half of all households with children had only one child (16). As for Norway, in 2016 it was most common for children to live with one sibling (46%), while 25% lived with two siblings and 8% lived with three or more (17), suggesting a preserved modal family size of 2 children.

Globally, the observed drop in TFR from the 1950s onwards might partly reflect societal changes that have been widely accepted (13, 18, 19). This includes a shift in women's social roles and consequential larger investments in women's education (13). Other social changes such as a shift from agriculture to urban living may also influence the preferable family size, especially in traditional agriculture-oriented regions, with less incentive for families to have more kids that could be a helping hand in farm-work (19). In the ongoing debate, several points have been suggested as key factors for the globally observed fertility decline: fewer deaths in childhood, more women in education and greater access to contraception (18). In Norway, decreased fertility has created political concern, facilitated by the most recent record low TFR (20). Following the year 2009, reports suggest the impact of economic activity, women's work experience and unemployment in the municipality on both postponement of first births and the decrease in higher order births (21). Although second order births remain stable (13, 15, 21), for higher order births, economic stability seems to be becoming increasingly important (21).

From the demographic perspective, differences across developed countries are largely driven by differences in the probability of having a second child (22). In their attempts to investigate demographic, sociological and economic factors that play a role in the decline of family size, Lutz and Skirbekk introduced the term "low fertility trap" to describe countries where it is common to have few children (mostly < 2) (23). In its simplified interpretation, low fertility hypothesis assumes that after some years of stability, ideal family size may enter a period of decline, particularly in those countries that experienced very low actual fertility rates (given that preferences can be influenced by the actual fertility) (23).

1.1.2 The effect of adverse pregnancy outcomes on later reproduction

Desired family size is influenced by various factors, both biological and socioeconomic (including social norms and related personal choices) (1, 24). It has been shown that preexisting poor health can shape women's decision towards future pregnancies (1, 4, 25), as can previous experience of adverse pregnancy outcomes (1, 4). Cesarean section (CS) rates are at increase (24, 26), and accompanying adverse pregnancy outcomes such as preterm delivery (PTD) and small for gestational age (SGA) continue to be public health challenges, despite health system improvements (26). Besides having high co-occurrence and

interrelation (26), it is suggested that these outcomes, along with preeclampsia (PE) are associated with reduced subsequent fertility (1, 12). The effect of CS is a subject of ongoing debate, with implications from some studies that it might pose physical consequences on subsequent fertility (27, 28). The association between subfertility and PTD has been suggested (29), while the recurrence risk of PTD proposes shared maternal-fetal genetic aspects (25, 26). Experience of PE is also found to decrease subsequent reproduction, however, whether the effects are due to biological or psychological consequences remains inconclusive (30, 31). As for perinatal loss, the inverse effect has been proposed, due to its association with increased reproduction (12, 24, 32). In epidemiologic studies, this concept is known as 'selective fertility' and has been described as the tendency to replace losses in order to obtain the desired number of children (32, 33). Besides observed increased reproduction following the death of an infant, this is further supported by decreased reproduction after twin pregnancies (32). Failure to consider this factor in epidemiologic studies when estimating the effects of pregnancy outcomes on future reproduction may lead to erroneous conclusions (33).

Psychological effect. Childbirth represents one of the milestones in women's life and can have a profound effect on women's psychosocial well being (34, 35). Previous traumatic birth experience can, therefore, make woman reluctant to conceive again (1, 4, 25, 35, 36). Fear of childbirth is one of the suggested reasons for avoiding future pregnancies after operative delivery (34). Aligned with this are studies that report reduced subsequent birth rate after CS (35, 37, 38). A study on traumatic birth experience suggested that younger women (<35 years of age) and partnered primiparas who rated their first birth experience as negative were less likely to have a subsequent birth compared with women who rated their experience as average or good (39). In contrast, a Swedish study on 451 primiparous women found that perceived negative birth experience, mode of delivery and adverse pregnancy outcomes were not significantly associated with not having another child (40). The authors attributed such finding to the context of childbirth in Sweden, where women and their partners can talk through their experience with a health care provider in case of an adverse event during the pregnancy or childbirth (40). A more extreme level of fear – tocophobia – has been defined as 'severe fear of childbirth' (36), and has variable prevalence rates (ranging 6 -14%), mostly due to lack of consensus on the definition of tocophobia (34, 35). Notably, tocophobia, vaginal trauma and prolonged labour have been increasingly reported

by women who were not aware of the body's natural ability to cope with childbirth, suggesting the importance of informativeness of women prior to labour (41).

1.1.3 Why Norway is an optimal setting for exploring low parity

As opposed to a large number of European countries that were being caught in the "low-fertility trap", Norway has for years kept its modal family size (2 children) on Nordic average (13). The embeddedness of fertility decisions in social contexts has already been recognized in a study of German fertility (42), while a Finnish study suggested that 'cultural normative' factors account for relatively high levels of higher order births in local social contexts (43). Kravdal describes several factors as a reason for stabile modal family size in Norway in comparison to most other rich countries (13):

1. *Norway's advantaged economic position.* Norwegian per capita gross domestic product is one of the highest in the world, which allowed the creation of a low-risk social environment concerning income, unemployment and social support. In addition, Norway has a low income inequality, where very few are considered poor with low trends of unemployment. This advantaged economic position allowed the government's generous policies and welfare arrangements, leading to low income insecurity for individual families and state support for parental leave. This has also shown its effect after the International Financial Crisis (2008), enabling Norway to contain the impact of the crisis, leading to quite milder recession compared to other European countries (44). Furthermore, widely spread social liberal ideas about gender equality and public responsibility for individual well-being, which are found to positively affect fertility, have strong roots in all the Nordic countries.
2. *Flexible higher education system* that allows students to leave and later re-enter schooling, with readily available educational loans and student daycare. Largely free high quality tertiary education along with paid parental leave tends to lower social pressure to restrict childbearing, which allows women in Norway to raise a family without compromising education and/or career.
3. While the *retreat from marriage* as a traditional family unit has been pronounced as in most rich countries, in Norway this has been largely *replaced by cohabitation*. The relatively high fertility among cohabiting couples might reflect the strong trust in the welfare state and liberal values. It is also possible that factors that might discourage

reproduction among cohabitants tend to be weaker in Norway than in many other countries.

Given that norms to some extent reflect current actual behaviour, higher fertility level in Norway compared to the European average tends to further promote high fertility (13). Aligned with this, despite the corresponding increase in the mean age of mothers with second births, there have been no observed significant change in second birth rates (21). This implicates that most women who become mothers in Norway, will continue their reproduction beyond one child (21). Similarly, in his demographic research in European countries (45), Frejka suggests that it is more common for women with one child to have a second child in Norway than in most other rich countries.

1.1.4 The higher CVD mortality of one-child mothers

Pregnancy exerts complex physiological influences on the female cardiovascular system and other organ systems. Such influences are fluctuations of serum sex hormones, hemodynamic changes, oxidative stress and changes in lipid and other blood profiles (1). It has become apparent that the event of pregnancy per se and women's overall reproductive patterns may have long-term implications for future health. The association between a number of children (parity) and CVD mortality (and/or morbidity) came into focus during the 1980s and 1990s, leading to one of the first important insights in this field (46). The majority (8, 47, 48, 49), but not all (50, 51) of the following epidemiological studies have reported a J-shaped association between parity and CVD mortality, showing the highest risk among nulliparous women and one-child mothers (with nadir of risk in 2 births). In agreement with this, several population-based Norwegian studies found that mortality for late middle-aged women was highest for the childless and mothers with one child (3, 4, 5, 6), with particularly excess CVD mortality among one-child mothers (up to 50%) (4, 5). A recent dose-response meta-analysis of cohort-studies confirmed a non-linear J-shaped association between parity number and CVD risk and reported a 4% increased risk of CVD in women with one lifetime birth (49). Efforts to elucidate the association between the number of children and the risk of female CVD have been inconclusive (1, 8). Explanations range from biological to social, and include: lifestyle risk factors associated with childrearing (7), sex hormone fluctuations, protective effect of future pregnancies (8), and lifestyle factors prior to conception such as elevated blood pressure and obesity (52) as well as metabolic irregularities triggered by

gestation (1). Biological condition as a possible explanation for the observed parity-CVD/mortality association was offered by early studies (53, 54) when different patterns of fertility were reported to be related to changes in post-pregnancy blood lipids and blood pressure levels. It is, however, still unclear if the biological factors are present in women with low parity (one-child mothers) before they conceive.

1.1.5 CVD in women

Historically, cardiovascular disease (CVD) was widely perceived as a public health problem for the male population (55). This was mostly due to the fact that women develop coronary heart disease about a decade later than men, while for myocardial infarction the occurrence is around 10-15 years later (56). The situation, however, reverses itself once women develop the disease. Case-fatality rates are higher for women, following both myocardial infarction and myocardial revascularization procedures: 23% of women and 18% of men die of a heart attack within one year after the event (55). Although death rates from CVD are declining, still more women die of CVD (1, 2). A recent Norwegian publication reported significant declines in the incidence of acute myocardial infarction across all age groups for men, while less steep decline in women is observed, significant only for the older population (above age 65) (2).

Only more recently have we begun to look more into similarities and differences between men and women concerning the structure and function of the cardiovascular system. The beginning of the research in this field dates from the late 1980s with the observation from Steingart's group (57) that women with symptoms suggestive of coronary artery disease were treated less aggressively than man. One reason for this could be that the majority of human data originated from studies of young (18-22 years), healthy, 70kg Caucasian males (58). While the data undoubtedly enabled increased knowledge of cardiovascular physiology, the information has been used to represent the behaviour of humans as a collective, rather than a selective (58). Even though the cardiovascular systems of males and females possess the same structural elements, how those component function to achieve homeostasis differs from subtly to profoundly (58).

Sexual dimorphism of cardiac function

There are clear sex specific differences in the cardiac chamber and coronary artery size, which are suggested to be largely (but not completely) explained by body size differences (59). Sex differences in the composition of the circulating blood have long been a known factor in clinical studies, with the most obvious being a lower number of red blood cells per plasma unit volume, and lower plasma protein levels in females than in males (58). Serum lipid levels also demonstrate sexual dimorphism, with high density lipoprotein (HDL) cholesterol and triglycerides (TGs) being lower in premenopausal woman than in men of the same age (58). Although this 'antiatherogenic' lipid profile was found to be associated with a lower incidence of CVD in these women (compared to the men of the same age), pro-atherogenic changes after menopause reverse the situation: lipid profile of women becomes correlated with a higher incidence of CVD (60). Total cholesterol levels increase with age in both sexes, however, the increase for men plateaus around age 45-50, while for women the increase continues sharply until age 60-65 (61).

Hormonal milieu plays an important role in sex specific differences in the cardiovascular system (62, 63). Not only that it modulates functional characteristics of the cardiovascular system (62), but it is suggested to be the reason for a lower likelihood of coronary artery disease in premenopausal women compared to their male counterparts (62). It is well established that estrogen exhibits immediate (non-genomic) effects (at the cell membrane level), as well as more delayed (genomic) effects (interaction through estrogen receptors at the cell nucleus level) (63). Among its known effects is regulation of vasomotoricity (stimulating production of vasodilatator nitrous oxide) and protective effects again vascular injury (reendothelialization of injured vessels) (64). Estrogen receptors are found both in the smooth muscle cells of the coronary arteries (65) and in endothelial cells (66), therefore it can increase nitrous oxide production by endothelial cells, inhibit their apoptosis (67) and promote their angiogenic activity (65). Estrogen shows an antiatherogenic effect on serum lipids: it raises levels of HDL and lowers total cholesterol, low density lipoprotein (LDL) and lipoprotein A levels. (68). Accordingly, menopausal transition imposes increased vulnerability for the women's cardiovascular system, as all the protective effects of estrogen are substantially diminished due to the drop in its level (68).

1.1.6 Lipids and fertility, the role and potential mechanisms

While the role of serum lipids in cardiovascular health is well established, showing low HDL and high TGs and LDL to be strong predictors of CVD (9), their role in reproduction is uncertain. It is, however, well established that both HDL and LDL deliver cholesterol to the corpus luteum for progesterone synthesis (69). The presence of HDL cholesterol and ApoB in follicular fluid from human oocytes, suggests that these lipids play a more direct role in reproduction (10, 11, 70). Studies have identified these lipid fragments in the follicular fluid as predictors of embryo quality (71) and have reported appreciably higher clinical pregnancy rate and the number of top-quality embryos in high ApoB patients undergoing fertility treatment, compared with low ApoB patients, even after exclusion of ovarian-related disorders (10). In addition, higher HDL levels with large and medium particle size subfractions were associated with poorer embryo quality, while smaller particle size, with more potent antioxidant activity, showed to be beneficial for good embryo quality during IVF (71). Fujimoto et al suggest that there is "evidence that the preimplantation embryo does not have active cholesterol biosynthesis capacity due to a lack of hydroxymethylglutaryl co-enzyme A reductase activity until the blastocyst stage. Thus, the cholesterol required for early embryo development appears to be solely reliant on the intracellular cholesterol levels present in the oocyte prior to fertilization" (69). Previous animal studies have reported the association between dyslipidemia and infertility showing sterility in HDL receptor-deficient female mice (72) while the Longitudinal Investigation of Fertility and the Environment study (LIFE) found concentrations of free cholesterol to be associated with human fecundity in both sexes (73). Posed explanations have been that abnormalities in HDL metabolism including change in structure, concentration or function compromise female fertility (10, 11, 70). It has been suggested that genetic polymorphisms that alter function in proteins engaged in cholesterol metabolism may affect human fertility (74, 75). One possible molecular mechanism could be through a mediating role of HDL on Paraoxonase 1 (PON1) activity. Paraoxonase (PON) is an HDL-associated enzyme that inhibits LDL oxidation, and thus protects cells from oxidative stress (76). Its stability and binding affinity are strongly influenced by changes in the shape and size of HDL particles (77). These changes may lead to decreased antioxidative capacity and consecutively – oxidative stress. Oxidative stress is associated with adverse cardiovascular and fertility outcomes, including atherosclerosis, polycystic ovary syndrome (PCOS), PE, endometriosis and infertility (75, 78). A recent study in women of reproductive age with upper normal ranges of thyroid-stimulating

hormone has suggested a direct link between unfavourable lipid profile and increased oxidative membrane damage (79).

Alterations in serum metabolites may, therefore, be reflected in the oocyte and embryo quality (69). It is suggested that a better understanding of the metabolic effects of various subfertility etiologies may help improve the likelihood of pregnancy (80). Recently, triglycerides to high density lipoprotein (TG/HDL) ratio has been proposed as a simple and reliable marker of insulin resistance given that increased serum TGs and low serum HDL levels often accompany metabolic syndrome (81). Beyond fertility implications, evidence demonstrates that determining the plasma concentration ratio of TG/HDL identifies a subset of apparently healthy individuals who display a cardio-metabolic risk profile that predisposes to CVD (81).

1.1.7 Reproductive health – underused opportunity to improve women's health

Pregnancy imposes great alterations in almost every organ of the women's body, to meet the demands of a growing fetus (82). This is accompanied by physiological metabolic and lipid alterations, reflecting the maternal metabolic response to support fetal growth (82, 83), with accompanying significant hemodynamic changes (84). These changes impose particularly great demands on the cardiovascular system, kidneys and the liver (84). Pregnancy may, therefore, act as a stress test (84, 85, 86) revealing vulnerable organ or the system that fails to adequately adapt its function during pregnancy (82). This has become apparent in women who conceive with chronic conditions (82), and also have implications for assessing common pregnancy complications as a possible indicator of subsequent CVD (86). Of relevance from the aspect of public health, pregnancy can also act to unmask subclinical conditions (or genetic predisposition) in apparently healthy women, which may be exaggerated in later life, when the effects of ageing and/or menopausal transition diminish the reserves of an impaired organ (system) (82). Recent joint recommendation from the American Heart Association and the American College of Obstetricians and Gynecologists highlights the importance of utilization of women's reproductive history as a significant tool and additional source of information to optimize early identification and risk assessment for CVD (86). Aligned with these guidelines, the American College of Obstetricians and Gynecologists and the Task Force on Hypertension in Pregnancy (composed of 17 experts in

the fields of obstetrics, maternal–fetal medicine, hypertension, internal medicine, nephrology, anesthesiology, physiology, and patient advocacy) provided additional recommendations suggesting regular yearly check-ups of CVD health and related blood parameters in women with PE (87).

2. Objectives

The overall aim of this thesis was to explore pre- and post-pregnant lipid levels and adverse pregnancy outcomes and their association with the probability of having no and only one lifetime pregnancy.

The specific aims were:

To explore the extent to which pre-pregnant serum lipid levels of total, LDL and HDL cholesterol, TG and TG/HDL ratio are associated with having no and one lifetime pregnancy (Paper I).

To estimate post-pregnant lipid levels in one-child mothers compared to mothers with two or more children and to assess these lipid's associations with the number of children. (Paper II).

To explore if PTD, PE, SGA and CS at first birth are associated with having one lifetime pregnancy, and assess the modifying effect of perinatal loss (Paper III).

3. Material and Methods

An overview of material and methods is outlined in Table 1, below.

	Paper I	Paper II	Paper III
Aims	To explore the extent to which pre-pregnant serum lipid levels of total, LDL and HDL cholesterol, TG and TG/HDL ratio are associated with having no and one lifetime pregnancy	To estimate post-pregnant lipid levels in one-child mothers compared to mothers with two or more children and to assess these lipid's associations with number of children	To explore if PTD, PE, SGA and CS at first birth are associated with having one lifetime pregnancy, and assess the modifying effect of perinatal loss
Design	Nationwide, population-based historic cohort study from Norway Prospectively collected information.	Nationwide, population-based historic cohort study from Norway Retrospectively collected information.	Nationwide, population-based historic cohort study from Norway Retrospectively collected information.
Data source	CONOR MBRN	CONOR MBRN	MBRN
Population	2 645 parous and 1 677 nulliparous women examined before first childbirth (if any). 1994-2003 (followed for a 2nd birth until 2008)	32 618 parous women examined after first childbirth. 1994-2003 (followed for a 2nd birth until 2008)	882 803 women giving (singleton) childbirth. 1967-2007 (followed for a 2nd birth until 2014)
Outcome	Number of born children	Number of born children	One lifetime pregnancy
Main exposure	Pre-pregnant lipid levels	Post-pregnant lipid levels	Preterm delivery, preeclampsia, small for gestational age, cesarean section and perinatal loss
Stratification by	BMI	BMI, self-perceived health	Marrital/cohabitating status
Adjustments	Age at examination, level of education, time since last meal, smoking, oral contraceptive use and BMI	Age at examination, year of the first birth, level of education, time since last meal, smoking, oral contraceptive use and BMI	Age at first birth, level of education, year of the first birth
Measure of association	Multinomial OR 95% CI	OR 95% CI	RR 95% CI

Table 1. Overview of material and methods in the thesis

3.1 Data sources

The current thesis was based on two main population-based data sources: the Cohort of Norway (CONOR) and the Medical Birth Registry of Norway (MBRN). We also had data on the level of educational attainment from the National Education Database that was used in paper III. All Norwegian residents receive a unique national identification number that enables linkage of these resources.

3.1.1 Cohort of Norway

CONOR is a population-based collection of health data and blood samples provided by participators older than 20 years of age residing in several different regions in Norway during 1994 to 2003, and obtained through several large health surveys (88).

Participants were invited to the surveys based on the 11-digit personal identification number and addresses obtained from the Population Registry of Norway (88, 89). Data collection followed a standard procedure. Letters of invitation were sent approximately 2 weeks before the time of appointment and included a questionnaire, and information about the aims of the study, examinations and procedures. At the examination, the questionnaire was collected from the attendees along with their written consent, and participants underwent a physical examination and a non-fasting blood sample was drawn. The examinations included standardized measurements of height, weight, systolic and diastolic blood pressure and non-fasting lipid levels (total cholesterol, HDL cholesterol, TGs). Trained personnel conducted all procedures (88).

The questionnaire consisted of about 50 questions that addressed a wide range of topics: self-reported health and disease including diabetes, asthma, coronary heart disease, stroke, family history of the disease, risk factors and lifestyle, social network and support, education, use of medications and reproductive history (88).

The administrative responsibility for CONOR was delegated to the Norwegian Institute of Public Health (NIPH) in 2002 and is research collaboration between the NIPH and the Universities in Bergen, Tromsø, Trondheim and Oslo (89).

3.1.2 The Medical Birth Registry of Norway

The MBRN is a population based database with a compulsory notification of all births (stillbirths and livebirths) from 16th week of gestation since 1967 (88). As part of the preventive health program of pregnancy in Norway, all women are examined and interviewed free of charge by a midwife and/or a general practitioner on a regular basis throughout pregnancy. Information on demographic characteristics, medical history, body measures, previous births and previous and current complications and interventions during delivery were collected. The obtained information was regularly updated in the antenatal chart during health visits. At the time of delivery, the antenatal chart is brought to the delivery department and all compulsory data were collected by the attending physician or midwife and transferred to the MBRN (88). Information about the infant (vital status, time of death, anthropometric measurements, Apgar scores and neonatal diagnoses) are also collected. The notification of perinatal death specifies the time of death and gestational age, and for stillbirths specifies the time of death in relation to labour (antepartum, intrapartum or unknown). Gestational age was estimated by last menstrual period until 1998, and on ultrasound estimates thereafter. Ultrasound estimates that are used originate usually from a routine scan in the second trimester. If the information on ultrasound is missing, gestational age estimates are based on the last menstrual period. Detailed data on in-vitro fertilization (IVF) were available from 1988 and were reported from all fertility clinics in the country. As from 1999, the MBRN notification form was extended and revised to include additional information about the mother, the infant and place of birth. Until 1999 maternal health and all outcomes were notified as a free text and coded at the MBRN using ICD-8. As from 1999, checkboxes for the most common maternal and infant risk factors were introduced, as well as for maternal previous health status. Checkboxes are suggested to contribute to more valid information to registries than written text (90).

The main maternal and fetal complications are notified by checkboxes, in addition to available free text in the notification form. This includes PE, CS, and perinatal death. PE is reported by checking one or two of the checkboxes (preeclampsia mild; preeclampsia, severe; preeclampsia, before 34 weeks) (before 1999, PE was notified as either ’preeclampsia’ or the combination of ’hypertension’ and ’proteinuria’) (91). Available checkboxes also include: HELLP syndrome, eclampsia, gestational hypertension

(without proteinuria), and gestational diabetes. Checkboxes for CS include options for planned, emergency and unspecified cesarean section.

Maternal health is reported by checking one of the following options: asthma, allergy, recurrent urinary infection, chronic renal disease, chronic hypertension, cardiovascular disease, rheumatoid arthritis, epilepsy, diabetes mellitus (type 1, type 2) and other.

Validation studies have shown a very good ascertainment of pre-gestational diabetes (92) and that most women labelled with gestational hypertension in the MBRN have hypertensive disorders of pregnancy (93). PE registration in MBRN (91) along with other disease and pregnancy complications have shown acceptable accuracy (93), however, the possibility of underreporting of milder forms of disease and diabetes type 2 (before 1999) might be possible (91).

3.2 Study populations and design

Paper I

This is a historical population based cohort study, with data obtained from CONOR and MBRN. The study population consisted of 2 645 women giving birth to their first child during 1994 – 2003 subsequent to their participation in CONOR (488 one-child mothers and 2157 women with ≥ 2 births of younger reproductive age 20-40) with linked data from the MBRN, and 1 677 nulliparous women (see Figure 3 for a flowchart with inclusions and exclusions for the study population). All parous women were examined before their first childbirth. This yielded a total of 4743 women in the study. Sub-analyses included only women with reported partners as a proxy for exposure to pregnancy (ever) (228 nulliparous and 216 mothers with ≥ 2 births).

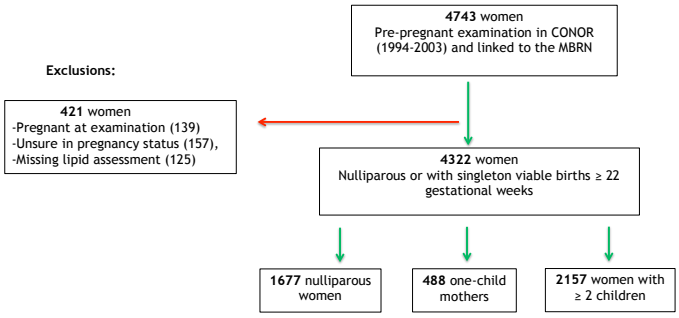


Figure 3. Flow-chart of participants in Paper I

Paper II

This is a historical population based cohort study, with a collection of data from CONOR and the MBRN. The study population consisted of 32 618 parous women (4 490 one-child mothers and 28 128 women with ≥2 children aged 20-69 years) examined after first childbirth as a part of CONOR (1994-2003) with linked data on reproduction and number

of children from the MBRN (1967-2008) (see Figure 4). To avoid misclassification of births that occurred before 1967, we included only women who reported no other births than those registered in the MBRN.

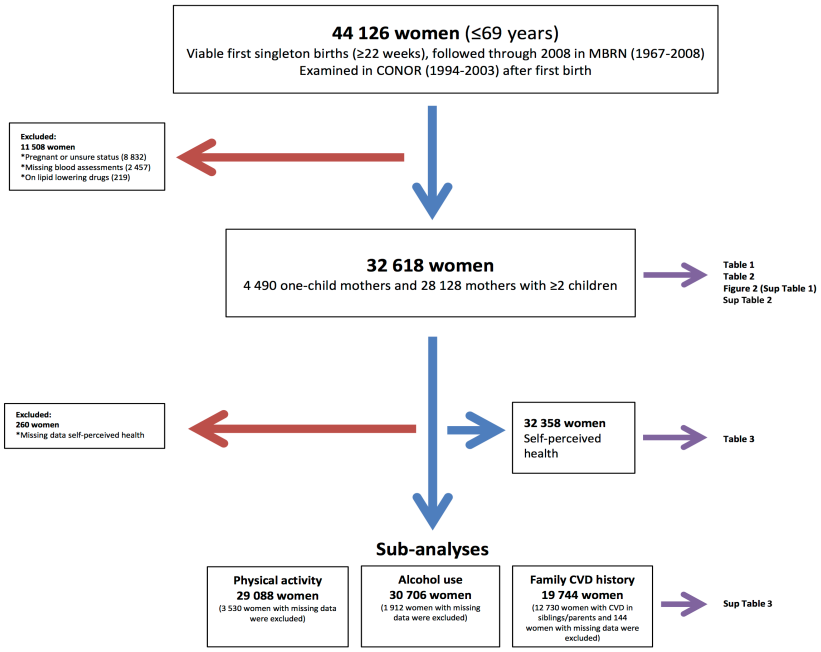


Figure 4. Flow-chart of participants in paper II

Paper III

This is a historical population based cohort, with data obtained from the MBRN and the National Education Database. We used personal identification numbers to link all births to their mothers, creating a sibship structure, with the mother as the observation unit. The

study population consisted of 882 803 mothers giving birth to their first singleton infant (≥22 gestational weeks) during 1967 to 2007 who were followed for the occurrence of second birth in the MBRN until 2014 (see Figure 5 for a flowchart with inclusions and exclusions for the study population).

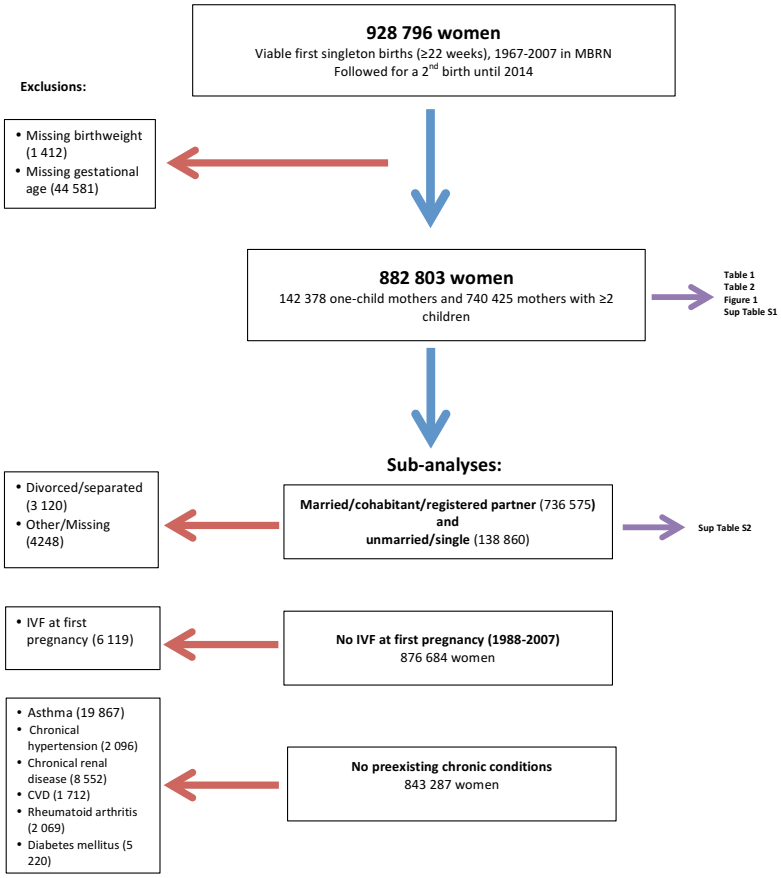


Figure 5. Flow chart of participants in Paper III

3.3 Exposures, outcomes and confounding factors

3.3.1 Exposures

Papers I and II: Non-fasting lipid levels

In CONOR lipid levels of total, HDL cholesterol, and TG levels were taken in non-fasting state and measured by an enzymatic method. We calculated LDL using the Friedewald formula (94): Total serum cholesterol minus HDL cholesterol minus one fifth of the TG concentration. Due to the lower precision of calculation with highly increased TG levels, LDL cholesterol levels were calculated only for participants with TG concentrations < 4.5mmol/l (94). TG/HDL-c ratio was calculated as: TG concentration divided by HDL cholesterol concentration. We additionally used non-HDL cholesterol levels (calculated as total cholesterol minus HDL cholesterol) as a useful tool in individuals with higher TG levels (95) (paper II). We used total (mmol/l), LDL (mmol/l) and HDL cholesterol (mmol/L), and TG (mmol/L) and TG/HDL-c ratio (mmol/l) levels as categorical variables, calculating lipid quintiles. Lipid quintiles were calculated accordingly, on a total sample prior to pregnancy in paper I as well as on a post-pregnancy sample (paper II).

Laboratory measurements (Papers I and II)

Non-fasting blood samples were obtained by trained personnel and analyzed on a Hitachi 911 Auto Analyzer (Hitachi, Mito; Japan) (88). Serum concentrations of total cholesterol, HDL cholesterol and TG were analyzed subsequent to sampling, with the use of reagents from Boehringer Mannheim (Mannheim, Germany). Total cholesterol and HDL cholesterol were measured by applying an enzymatic colourimetric cholesterol esterase method, with HDL cholesterol measured after precipitation with phosphortingsten and magnesium ions. An enzymatic colourimetric method was applied for measuring TG, while glucose was measured by using an enzymatic hexokinase method (96). The day-to-day coefficients of variation were: total cholesterol: 1.3%-1.9%; HDL cholesterol: 2.4%; TG: 0.7%-1.3% and glucose: 1.3-2.0%.

Paper III: Adverse outcomes in the first pregnancy

All exposure variables were obtained from the MBRN. Perinatal death included stillbirths and neonatal deaths within 7 days after delivery. Stillbirth was defined as stillborn with gestational age ≥ 22 completed gestational weeks. PTD was defined as delivery occurring before 37 completed weeks. SGA was defined as infant birth weight below the tenth percentile by gestational age and sex, based on a previous standard using data from MBRN (97). PE was defined as any recording of blood pressure of ≥ 140/90 mmHg after 20 weeks of gestation with proteinuria ≥ 0.3 g/L per 24 hours, and included all forms of PE, eclampsia and syndrome of hemolysis, elevated liver enzymes and low platelet count (HELLP), in accordance with recommendations by the Norwegian Society of Gynecology and Obstetrics (98). To account for time trends of PTD, SGA, PE and CS, we analyzed our data by the following periods: 1967-1976, 1977-1986, 1987-1996, and 1997-2007. We additionally examined the characteristics of the included women (maternal age, educational level, cohabitating status) during the same periods.

3.3.2 Outcomes

In *paper I*, the main outcomes were having no and one lifetime pregnancy (relative to 2 or more pregnancies). Using the unique national identification number, each woman was linked to all her subsequent births (if any) in the MBRN after participating in CONOR (prior to pregnancy). Women reporting no children in CONOR at the time of examination and with no valid records in the MBRN were considered having no pregnancies. We defined one-child mothers as women being 6 years out from their first pregnancy and with no additional births registered in the MBRN. To extend each woman's likelihood of completing her birth record, we separately examined women who were 7 years out from their first pregnancy. About 95% of Norwegian women will complete their second pregnancy within 7 years (3). In a subanalysis we used the presence of a partner (ever) as a proxy for exposure to pregnancy among nulliparous women, (nulliparous vs. women with ≥ 2 births), including only women with a reported partner (ever).

In *paper II*, the outcome was the probability of having one lifetime pregnancy (relative to 2 or more pregnancies). One child mothers were defined as women being 7 years out

from their first pregnancy and with no additional births in the MBRN, who participated in CONOR after their first childbirth. To minimize the effect of ageing, we included only women ≤69 years of age.

In *paper III*, the main outcome was the risk of having one lifetime pregnancy (relative to 2 or more pregnancies). One child mothers were defined as women being 7 years out from their first pregnancy and with no additional births registered in the MBRN (i.e. we included first births that occurred before 2007 and all women were followed until 2014 for the occurrence of a second pregnancy).

3.3.3 Potential confounders

From CONOR (Papers I and II)

In *Paper I,* the covariates were obtained from CONOR and based on a sample prior to pregnancy. These included age at examination, level of education (categorized in: <11 years and >11 years of education), smoking (current smoker: yes, no), time since last meal (linear term), oral contraceptive (OC) use (now, previously, never) and body mass index (BMI) (linear term). Age at examination/participation in CONOR was used as a continuous variable in all analyses in papers I and II. Age is associated with both lipid levels and female fertility (68, 78), and these data were complete. Attained level of education was obtained from standardized questionnaires and was missing for 49 (1.13%) women. Educational level is a commonly used measure of socioeconomic status in epidemiologic research (99), and can, therefore, serve as a proxy for lifestyle factors. Smoking questions included two options (daily smoker now or not a daily smoker now). We classified women as 'current smoker' or 'not a current smoker' (yes or no). There was 20 (<0.5%) women with missing data on smoking. Smoking adversely influences female fertility (100) with most of its effects attributed to HDL cholesterol decrease (101). However, the smoking status of participants was only available at enrolment of the study, and this represents a limitation. Frequency of smoking (number of cigarettes per day), as a measurement of smoking intensity, was not taken into account due to a high number of missing data on this variable. Time since last meal was used as a continuous variable to account for temporal proximity of food intake, and these data were complete in the pre-pregnancy sample. Recent food intake can affect TG levels, while cholesterol

levels seem to be less sensitive (102). OC use was assessed by questions on the use of contraceptive pills, which included three options (use or ever used contraceptive pills: now, previously, never). OCs are shown to affect both serum level of TG (68, 73) and female fertility. There were 20 (<0.5%) women with missing data on OC use. Body mass index (BMI) was used as a continuous variable. BMI was calculated as kilograms per meter squared (kg/m^2) and both body weight (kg) and height (cm) were measured according to a standard protocol in CONOR. These measurements were manually recorded by the year 2000, and with an electronic Height and Weight Scale thereafter (96). BMI is a factor known to affect serum lipid levels and is also found to be associated with lower fertility (103, 104). We also assessed the effect of BMI by stratifying our results by women's pre-pregnancy BMI (paper I), as well as post-pregnant BMI (paper II).

In *Paper II*, we used the same covariates as in *paper I*, however, based on a sample after the first pregnancy. In addition, we used the variable 'year of first birth'. The temporal proximity of childbirth is associated with both lipid levels and chances of subsequent pregnancy, as recent pregnancy affects serum lipid levels and conception (83). Besides accounting for time elapsed since first birth, year of first birth was also used as a proxy for generational/environmental factors (105). This covariate was not a significant predictor in the pre-pregnancy sample (Paper I), and was, therefore, not included in the model.

Questions on self-perceived health included 4 options (What is your current health status: poor, not so good, good, very good). Answers 'poor' and 'not so good' were classified as 'bad', while 'good' and 'very good' were classified as 'good' perceived current health. Self-perceived health (good and bad), education (<11years and >11 years) and BMI (<25 and ≥25) were also assessed in stratified analyses. Missing data were low for the majority of parameters, and were as follows: education 276 (0.8%), smoking 230 (0.70%), time since last meal 1416 (4.3%), OC use 1319 (4.0%), BMI 51 (0.16%), self perceived health 220 (0.8%).

In a subanalysis, we explored the family history of CVD by using women with reported heart attack and/or angina in siblings and/or parents (missing data: 0.4%). Information on alcohol use and vigorous physical activity was missing for 5.8% and 10.8% of women, respectively, and was therefore only included in subanalyses.

From the MBRN/the National Education Database (paper III)

In *Paper III*, the covariates included maternal age at first birth, education and year of childbirth. Maternal age at first birth was used as a continuous variable. Maternal age is a factor associated with both female fertility and adverse pregnancy outcomes (26). The educational level contains information on the highest attained education. Data on education was missing for 1.1% of women, while maternal age and year of childbirth were complete. Year of firstchildbirth was used as a continuous variable and was applied to account for potential cohort effects (105). Marital status included categories: married, cohabitant/registered partner (registered from 1977 and 2008, respectively), unmarried/single, divorced/separated (including widowed) and was included in a subanalysis. Missing data for this variable was 0.4%.

3.4 Statistical analyses

Paper I

Analyses were performed using the Statistical Package for Social Sciences (SPSS, version 22.0 and 23.0, Chicago, Illinois). Characteristics of the analyzed women were presented as means with standard deviations for continuous data and as numbers with percentages for categorical data. Differences between nulliparous women, one-child mothers and mothers with two or more children, as well as pre-pregnant health status were analyzed by Chi-square tests and t-tests where appropriate. Linear associations across pre-pregnant lipid levels (in quintiles) for no and one lifetime pregnancy were assessed by p-values for trend. Odds ratios (OR) with 95% confidence interval (CI) of no and one lifetime pregnancy by lipid levels and TG/HDL-c ratio, when compared to women with two or more pregnancies were calculated using multinomial logistic regression and adjusted for mother's age at examination, level of education (categorized in: <11 years and >11 years of education), smoking (current smoker: yes, no), time since last meal, OC use (now, previously, never) and BMI (linear term). To test the effect of (pre-pregnant) BMI, we stratified the main analyses by BMI (<25 and ≥ 25). To avoid influence from age at first delivery on the number of children, we excluded women older than 34 years at the time of first delivery in a subanalysis. Additionally, we performed sensitivity analyses including only mothers who were 22-30 years old at the time of first delivery. Using the presence of a partner as a proxy for exposure to pregnancy among nulliparous women, we performed logistic regression in a sub-analysis (nulliparous vs. women with ≥ 2 births) including only women with a reported partner.

Paper II

Statistical analyses were done using SPSS, version 25, Chicago, Illinois. Baseline characteristics were presented as means with standard deviations (continuous data) and numbers with percentages (categorical data). Differences between lipid quintiles were assessed by p values (Wald test) and between one-child mothers and mothers with two or more children, using Chi-square test and t-test, where appropriate.

We used logistic regression to calculate ORs for one lifetime pregnancy by lipid levels. Estimates were adjusted for mother's age at examination (linear term), year of first birth (linear term), BMI (linear term), OC (now, previously, never), smoking (at examination:

yes, no), education (≤11 years (low), >11 years (high)) and time since last meal (linear term). Besides accounting for time elapsed since first birth, year of first birth was also used as a proxy for generational/environmental factors (105). OC use was defined as current use of OC, previous use or never. Effect of BMI (<25 and ≥25), self-perceived health (good and bad) and education (high and low) were also assessed in stratified analyses. Answers 'poor' and 'not so good' were classified as 'bad', while 'good' and 'very good' were classified as 'good' perceived current health. We performed a sensitivity analysis on women <40 years of age to explore the effect of menopause on women's lipid profile. Missing data were low for the majority of parameters, and were excluded from the main analyses, except for the OC use. Due to higher numbers of missing values for serum glucose, this variable was excluded from further analyses.

We compared the occurrence of IVF in the first pregnancy, diabetes, use of antihypertensive medications, PCOS, and thyroid disease between one-child mothers and women with two or more children. We also excluded women using antihypertensives in the main analyses.

In subanalyses, we explored the impact of past-year physical activity (≤1 hour per week and ≥1 hour per week) and alcohol use (≤1 time per month and >1 time per month). We also excluded women with reported heart attack and/or angina in siblings and/or parents, with the additional exclusion of women with diabetes in parents.

In order to assess how robust the associations are to potential unmeasured confounding, we calculated E-values (106) for both the main analyses and sensitivity analysis on women <40 years of age. The E-vale is defined as "the minimum strength of the association, on the risk ratio scale, that unmeasured confounder would need to have with both the exposure and the outcome to fully explain away this exposure-outcome association, conditional on the measured covariates" (106).

Paper III

Statistical analyses were performed using Stata Software, version 15 (StataCorp. College Station, TX, USA). Characteristics of the included women were presented as numbers

and proportions. Differences between one child mothers and mothers with two or more children were analyzed by t-test and Chi-square tests. For each of the adverse outcomes of pregnancy (PTD, SGA, PE and CS) a four-category variable was created: 1) women with the outcome and a live infant, 2) women without the outcome and with loss 3) women with the outcome and loss and 4) women with no adverse outcome and no loss (reference category). Each appropriate category of complication included only the complication in question (stated) and no other observed complications. We used generalized linear models for the binary family to calculate crude and adjusted relative risks (RRs) with 95% CI for having one lifetime pregnancy in women experiencing the adverse pregnancy outcomes compared to women without these conditions. Analyses were adjusted for maternal age as a six categorical variable (years: <20, 20-24, 25-39, 30-34, 35-39 and >40), attained education as a two categorical variable (years: <11 and > 11) and year of first childbirth (continuous). We also constructed a composite variable that combined the effect of two, three and all four outcomes on the risk of having one lifetime pregnancy, and stratified this analysis by perinatal loss. We explored possible interactions between the observed outcomes and perinatal loss by examining the statistical significance of interaction terms. Due to an overall higher risk of pregnancy complications among women with multiple pregnancies (24), we included only women with singleton first births in our analyses. In subanalyses, we excluded women with preexisting diabetes, previous hypertension, IVF pregnancies (from 1988) and women with chronic conditions (asthma, chronic renal disease, cardiovascular disease and rheumatoid arthritis) and performed stratified analyses on marital/cohabitant status (to examine the effect of having a partner). We truncated the data to allow all included women at least a 7-year timeframe to complete their birth record (i.e. we included first births that occurred before 2007, and all women were followed until 2014 for the occurrence of a second pregnancy) (3). Due to the low number of missing cases (1.1% for education and 0.48% for marital status), these were handled by a listwise deletion in the analyses. Additional exclusion of women with missing data on education and cohabitation showed no effect on our results. The proportion of missing data for other variables were low: birthweight (0.16%) and gestational age (5.0%), and these were excluded from our calculations for the 4-level variable.

3.5 Ethical considerations

All three studies were approved by the regional ethical review board REK-Vest (Ref numbers: *Paper I and II:* 2013/118; *Paper III:* 2009/1868) and access to data was granted by the Steering Committee for CONOR and by the MBRN.

Our studies used banked blood samples collected in CONOR, and subjects were not re-contacted for the analysis. Written informed consent included use for research and linkage to health registries, and was obtained for each participant. Personal identification numbers are omitted from data when used in research purposes.

4. Summary of main results

4.1 Paper I

There were 4 743 women with a baseline assessment of lifestyle factors in CONOR (1994-2003) that were linked to the MBRN. We excluded 421 women with pregnancy at the time of examination (n =139), unsure pregnancy status (n =157) and missing lipid assessments (n =125). Thus, 4 322 women were included in the analyses (1 677 nulliparous, 488 one-child mothers and 2 157 women with ≥ 2 births). Sub-analyses included only women with reported partners (228 nulliparous and 216 mothers with ≥ 2 births).

Assessed in quintiles, higher pre-pregnant TG and TG/HDL ratio levels were associated with increased risk of one lifetime pregnancy compared to having ≥ 2 children. Compared to the highest quintile, women in the lowest quintile of HDL cholesterol had an increased risk of one lifetime pregnancy (OR 1.7 95% CI 1.2-2.4), as were women with the highest LDL cholesterol, TG and TG/HDL ratio quintiles (compared to the lowest) (OR 1.2 95% CI 0.8-1.7; OR 2.2 95% CI 1.5-3.2; and OR 2.2 95% CI 1.5-3.2, respectively). Similar effects were found in women with BMI ≥ 25 and the highest LDL and total cholesterol levels in risk of lifetime nulliparity.

Nulliparous women were older at the time of examination, had higher BMI and were more frequent smokers compared with women with two or more births. A higher proportion of nulliparous women had >11 years of education. One-child mothers had higher mean age both at the examination and at delivery (29.5 vs. 26.7 and 32.3 vs. 29.9, respectively), were more often smokers and had lower education than mothers with ≥ 2 births. The mean BMI prior to pregnancy was higher in one-child mothers (24.2 vs. 23.5), whereas mean years from examination to first delivery were similar for the two groups. Women with no and one child were less frequent users of OCs at the time of examination compared to mothers with ≥ 2 births (27.4%, 34.6% and 48.9%, respectively).

The proportion of diabetes at first delivery in one-child mothers was higher than in women with two or more births (1.4% versus 0.9%, p =0.30). A significantly higher number of one-child mothers had IVF in their first pregnancy (7.2% versus 2.6% in women with ≥ 2 births, p< 0.001).

Similar effects of pre-pregnant lipids as in one child mothers were observed when subanalysis (nulliparous vs. ≥ 2 births) were performed on women who had a partner (as a proxy for ever being exposed to pregnancy). In women with a partner, risk of having no children was increased among women in the highest quintiles of TG and TH/HDL-c ratio (compared to the lowest quintiles) and also for those in the lowest HDL quintile (compared to the highest) (OR 1.9, 95% CI 0.9-4.2; OR 2.0, 95% CI 1.0-4.1; and OR 1.6, 95% CI 0.7-3.6, respectively).

4.2 Paper II

We used data on 32 618 parous women (4 490 one-child mothers and 28 128 women with ≥2 children) examined after first childbirth as part of CONOR (1994-2003) with linked data on reproduction and number of children from the MBRN (1967-2008). ORs for one lifetime pregnancy for the highest quintiles of LDL and total cholesterol (compared to lowest quintiles) were 1.30 (95%CI: 1.14-1.45) and 1.43 (95%CI: 1.27-1.61), respectively. Additional analyses on non-HDL cholesterol showed similar results as for LDL levels. Sensitivity analysis (women <40 years) showed no appreciable change in our results. In stratified analyses, estimates were slightly stronger in overweight/obese, physically inactive and women with self-perceived bad health

One-child mothers had significantly more IVF in the first pregnancy (1.3% vs. 0.1%, p<0.001), were more frequent users of antihypertensive medications (3.6% vs. 2.9%, p=0.01), had slightly higher proportion of stroke (0.6% vs. 0.4%, p=0.05) and a significantly lower proportion of thyroid disease (0.5% vs. 1.0%, p<0.001), compared to women with two or more children. Exclusion of all women with thyroid disease from our main analyses did not affect the results. Diabetes (1.1% vs. 0.9%) and history of heart attack (0.2% vs. 0.1%) were not significantly different in one-child mothers and women

with two or more births. Exclusion of women on antihypertensive therapy did not alter the main results.

E-value calculations showed that an unmeasured confounder would need to have nearly four times as large an effect as maternal age (covariate with the strongest effect in the adjusted model, with Exp (B)=1.13), and be associated with both the exposure and the outcome to completely explain away the observed associations (106).

After excluding 12 730 women with reported CVD in parents or siblings and 144 women with missing information, risks of one lifetime pregnancy by lipid quintiles increased slightly across LDL and total cholesterol levels. Additional exclusion of diabetes in parents did not affect the results. Stratified analyses on alcohol use showed the only slight modifiable effect of alcohol on lipid levels.

4.3 Paper III

We identified 882 803 women giving birth to their first singleton infant (≥22 weeks) between 1967 and 2007, of which 16 % had only one lifetime pregnancy. These women were older at first delivery, had less education and a higher proportion of unmarried compared to women with two or more births. In women with pregnancy complications where the infant survived the perinatal period, RRs for one lifetime pregnancy were increased (PTD: 1.21 [1.19-1.22], SGA: 1.13 [1.12-1.15], PE: 1.09 [1.07-1.11], CS: 1.24 [1.23-1.25]), but significantly reduced if the child was lost (PTD: 0.63 [0.59-0.68], SGA: 0.57 [0.51-0.63], PE: 0.69 [0.59-0.80], CS: 0.67 [0.56-0.79]), compared to women with no perinatal loss and no adverse outcome.

Tests of interaction between PTD, SGA and CS at first birth and loss showed a significant effect on the risk of having one lifetime pregnancy (P=0.001, P=0.005 and P<0.001, respectively). We found no significant interaction between PE in first birth and loss (P=0.47).

Exclusion of women with preexisting diabetes, hypertension and IVF pregnancies (from 1988) did not substantially change the results. Additional exclusion of asthma, chronic renal disease, cardiovascular disease and rheumatoid arthritis showed only slight effect

for women with a surviving child (PE: (crude) RR 1.15, 95% CI 1.13-1.17 and CS: (crude) RR 1.65 95% CI 1.63-1.68), while for women with no loss results remained unaltered. Stratification by marital/cohabitating status showed a decrease in RRs of having one lifetime pregnancy among unmarried and single women with observed complications, implicating effect of having a long-term partner. In our examination of changes in maternal characteristics throughout 4 decades of MBRN, we found an increase in the proportion of one child mothers (first vs. last decade: 14.4 % vs. 18.4%) with more than doubled proportion of women aged ≥35 at first birth. In the same group, a decrease in the number of single women for almost one third was accompanied with a 7.6% increase in education level. Similar to the results for the separate effects of the outcomes, the analyses for the composite variable showed that the increased risk of having one lifetime pregnancy consistently varied by the survival of the infant in the perinatal period.

5. Discussion

5.1 Methodological considerations and limitations

5.1.1 Design

The main advantage of registry data is that they are readily available (107) and comprise a collection of data for several decades, offering unusual possibilities for research across generations (107). As suggested in a recent commentary (107), the MBRN along with other Nordic Medical Birth and population registries and tissue biobanks, are considered "a potential goldmine for future clinical and epidemiological research". The lack of biological material is a disadvantage in ordinary register-based research (107), however, in this thesis we were able to link national cohort data (CONOR) with biological material (papers I and II) and register data from the MBRN, bringing a new possibility to explore the outcomes of interest. Women who gave birth at the time of the establishment of the MBRN (1967) have now reached the age of 60-80 years, enabling us to investigate the relation between prepregnant health, health in midage, as well as the effect of reproductive history and pregnancy complications in this age group. This, however, may not apply for the younger cohorts of women (i.e. Paper I), which is a common limitation in the registry-based studies. Another limitation of registry-based design is that data consists of predefined variables (not available for researchers to influence data collection), however, this is outweighed by the great number of study objects available for research. This makes the detection of moderate risk factors possible (paper III) (107), and also provides a unique opportunity to explore the effect and associations of rare events (perinatal death, PTD) (paper III).

5.1.2 Precision

As precision refers to the lack of random error, options to increase precision include increasing study size and modifying study designs (108). Since the confidence intervals reflect both the strength and the direction of the association, these parameters also offer information on precision and random variation of the estimates (109). In *papers II* and *III*, the study sizes were large, with generally precise association measures and narrow

confidence intervals. For *paper I*, the study size was smaller with wider confidence intervals, however, the precision of the estimates was not problematic. In sub-analyses, fewer participants may have hampered the results.

5.1.3 Validity

Internal validity

Internal validity can be referred as “the degree to which a study is free from bias or systematic error” (110), and implies the validity of a conclusion to the source population (109). There are three main sources of bias that can violate the internal validity: information bias, selection bias and confounding (109).

Information bias (misclassification)

Information bias or misclassification occurs when there are measurement errors in the information about study participants, the exposures or the outcomes. These errors can lead to non-differential or differential misclassification. Rothman describes these in the following way: "For exposure misclassification, the misclassification is nondifferential if it is unrelated to the occurrence or presence of disease; if the misclassification of exposure is different for those with and without the disease, it is differential. Similarly, misclassification of disease [outcome] is nondifferential if it is unrelated to the exposure; otherwise, it is differential" (109). In large registry-based data, despite registry quality controls, errors in registration of both outcomes and exposures may be seen. However, any misclassification of this type would most likely be non-differential.

Misclassification of exposures

Very few women in our samples had a lack of information about the main exposures (lipid assessments and pregnancy complications). Lipid assessments were obtained from CONOR, where trained and experienced personnel collected and measured levels of

total, HDL cholesterol, and TG levels, taken in a non-fasting state. Laboratory measurements were performed by an enzymatic method (88). Due to better capture of dose-response effect, we used quintile distribution instead of cut-off point or continuous variable. Accordingly, quintiles were calculated on a total sample of women prior to pregnancy (paper I), and on a total sample after pregnancy (paper II). In *paper I*, we had missing data on 16 cases of LDL, 2 cases of TG, and 2 cases of TG/HDL ratio. In *paper II*, all missing lipid level assessments were excluded from the analyses, and additional missing value exploration has not shown any disturbing patterns.

Non-fasting state of participants when collecting lipid levels is stated as a limitation in this study, as TG levels are more sensitive to recent food intake, while cholesterol levels seem to be less affected (102). However, adjustments for time since last meal did not show appreciable changes in results in Paper I and Paper II. In addition, since it is unlikely that women's fasting status is related to the number of children, any bias arising from this is likely non-differential. Non-fasting lipids have successfully been used in lipid and CVD research (73, 111, 112).
Moreover, non-fasting TG levels have been suggested to show a better predictive capability for CVD in women than fasting TG levels (113). Measurement errors during blood sampling and laboratory assessments cannot be excluded. However, the automatization of the process and standardization of the laboratory measurements, along with sampling performed by highly trained and experienced personnel (88, 96) minimizes the likelihood of bias.

In *paper III*, information on the exposures was extracted from the MBRN. National guidelines for diagnosis and monitoring of maternal and fetal complications have been updated regularly since 1995, but lack of adherence may be present. (114). Although we had limited registration of ultrasound based gestational age (from 1999 onwards), possible errors in gestational age estimates (last menstrual period vs. ultrasound-based) are not likely to be substantial in MBRN (25). Validation studies showed good ascertainment of PTD with PPV 90-93% (93). Although overall reporting of PE was satisfactory (91), the underreporting of milder cases might be present. While the experience of PE might be linked with the number of children finally born by a woman, for the mild forms, unrecognized by either a mother or health personnel, this is not likely

to be the case. Therefore, if present, this would represent a non-differential misclassification.

Misclassification of outcomes

Given the lack of information on women's reproductive planning in our data in paper I, the heterogeneity of causes for childlessness among nulliparous women may be present (including women who are voluntary childless or have not been exposed to pregnancy (ever)). Thus, the increase in risk of having no children may be underestimated in paper I. We tried to address this in subanalyses including only women with a reported partner as a proxy for exposure to pregnancy among nulliparous women. We found that the results for nulliparous women were similar to our main results for one-child mothers. Women with a reported partner had a higher risk of having no children (compared to partnered women with ≥ 2 births) if their TG and TG/HDL-c ratio levels were in the highest quintiles and HDL in the lowest quintile. These findings support the role of serum lipids in lifetime nulliparity among women with partners.

In *paper I* we defined one-child mothers as women being 6 years out from their first pregnancy and with no additional births registered in the MBRN. It is possible that this selection may include some mothers that gave birth to a second child during the 7^{th} year, thus being a 2+-child mother. Misclassification of the outcome will generally bias the results towards the null, so the true effect may be greater. However, truncation of data to extend the time to 7 years did not appreciably alter the results (subanalysis). Thus, we conclude that the misclassification of one-child mothers is not likely to have occurred.

In *paper II,* we used the same principle of identifying one-child mothers and 2+ child mothers as in paper I. The 7-year follow up on successive delivery allows sufficient observation time for new childbirths to occur (3). The big sample size and long follow up for the occurrence of a second birth further minimized the risk of misclassification of outcomes. In *paper III*, identification of one-child mothers and mothers of 2+ children was based on a large sample size from MBRN. We had a complete registration of all births linked to the mother through her unique personal identification number. One-child mothers were defined as women being 7 years out from their first pregnancy and with no additional births registered in the MBRN.

Potential confounders

As defined by Rothman (109), confounding refers to a mixing of the effect of the exposure with the effect of another variable on the outcome. Confounder, thus, represents variables that are associated with the exposure and with the outcome but is not presumed to be a causal consequence of the exposure (109).

Confounders may be stratified on or adjusted for to block confounding paths (108). For the studies included in this thesis, confounders were identified a priori, based on existing literature.

In *paper I,* adjusting for age at examination, level of education, smoking, OC use, and BMI led to the strengthening of results while adding time since last meal to the model showed minimal effect. Time from examination to first birth showed not to be a significant predictor (and showed no effect on our results), and was therefore not included in the model. To study more closely the effect of prepregnancy BMI, we stratified analyses by BMI. This led to the strengthening of results in the strata of women with BMI ≥25, while the risks remained, albeit slightly attenuated in BMI<25.

In *paper II*, we both stratified analyses and adjusted for a range of potential confounders, similar to paper I, with additional adjustments for year of the first birth. We found that age at examination, OC use and BMI attenuated the results, while adding smoking, education and year of the first birth strengthened the results. Time since last meal only slightly attenuated the risks. We further stratified analyses by BMI, family history of CVD, selfperceived health status and education. We explored the effect of menopause in a sensitivity analysis, selecting the women below 40 years of age. This provided only slight attenuation from our main results. In subanalyses, we stratified by alcohol use and vigorous physical activity. The physical activity showed decreasing effects on lipid levels. Alcohol use showed a stronger effect on LDL levels, while for the total cholesterol levels we found OR alteration in low frequency users and decreased OR for high frequency users. This may reflect a reluctance to report drinking frequency in the low frequency group or that abstinence from alcohol is marker of other unmeasured risks (111).

The common limitation for all three studies in this thesis was the lack of information on women's family decision planning. Unfortunately, these types of data are not routinely collected in birth registries and health surveys and are available only in appropriate specialized clinics and/or surveys, which usually suffer from small sample size. In *paper I*, we tried to address this by using the presence of a partner and/or marital status as a proxy for exposure to pregnancy. In addition, covariates in *papers I* and *II* are measured at one point in time (CONOR) and changes in risk factors over time could not be assessed (i.e. the smoking status of the participants was available only at the enrollment in the study). Furthermore, we lacked information on apolipoprotein E genotype, c-reactive protein (CRP), sex hormone status dietary intake or stress and thyroid tests/antibodies, factors that are all found to affect female fertility (79) and residual confounding by those factors cannot be excluded. However, we additionally calculated E-value to assess the robustness of the associations to potential unmeasured confounding (Paper II). In addition, the exclusion of women with thyroid disease in Paper II showed no effect on our results. Lastly, we had only 4 cases of PCOS in our samples, thus under-reporting might be present.

In *paper III* we adjusted for a limited number of potential confounders. For women with complications and no loss, change of RR towards the null after adjustments suggests that the observed associations between adverse outcomes and the probability of one lifetime pregnancy were partially explained by adjusted factors, with maternal age at first birth changing the estimates the most. The associations, however, became stronger after adjustments for women with complications and a loss. Smoking and BMI have not been registered in the MBRN before 1999 and 2006, respectively. Due to truncation of our data to first births before 2007, we did not include smoking and BMI in adjustments in order not to decrease the power of the study. The lack of adjustments for smoking and BMI represents a limitation in the study. Level of education may serve as a proxy for BMI as higher education correlates with lower BMI (115) and adjustments for education may partially have accounted for BMI. Smoking is associated with a higher risk of pregnancy complications and stillbirth (100). However, we do know that smoking among pregnant women in Norway has declined tremendously during the last decades (111). The unmeasured confounding will always be an issue in observational studies, however, the stability of our results over the 4 examined decades, despite increasing obesity rates and smoking decline during the last decades (115), suggests that BMI and smoking are

not the primary determinants of the observed association. Other limitation included lack of information on fertility treatments other than IVF. Residual confounding by CS indication cannot be excluded, however, exclusion of women with selected chronic conditions makes these less likely as indications. A Norwegian study found the results consistent in both high and low risk cesarean delivery, implicating cesarean delivery as a reason for reduced fertility, and not the indications that led to it (24). Validation studies showed good ascertainment of pre-gestational diabetes, however, the possibility of underreporting of type 2 diabetes (until 1999, when the new form was introduced) might be present (92).

Mediation

In epidemiologic studies, the concept of confounders and mediators are not always readily distinguishable (116). In that respect, some confounders may be potentially also regarded as mediators. One such variable in our studies might be BMI. In papers I and II, we decided not to do a mediation analysis, since the criteria for a mediator was not fulfilled (i.e. the mediator is a presumed causal consequence of a predictor) (116), given that BMI status preceded blood lipid measurements. Instead, to study more closely the effect of BMI, we stratified by prepregnancy (paper I) and postpregnancy BMI status (paper II) in our studies.

Interaction

Interaction can be defined as “variation in the selected effect measure for the factor under study across levels of another factor” (110). In paper III, we explored possible interactions between the observed outcomes (PTD, SGA, PE and CS) and perinatal loss by examining the statistical significance of interaction terms. Tests of interaction between PTD, SGA and CS at first birth and perinatal loss showed a significant effect on the risk of having one lifetime pregnancy. There was no evidence of significant interaction between PE in first birth and perinatal loss.

E-value calculation

VanderWeele and Ding (106) recently proposed a standardized approach to sensitivity analyses for confounding. They introduced the E-value, defined as "the minimum strength of the association, on the risk ratio scale, that unmeasured confounder would need to have with both the exposure and the outcome to fully explain away this exposure-outcome association, conditional on the measured covariates" (106).

We performed E-value calculations accordingly (for the common outcomes - >15% prevalence) (106):

$$E - value = RR^{*} + \text{sqrt}\{RR^{*} \times (RR^{*} - 1)\}$$

* When the outcome is common (more than 15%), an approximate E-value can be obtained by replacing the risk ratio with the square root of the odds ratio, i.e. $RR^{*} \approx \text{sqrt}(OR)$, in the E-value formula (106).

In paper II, in order to assess how robust the associations are to potential unmeasured confounding, we calculated E-values for both the main analyses and sensitivity analysis on women <40 years of age.

Selection bias

Selection bias occurs when the subjects studied are not representative of the target population about which conclusions are about to be drawn (109). It refers to an error caused by influencing factors that affect the selection of study subjects or participation in the study (109). *Paper III* was based on the MBRN, which is a nationwide register based on a compulsory notification of all births (with a very low dropout rate) (107). Therefore, the possibility of selection bias in this study is very low. In *papers I* and *II*, the design of the studies included linkage between CONOR and MBRN, creating a cohort of women. One of the two main sources of selection bias in cohort studies is the loss to follow up. In *paper I* (pre-pregnancy sample), selection bias would, thus, arise if the women who later

had one lifetime pregnancy were more likely to emigrate than future mothers of 2 or more children, prior to their first births. No notable differences in observed emigration status were observed between women with one lifetime pregnancy and those with 2 or more pregnancies, and the same was true for *paper II* (post-pregnancy sample). Therefore, it is unlikely that loss to follow up would introduce systematic bias in our results, while a life-long personal identification number (given to all Norwegian permanent residents) minimizes the other sources of loss to follow up. Another common source of selection bias is non-participation. The overall participation rate in CONOR was 58%, which is qualified as fairly good (88). However, as we only observe those who agree to participate in the study, this may be affected by a possibility that individuals who choose to take part in a study may have different characteristics compared to the general population. Although non-participants are more likely to belong to lower social class, have lower health awareness and lifestyle factors (117), it has been reported that large population-based studies are generally robust against bias arising from self-selection of participants (117).

External validity

External validity or generalizability is defined as "the degree to which results of a study may apply, be relevant or be generalized to populations or groups that did not participate in the study" (110).

Paper II and *III* in this thesis are large registry based studies. Given the nationwide character of birth notification in the MBRN, our results should be applicable to all Norwegian women who gave childbirth. However, using national population data implies possible differences from other populations concerning race and ethnicity, lifestyle and health status. Regarding the effect of observed pregnancy complications on women's future reproduction, it is likely that industrialized countries with a similar level of obstetric interventions and facilities for treating preterm babies will show similar results. Existing social policies supportive of high reproduction and other similarities within Nordic countries makes the results from this thesis highly applicable to this region, however, caution is needed with regard to the countries with restrictive reproductive policies (i.e. former 'one-child policy' in China). Due to a smaller sample size in *paper I*,

the same caution applies regarding the generalizability of results to other populations of reproductive aged women. However, given the increased risk of CVD in women with low parity worldwide (32, 33, 34), and our indications of possible biological underpinnings of this association, it is our belief that our results are relevant to other respective populations of women from different parts of the world.

5.2 Discussion of main results

5.2.1 Paper I

We found that women's pre-pregnant lipid levels were associated with having one lifetime pregnancy. Women with high levels of LDL, TG and TG/HDL-c ratio as well as low HDL levels, measured years before conception, were at increased risk of having only one lifetime pregnancy. High levels of LDL and total cholesterol were associated with having no children, while in overweight and obese women this was true for all the examined lipids.

Currently, different factors affecting fertility are vigorously discussed in the literature (1, 7, 8, 52). Our findings contribute to the debate by providing a possible biological underpinning for a joint mechanistic pathway for reduced fertility and cardiovascular conditions (70). Findings suggest that the previously observed association between low parity and increased CVD risk may be confounded by preexisting adverse lipid levels, as unfavourable lipid profiles are found to be related to both subfertility and later CVD.

Our findings are in line with both animal and human studies that link fertility with lipids (70, 72, 73, 118). The LIFE study has reported concentrations of free cholesterol to be associated with human fecundity in both sexes (73). Due to our focus on metabolic aspects of lipid profile in relation to fertility (and possible CVD risk), we have chosen TG/HDL ratio over traditional lipid ratios due to its stronger correlation with insulin resistance (119). Recent insights of TG/HDL-c ratio as a reliable indicator of insulin resistance and atherogenicity (120) highlight its ability to identify insulin resistance in apparently healthy individuals (81). Therefore, the observed higher levels of TG/HDL-c ratio in our study may be indicative of possible preexisting metabolic risk factors among women with one lifetime pregnancy, as well as in nulliparous women (overweight and obese, nulliparous with a reported partner). These findings also mirror increasing rates of infertility in both sexes among the population with metabolic syndrome (73). This notion is further supported by the higher proportion of diabetes in one-child mothers of reproductive age and a substantially higher proportion of IVF treated pregnancies, which remains after exclusion of women older than 34 years at the time of first delivery.

Obesity is a well-established risk factor for both metabolic and fertility irregularities (103, 104). To look more closely into the effect of obesity, we stratified analyses by

BMI. As expected, we observed stronger effects among overweight and obese women, however, the higher risk of having only one child remained in normal weight women (BMI< 25) with the lowest HDL quintile and the highest TG and TG/HDL-c ratio quintiles. These findings reflect observations of metabolic irregularities among normal weight women as an independent risk factor for fertility disorders (121, 122). Recent genetic reports show that metabolic risk appears to be generated by different pathways in normal weight and obese subjects (123), proposing the so-called Metabolically Unhealthy Normal Weight phenotype.

Women with one lifetime pregnancy had generally poorer lifestyle factors (BMI, smoking), were older and less educated. It is possible that unfavourable socioeconomic status, reflected in lower educational level (4), could be a hindering factor for further reproduction. However, a study exploring age at first birth, parity and post-reproductive mortality suggests that late childbearing in itself may be a signal of the preexisting poor health of a woman (124). Recent reports show that infertility diagnosis or undergoing fertility treatment is associated with increased risk of maternal morbidity compared to women without fertility problems (125).

Given the lack of information on women's reproductive planning in our data, the possibility for variability in reasons for childlessness among nulliparous women in this cohort could be present. This could also explain the observed risk differences between nulliparous women and one-child mothers in our main results, as the risk may be diluted by low risk groups of women who did not want children (126) or have not been exposed to pregnancy (ever). We tried to address this in a subanalysis by including only women with a reported partner as a proxy for being exposed to pregnancy. We observed results similar to our main results for one-child mothers, suggesting the role of serum lipids in lifetime nulliparity for partnered women.

We lacked data on Apolipoprotein E genotype, CRP and thyroid tests/thyroid antibodies, factors that all are found to affect female fertility (79). We also had no available assessments of duration or temporal proximity of OC use, dietary intake or stress, therefore unmeasured confounding cannot be ruled out. Smoking adversely influences female fertility (100) with most of its effect attributed to HDL cholesterol decrease (101). We accounted for this in our analyses; however, smoking status of participants was only available at enrollment.

5.2.2 Paper II

Given that lipid levels are susceptible to change during women's lifespan, influenced by both pregnancy and menopause (127, 128), we wanted to explore women's lipid status after childbirth. The ideal situation for testing our hypothesis would be to assess women that were analyzed several times both before and after their pregnancies. However, since this was not possible to achieve in our sample, we used a large sample of roughly 33 000 women with post-pregnancy measures to study their lipid profile. These women were different from the women examined in paper I.

We found that women with one lifetime pregnancy had significantly higher mean values in nearly all the observed lipids (except TG/HDL ratio) compared to mothers with two or more children (measured more than a decade after childbirth). Therefore, we aimed to further assess if lipid levels in these women were associated with their lifetime parity status, namely, the probability to have one child compared to having two or more children. We observed that women with LDL cholesterol greater than 4.57 mmol/l (highest quintile) and total cholesterol level greater than 5.70 mmol/l (two highest quintiles), measured more than a decade after first childbirth, had a higher probability of having only one child compared to women with the lowest quintile levels.

Several studies have investigated women's lipid trajectories after childbirth, however, the majority of studies looked at the effects in reference to nulliparity (127, 129, 130, 131). Although relevant from the aspect of total parity, this design has limited the ability of prior studies to identify the high-risk group of women having only one-child (as a feature of subfecundity). Some previous studies have reported no consistent association between parity and LDL/TG levels (130, 132), while others, with longer follow-up, have found an association between declining total cholesterol levels by parity (132) and associations between primiparity and levels of total cholesterol and LDL (131). A recent study reported the most pronounced adverse lipid changes in women following first birth, emphasizing the effect of a first pregnancy on women's lipid profile (127).

Our finding for non-HDL cholesterol further reinforces the role of lipids in relation to fertility. Since measurement of non-HDL cholesterol may be particularly useful in individuals with high TG levels (where Friedwald calculation for LDL cholesterol may have poorer performance) (94), the similarity of our findings for LDL and non-HDL cholesterol is suggestive of Friedwald's calculation optimal performance. Given that

non-HDL cholesterol represents a sum of all ApoB-containing lipoprotein populations (95), its role in reproduction may involve ApoB related factors (10), while its predictive value for CVD risk is at least equivalent to or even more robust than LDL concentrations (95).

The lack of association between post-pregnancy TG/HDL ratio in midlife women and the number of children could suggest that metabolic health might have more influence on fertility in women of younger reproductive age. In older women, metabolic factors might be overweighted by arterial remodelling (128) and other factors related to a still unclear role of HDL decline by higher parity (127, 128, 129). Although age-related factors are suggested to influence the change of HDL fractions in follicular fluid (11), several studies have reported the highest magnitude of the HDL drop associated with first birth, independent of maternal age (121, 125, 133). While HDL concentrations in the follicular fluid have been found to correlate with plasma levels (10), exactly how HDL content is influenced by pregnancy or may influence fertility potential is still unclear (11).

Possible mechanisms behind the pregnancy-lipid status effect

Possible explanations for the effects of pregnancy on lipid levels could include genetic differences or incipient dyslipidemias, which may induce excessive alterations in levels of lipoproteins associated with pregnancy (1, 130, 134). Progesterone during pregnancy may act to reset lipostat in the hypothalamus (134) and the placenta may convey an active role on maternal lipoprotein metabolism through fetal polymorphisms (inherited from the father) (135). It is possible, that in some women with preexisting dyslipidemia, placental influence (expressed from paternal inherited allele) will either partly compensate for or exaggerate maternal lipid profile (135). Interestingly, it has been observed that in several cases of familial lipoprotein lipase deficiency, TG levels during gestational follow up never reached concentrations higher than those observed during other periods of life (135). Hormonal changes accompanying pregnancy, related fat retention and/or redistribution and lifestyle/behavioural practices may also introduce long-term changes in lipid metabolism (1, 130, 134), particularly in predisposed women.

Menopause. Besides pregnancy and childbirth, another important milestone in women's life that can affect lipid profile is a menopausal transition (136). Estrogen deprivation in menopause may lead to increased total and LDL levels (68, 136). Due to a generally higher number of missing data for this variable, we examined the menopausal effect in a sensitivity analysis, including only women < 40 years of age. We observed that the results were only slightly attenuated from our main results, suggesting that menopause is not the major driver of the observed associations.

Large sample size in this study allowed us to further explore the effects of various factors on the association between post-pregnant lipid levels and the number of children, such as: education, self-perceived health, family history of CVD, alcohol use and physical activity. Self-perceived health status is considered a strong predictor of circulatory diseases and mortality and may convey additional knowledge that is not captured by available clinical measurements (137). Indirectly, it may also provide useful insights about possible psychosocial factors, given that women with unfavourable psychosocial status are less likely to rate their health as good (137). Stratification by these factors showed the robustness of the associations while finding for physical activity is in agreement with reports of the modifiable effect of physical activity on lipid status (138).

Similar to paper I, assessments of the duration of OC use, dietary intake or stress were not available. Non-fasting lipid status is a limitation, however, adjustments for time since last meal have not substantially changed our results. Additionally, the similarity of our findings for non-HDL and LDL cholesterol further supports this notion, given that non-HDL levels are shown to be mostly unaffected by the recent food intake (95). Although detailed information on various environmental factors is unfortunately not registered in the MBRN, we tried to address this by using 'year of first birth' to account for potential cohort/generational/environmental effects (105). As for the possible effect of PCOS underreporting on our findings, one may speculate that underreporting of PCOS in our cohort might result in overestimation of the observed effect, however, it remains highly questionable whether this would substantially influence our results with the estimated 6% prevalence in the total sample (139). Extensive elaboration on this is further complicated by discrepancies in PCOS reporting, in part due to the use of various definitions of the syndrome and its subphenotypes, as well as differences between study cohorts and

ethnicities (139). Furthermore, we calculated E-values for both the main analyses and sensitivity analysis on women <40 years of age. The E-value calculations showed that an unmeasured confounder would need to have nearly four times as large an effect as maternal age (covariate with the strongest effect in the adjusted model, Exp (B)=1.13) and be associated with both the exposure and the outcome to completely explain away the observed associations (106). Although a strong unmeasured confounding factor could explain the association, a degree of confounding this strong seems unlikely, having in mind our generally stable results from various stratified analyses. We observed persistent higher ORs for both the strata of women who rate their health as good and those highly educated, which suggests that women's health and education/socioeconomic status are not the main drivers of this association.

5.2.3 Paper III

We found that the risk of having one lifetime pregnancy was moderately increased for women with adverse outcomes however, largely dependant on perinatal survival of the infant. Women with PTD, SGA, PE or CS and a surviving child had moderately increased risk of having only one child compared to women without adverse outcome and no loss in the first pregnancy. However, having the same outcomes and losing the child significantly reduced the risk of having one lifetime pregnancy, irrespective of the outcome. In line with previous studies (24, 32, 33), results potentiate 'selective fertility' as an important force in reproduction.

We found increased risk of having one lifetime pregnancy in women with CS whose child survived and a markedly decreased risk if the child was lost. In line with several previous studies, this suggests that maternal choice plays an important role (12, 24, 29, 140), while contrasts findings that report no association with reduced fertility (141, 142), or concluded physical consequences of CS on fecundity (27, 28).

Similar to this, when we explored the effect of the combined variable (combining 2, 3 and all the 4 outcomes), we observed that the increased risk of having one lifetime pregnancy consistently varied by the survival of the infant in the perinatal period. We

found decreased risk differences in a scenario with 3 combined outcomes (PE-PTD-CS and PTD-SGA-CS). Here, the higher risk of having one child remained irrespective of child loss, which suggests condition severity (26) and/or underlying health factors that might discourage or prevent future pregnancies.

We further observed low dependency of preterm PE on perinatal survival (supported by non-significant interaction between PE in first birth and perinatal loss). Recent reports of 3.6 % recurrence of PTD in women with previous early onset PE (<34 weeks) suggest these women's predisposition to both PTD and PE from underlying conditions (30). It has been reported that the prevalence for the most chronic diseases increased since the 1980s (143) and we explored the effect of diagnosed condition in a subanalysis by excluding women with asthma, chronic renal disease, cardiovascular disease and rheumatoid arthritis (in addition to excluding chronic hypertension and preexisting diabetes). We found that, for women with a surviving child, crude risks for having one child only slightly decreased from our main results for women with PE and CS, suggesting that maternal diagnosed condition could not explain away the observed association. For the woman whose infant died during the perinatal period, the crude risks for having one child remained unaltered in the same analysis. Less likelihood for a continuation of reproduction after a loss in preterm PE could reflect a possibility of residual confounding by subclinical or underlying health factors other than clinical conditions evaluated in our study (i.e. the possibility of genetic and/or metabolic conditions (120)). The traumatic experience of preterm birth combined with maternal ongoing health problems after PE could also contribute to women's reluctance to conceive again (30).

Our extensive registry-based study adds valuable information about women's reproductive patterns during the lengthy period spanning over 4 decades. Additionally, we tried to quantify the degree to which a perinatal loss contributes to having a next pregnancy in a variety of clinical scenarios. From the aspect of methodology, we show that not accounting for perinatal loss when estimating the impact of pregnancy complications on future reproduction would be misleading. The MBRN, however, as the majority of registries, lack data on women's family planning decisions, which is an acknowledged limitation of this study. Other limitations included lack of information on BMI, smoking, fertility treatments other than IVF and limited registration of ultrasound

dates (from 1999). Level of education may serve as a proxy for BMI (higher education correlates with lower BMI (115)) and adjustment for education may partially have accounted for BMI. A Danish study reported stronger effect of BMI on elective than on emergency CS (37). Residual confounding by CS indication cannot be excluded, however, exclusion of women with the aforementioned chronic conditions makes these less likely as an indication. A Norwegian study found the results consistent in both high and low risk cesarean delivery, implicating cesarean delivery as a reason for reduced fertility, and not the indications that lead to it (24). Additionally, possible errors in gestational age estimates (last menstrual period vs. ultrasound-based) are not likely to be substantial in the MBRN (25). Validation studies showed good ascertainment of pre-gestational diabetes and PE (91, 92), however, the possibility of under-reporting of type2 diabetes and over-reporting of preexisting hypertension with spontaneous normalization might be present.

6. Conclusions

Paper I

Unfavourable pre-pregnant lipid levels were associated with having no and one lifetime pregnancy. These findings provide a possible biological underpinning for a joint mechanistic pathway for reduced fertility and cardiovascular conditions.

Paper II

Mean lipid levels measured after childbirth in women with one child were significantly higher compared to mothers with two or more children and were associated with a higher probability of having only one child. The findings corroborate an association between serum lipid levels and one lifetime pregnancy (as a feature of subfecundity), emphasizing that these particular women may be a specific predetermined risk group for cardiovascular-related disease and death.

Paper III

Associations between adverse outcomes of pregnancy and the risk of having one lifetime pregnancy were strongly modified by child survival in the perinatal period.

7. Future implications

Although examining parity in relation to maternal morbidity across time periods remains challenging due to the shift in women's societal roles, still elevated CVD risk and mortality among one child mothers requires increased attention, from clinical, observational and experimental studies. Observational studies can contribute with important knowledge on factors associated with low parity (as a feature of subfertility). Findings from this thesis add biological underpinning to the observed association between one lifetime pregnancy and increased CVD mortality. In order to get an in-depth view, future observational studies should aim to investigate women sampled both before and after the first pregnancy. Large-scale experimental and epigenetic studies are needed to explore the mechanisms underlying this association.

Our findings contribute with the knowledge about women's post-complication reproductive patterns during the lengthy period over 4 decades. More comprehensive understanding of the effect of adverse pregnancy events on future reproduction necessitates exploration of the specific factors such as social, cultural and health system differences that influence the event, which was beyond the scope of this thesis. The qualitative approach should be applied for identifying exact reasons behind women's reproductive decisions.

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9. Papers I-III

I

BMJ Open

Women's prepregnancy lipid levels and number of children: a Norwegian prospective population-based cohort study

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ABSTRACT

Objective To study prepregnancy serum lipid levels and the association with the number of children.

Design Prospective, population-based cohort.

Setting Linked data from the Cohort of Norway and the Medical Birth Registry of Norway.

Participants 2645 women giving birth to their first child during 1994–2003 (488 one-child mothers and 2157 women with ≥2 births) and 1677 nulliparous women.

Main outcome measures ORs for no and one lifetime pregnancy (relative to ≥2 pregnancies) obtained by multinomial logistic regression, adjusted for age at examination, education, body mass index (BMI), smoking, time since last meal and oral contraceptive use.

Results Assessed in quintiles, higher prepregnant triglyceride (TG) and TG to high-density lipoprotein (TG:HDL-c) ratio levels were associated with increased risk of one lifetime pregnancy compared with having ≥2 children. Compared with the highest quintile, women in the lowest quintile of HDL cholesterol levels had an increased risk of one lifetime pregnancy (OR 1.7, 95% CI 1.2 to 2.4), as were women with the highest low-density lipoprotein (LDL) cholesterol, TG and TG:HDL-c ratio quintiles (compared with the lowest) (OR 1.2, 95% CI 0.8 to 1.7; OR 2.2, 95% CI 1.5 to 3.2; and OR 2.2, 95% CI 1.5 to 3.2, respectively). Similar effects were found in women with BMI≥25 and the highest LDL and total cholesterol levels in risk of lifetime nulliparity.

Conclusion Women with unfavourable prepregnant lipid profile had higher risk of having no or only one child. These findings substantiate an association between prepregnant serum lipid levels and number of children. Previously observed associations between low parity and increased cardiovascular mortality may in part be due to pre-existing cardiovascular disease lipid risk factors.

Strengths and limitations of this study

- ► This is a large population-based study with data collected before pregnancy.
- ► Linkage with the Medical Birth Registry of Norway provided complete registration of total reproduction.
- ► Limitations include lack of data on family planning, dietary intake, duration of oral contraceptive use, *APOE* genotype, low-grade inflammation and thyroid status.
- ► Non-fasting lipid measurements were used; however, adjustments in our analyses for time since last meal did not change the results.

INTRODUCTION

Cardiovascular disease (CVD) is an important public health problem and remains the number one cause of death in women.[1] Reproductive history is important in evaluating health risks in women, as pregnancy may unmask a woman's predisposition for CVD.[1] Several studies have reported increased CVD mortality among women with no or only one lifetime pregnancy.[2–4] Efforts to elucidate the association between the number of children and the risk of female CVD have been inconclusive.[1 3] Proposed explanations are lifestyle risk factors associated with childrearing,[5] sex hormone fluctuations, protective effect of future pregnancies,[3] lifestyle factors prior to conception such as elevated blood pressure and obesity,[6] as well as metabolic irregularities triggered by gestation.[1] Detection of high-density lipoprotein (HDL) cholesterol and apolipoprotein B (ApoB) in follicular fluid from oocytes[7 8] suggests a relation between lipids and female reproductive function. More recent studies have reported associations between lipids and fertility in both sexes.[9] Low parity (as a feature of subfecundity) and cardiovascular events may share common pathophysiological mechanisms.[10]

While the role of serum lipids in cardiometabolic health is well established, showing low HDL and high triglycerides (TGs) to be strong predictors of CVD,[11] their role in reproduction is uncertain. It is also uncertain whether women with no or one lifetime pregnancy have a higher CVD risk to begin with, or whether future pregnancies may reduce the CVD risk.

We pursued this question by exploring the extent to which prepregnant serum lipid levels of total, HDL and low-density lipoprotein (LDL) cholesterol, TG and TG:HDL-c ratio are associated with having no and one lifetime pregnancy.

MATERIALS AND METHODS

Study design and population

We used linked data from the Cohort of Norway (CONOR) and the Medical Birth Registry of Norway (MBRN). CONOR is a population-based collection of health data and blood samples provided by participants older than 20 years of age residing in several different regions in Norway during 1994–2003.[12] Our subset included women with no children at the time of examination with standardised measurements of height, weight and non-fasting lipids levels. Lifestyle factors were obtained through an extensive questionnaire that collected self-reported information on smoking, oral contraceptive (OC) use, self-reported status on receipt of social security benefits, attained level of education and various lifestyle factors.[12] Education in Norway consists of primary school (7 years), lower secondary school (3 years), upper secondary school (3 years) and higher education. The first 10 years are obligatory.

The MBRN has since 1967 recorded data on all deliveries in the country after 16th week of gestation.[13] Based on mandatory notification, midwives and doctors report information using standard forms throughout pregnancy and at the time of delivery. The registry includes demographic information, mother's health prior and during pregnancy, complications in pregnancy and perinatal outcome. Using the unique national identification number given to all Norwegian citizens, each woman was linked to all her subsequent births (if any) after participating in CONOR. Women reporting no children in CONOR at the time of examination and with no valid records in the MBRN were considered having no pregnancies.

Women with baseline assessment of lifestyle factors in CONOR were linked to the MBRN. We defined one-child mothers as women being 6 years out from their first pregnancy and with no additional births registered in the MBRN.

Preconception measurements

Non-fasting blood samples were analysed on a Hitachi 911 Auto Analyzer (Hitachi, Mito, Japan).[12] Applied reagents were from Boehringer Mannheim (Mannheim, Germany). Serum concentrations of total cholesterol, HDL cholesterol and TG were analysed subsequent to sampling. The total cholesterol, HDL cholesterol levels and TGs were measured by an enzymatic method. The day-to-day coefficients of variation were 2.4% and 0.7%–1.3% for total cholesterol, HDL cholesterol and TG, respectively. To calculate LDL, we used the Friedewald formula[14]: total serum cholesterol minus HDL cholesterol minus one-fifth of the TG concentration. LDL cholesterol levels were calculated only for participants with TG concentrations below 4.5 mmol/L.[6 14] Accordingly, the TG:HDL-c ratio was expressed as mmol/L.

Trained personnel measured the height and weight, with the participants wearing light clothes and no shoes; measurements were taken as follows: height to the nearest 1.0 cm and weight to the nearest 0.5 kg. Body mass index (BMI) was calculated as weight in kilogram/(height in metres)^2.

Patients and public involvement

Patients or the public were not directly involved in this study. The detailed explanation of the recruitment process and the obtainment of written informed consent for CONOR were provided elsewhere.[12]

Statistical analyses

The characteristics of the analysed women were presented as means with SD for continuous data and as number with percentages for categorical data. Differences between nulliparous women, one-child mothers and mothers with two or more children, as well as prepregnant health status, were analysed by χ^2 tests and t-tests where appropriate. Linear associations across prepregnant lipid levels (in quintiles) for no and one lifetime pregnancy were assessed by p values for trend. ORs of no and one lifetime pregnancy by lipid levels and TG:HDL-c ratio, when compared with women with two or more pregnancies, were calculated using multinomial logistic regression and adjusted for mother's age at examination, level of education (categorised in <11 years and >11 years of education), smoking (current smoker: yes, no), time since last meal, OC use (now, previously, never) and BMI (linear term). To extend each woman's likelihood of completing her birth record, we separately examined women who were 7 years out from their first pregnancy. About 95% of Norwegian women will complete their second pregnancy within 7 years.[4] To test the effect of (prepregnant) BMI, we stratified the main analyses by BMI (<25 and ≥25). To avoid influence from age at first delivery on the number of children, we excluded women older than 34 years at the time of first delivery in a subanalysis. Additionally, we performed sensitivity analyses including only mothers who were 22–30 years old at the time of first delivery. Using presence of a partner (ever) as a proxy for exposure to pregnancy among nulliparous women, we performed logistic regression in a subanalysis (nulliparous vs women with ≥2 births) including only women with a reported partner (ever). All analyses were performed using the Statistical Package for Social Sciences (SPSS V.22.0 and V.23.0).

RESULTS

There were 4743 women with baseline assessment of lifestyle factors in CONOR (1994–2003) that were linked to the MBRN. We excluded 421 women with pregnancy

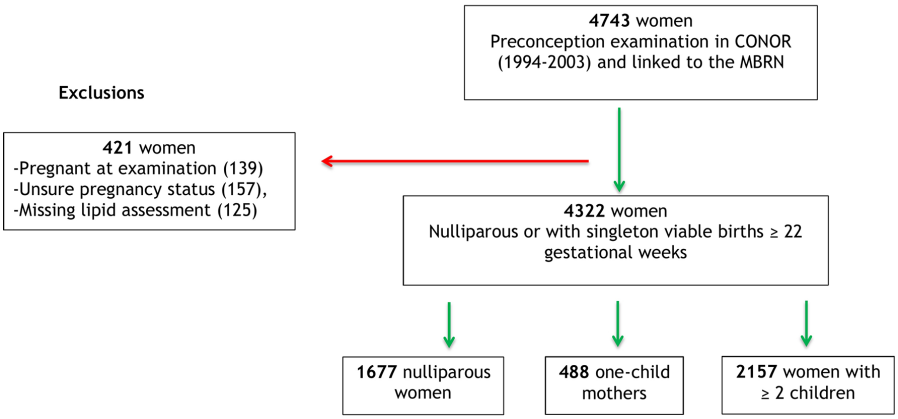

Figure 1 Norwegian women examined in the Cohort of Norway (CONOR) before conception of their first pregnancy and with linked data from the Medical Birth Registry of Norway (MBRN).

at the time of examination (n=139), unsure pregnancy status (n=157) and missing lipid assessments (n=125). Thus, 4322 women were included in the analyses (1677 nulliparous, 488 one-child mothers and 2157 women with ≥2 births; see figure 1). Subanalyses included only women with reported partners (228 nulliparous and 216 mothers with ≥2 births).

The characteristics of the included women are given in table 1. Nulliparous women were older at the time of examination, had higher BMI and were more frequent smokers compared with women with two or more births. A higher proportion of nulliparous women had >11 years of education. One-child mothers had higher mean age both at examination and at delivery (29.5 vs 26.7 and 32.3 vs 29.9, respectively), were more often smokers and had lower education than mothers with ≥2 births. The mean BMI prior to pregnancy was higher in one-child mothers (24.2 vs 23.5), whereas the mean years from examination to first delivery were similar for the two groups. Women with no and one child were less frequent users of OCs at the time of examination compared with mothers with ≥2 births (27.4%, 34.6% and 48.9%, respectively).

The proportion of diabetes at first delivery in one-child mothers was higher than in women with two or more

Table 1 Characteristics of 4322 Norwegian women in the Cohort of Norway, 1994–2003, with no, 1 or ≥2 children

Mean values	1677 no child	488 one child	2157 ≥2 children
Age (SD) at examination	30.5 (2.1)	29.5 (5.2)	26.7 (4.0)
Age (SD) at first delivery	–	32.3 (4.9)	29.9 (3.8)
Years (SD) from examination to first pregnancy	–	3.7 (2.1)	4.1 (2.3)
BMI (SD) at examination*	24.8 (5.1)	24.2 (4.5)	23.5 (3.4)
OC use*			
Now	455 (27.4)	168 (34.6)	1047 (48.9)
Previously	724 (43.5)	239 (49.2)	779 (36.4)
Never	484 (29.1)	79 (16.3)	317 (14.8)
Smoking at examination*			
Yes	537 (32.2)	182 (37.4)	462 (21.5)
No	1132 (67.8)	304 (62.6)	1685 (78.5)
Education*			
<11 years	312 (18.8)	127 (26.3)	300 (14.1)
≥11 years	1344 (81.2)	356 (73.7)	1834 (85.9)

Values are numbers (percentages) unless stated otherwise.
*Missing data on smoking: 8 nulliparous, 2 one-child mothers and 10 women with ≥2 children; BMI, 10 nulliparous; education, 21 nulliparous, 5 one-child mothers and 23 women with ≥2 children; OC use: 4 nulliparous, 2 one-child mothers and 14 women with ≥2 children.
BMI, body mass index; OC, oral contraceptive.

Table 2 ORs with 95% CI for no and one lifetime pregnancy (reference: women with ≥2 pregnancies) by prepregnant lipid quintiles in 4322 women in the Cohort of Norway, 1994–2003

Lipid quintiles* in mmol/L	n (%)	n[a] (%)	n[b] (%)	Nulliparous		One-child mothers	
				N	OR (95% CI)	OR (95% CI)	P for trend
LDL cholesterol†							0.82
<2.42	449 (56.9)	261 (33.0)	80 (10.1)	790	1.0 (reference)	1.0 (reference)	
2.43–2.84	433 (52.3)	309 (37.4)	85 (10.3)	827	1.1 (0.9 to 1.4)	1.0 (0.7 to 1.4)	
2.85–3.24	454 (52.2)	325 (37.4)	90 (10.4)	869	1.0 (0.8 to 1.3)	0.9 (0.7 to 1.3)	
3.25–3.76	426 (48.0)	353 (39.8)	108 (12.2)	887	1.1 (0.7 to 1.2)	1.1 (0.8 to 1.6)	
>3.77	391 (41.9)	421 (45.1)	121 (13.0)	933	1.2 (0.7 to 1.1)	1.2 (0.8 to 1.7)	
Total cholesterol							0.26
<4.19	432 (55.0)	259 (33.0)	94 (12.0)	785	1.0 (reference)	1.0 (reference)	
4.20–4.61	456 (54.6)	304 (36.4)	75 (9.0)	835	1.2 (0.9 to 1.5)	0.8 (0.5 to 1.1)	
4.62–5.0	434 (52.2)	306 (36.8)	91 (11.0)	831	1.0 (0.8 to 1.3)	0.9 (0.6 to 1.2)	
5.1–5.63	442 (45.7)	415 (43.0)	109 (11.3)	966	1.3 (1.0 to 1.7)	1.0 (0.7 to 1.4)	
>5.64	393 (43.4)	393 (43.4)	119 (13.1)	905	1.2 (0.9 to 1.6)	1.0 (0.7 to 1.4)	
TG							0.01
<0.66	429 (48.6)	372 (42.2)	81 (9.2)	882	1.0 (reference)	1.0 (reference)	
0.67–0.86	447 (49.8)	350 (39.0)	100 (11.1)	897	0.9 (0.7 to 1.1)	1.2 (0.8 to 1.7)	
0.87–1.09	455 (54.4)	294 (35.1)	88 (10.5)	837	0.9 (0.7 to 1.2)	1.3 (0.9 to 1.9)	
1.10–1.45	452 (53.6)	303 (35.9)	88 (10.4)	843	1.0 (0.8 to 1.3)	1.4 (1.0 to 2.0)	
>1.46	373 (43.3)	358 (41.6)	130 (15.1)	861	1.1 (0.9 to 1.5)	2.2 (1.5 to 3.2)	
HDL cholesterol							0.18
<1.20	326 (47.5)	263 (38.3)	97 (14.1)	686	1.0 (0.8 to 1.3)	1.7 (1.2 to 2.4)	
1.21–1.40	271 (44.7)	260 (42.9)	75 (12.4)	606	1.0 (0.8 to 1.2)	1.2 (0.8 to 1.7)	
1.41–1.60	634 (53.0)	431 (36.1)	130 (10.9)	1195	1.0 (0.7 to 1.3)	1.2 (0.9 to 1.6)	
1.61–1.84	443 (49.7)	356 (40.0)	92 (10.3)	891	0.9 (0.7 to 1.2)	1.1 (0.8 to 1.5)	
>1.85	483 (51.2)	367 (38.9)	94 (10.0)	944	1.0 (reference)	1.0 (reference)	

The estimates were obtained by multinomial logistic regression and adjusted for age at examination, educational level, smoking, time since last meal, oral contraceptive use and body mass index (linear term).
Number of women: with ≥2 children (n, reference group), nulliparous women (n[a]), one-child mothers (n[b]) and total number of women within the category (N).
*Quintiles calculated on a total sample prior to pregnancy.
†Missing data within lipids on 16 cases of LDL and 2 cases of TG.
HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides.

births (1.4% vs 0.9%, p=0.30). Polycystic ovary syndrome (PCOS) was rare and we only had three cases in our material. A significantly higher number of one-child mothers had in vitro fertilisation (IVF) in their first pregnancy (7.2% vs 2.6% in women with ≥2 births; p<0.001) (data not shown). This latter finding remained after excluding mothers older than 34 years at first delivery.

ORs with 95% CIs for no and one lifetime pregnancy (vs ≥2 lifetime pregnancies) by lipid levels (in quintiles) are presented in table 2 and figure 2. Significant trends in ORs for one lifetime pregnancy across TG and TG:HDL-c ratio quintiles were observed (p trend=0.01). The OR for having one lifetime pregnancy for women with the highest TG quintile compared with the lowest quintile was 2.2 (95% CI 1.5 to 3.2). The ORs for having one lifetime pregnancy for women with TG:HDL-c ratio levels in the two highest quintiles were 1.7 (95% CI 1.2 to 2.5) and 2.2 (95% CI 1.5 to 3.2), respectively, compared with the lowest quintile. There were no significant trends for LDL cholesterol, total cholesterol or HDL cholesterol, although the ORs of one lifetime pregnancy for the lowest HDL quintile were 1.7 (95% CI 1.2 to 2.4) and for the highest LDL quintile 1.2 (95% CI 0.8 to 1.7). We found no increased risk of being nulliparous by serum lipid levels except for the highest LDL and total cholesterol levels, and these estimates were not persuasive (OR 1.2 (95% CI 0.9 to 1.6) and 1.2 (95% CI 0.9 to 1.5), respectively). Truncation of data to extend the time for each woman to complete her birth record (to 7 years) did not appreciably alter the results, neither did

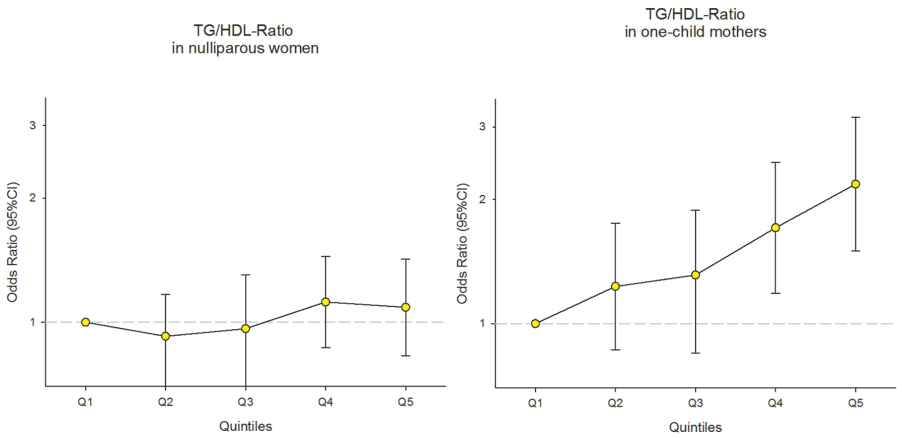

Figure 2 ORs with 95% CI for no and one lifetime pregnancy (reference: women with ≥2 pregnancies) by TG:HDL-c ratio quintiles in 4322 women in the Cohort of Norway, 1994–2003. The estimates were obtained by multinomial logistic regression and adjusted for age at examination, educational level, smoking, time since last meal, oral contraceptive use and body mass index (linear term). TG:HDL-c, triglycerides to high-density lipoprotein ratio.

exclusion of women older than 34 years at the time of first delivery nor the additional restriction of our analyses to mothers aged 22–30 years at first pregnancy. The similar effects of prepregnant lipids as in one-child mothers were observed when subanalyses (nulliparous vs ≥2 births) were performed on women who had a partner (as a proxy for ever being exposed to pregnancy). For women with partner, the risk of having no children was increased among the women in the highest quintiles of TG and TG:HDL-c ratio (compared with the lowest quintiles) and also for those in the lowest HDL quintile (compared with the highest) (OR 1.9, 95% CI 0.9 to 4.2; OR 2.0, 95% CI 1.0 to 4.1; and OR 1.6, 95% CI 0.7 to 3.6, respectively).

Stratified analyses by BMI are presented in table 3. In women with BMI≥25 there were significant trends in ORs of having no children or one child across increasing levels of prepregnant total cholesterol, TG and TG:HDL-c ratio quintiles (p trend=0.04 and <0.001, respectively). The adjusted ORs of one lifetime pregnancy for women with BMI≥25 and TG levels in the two highest quintiles were 2.1 (95% CI 0.9 to 4.8) and 3.5 (95% CI 1.6 to 7.4), and for the two highest TG:HDL-c ratio quintiles 3.1 (95% CI 1.3 to 7.4) and 4.3 (95% CI 1.9 to 10.0) compared with women in the lowest respective quintile. The risk of one lifetime pregnancy was also significantly increased for women with BMI≥25 and the highest LDL and total cholesterol, as well as the lowest HDL quintiles (OR 1.8 (95% CI 0.8 to 3.8), 1.2 (95% CI 0.6 to 2.4) and 2.6 (95% CI 1.3 to 5.3), respectively). Similarly, the ORs of having no pregnancy (in women with BMI≥25) were 1.7 (95% CI 1.0 to 3.0), 2.8 (95% CI 1.7 to 4.7) and 3.6 (95% CI 2.1 to 6.1) for women with the highest LDL, TG and TG:HDL-c ratio quintiles, respectively, compared with women with the lowest quintile. An increased risk of having no children was also found for the overweight and obese women with the lowest HDL quintile (OR 1.9, 95% CI 1.2 to 3.0). Unlike in one-child mothers, the risk of having no pregnancy among overweight and obese women with higher total cholesterol levels only slightly changed from the main results. In women with prepregnant BMI<25, there were significant trends in the risk of having one lifetime pregnancy across increasing levels of prepregnant TG (p trend=0.04), TG:HDL-c ratio (p trend=0.04) and HDL quintiles (p trend=0.05). There were increased risks of one lifetime pregnancy in the highest TG quintile (OR 1.9, 95% CI 1.2 to 3.0) and the two highest TG:HDL-c ratio quintiles (OR 1.6, 95% CI 1.0 to 2.4; and OR 1.8, 95% CI 1.2 to 2.8, respectively), as well as the lowest HDL quintile (OR 1.7, 95% CI 1.1 to 2.6). The risks of no and one lifetime pregnancy with higher LDL and total cholesterol levels only slightly changed compared with our main results.

DISCUSSION

Prepregnant lipid levels were associated with having one lifetime pregnancy. Women with high levels of LDL, TG and TG:HDL-c ratio, as well as low HDL levels, measured years before conception, were at increased risk of having only one lifetime pregnancy. High levels of LDL and total cholesterol were associated with having no children, while in overweight and obese women this was true for all the lipids examined.

These findings provide a possible biological underpinning for a joint mechanistic pathway for reduced fertility and cardiovascular conditions.[10] Our study suggests that the previously observed association between low parity and increased CVD risk may be confounded by pre-existing adverse lipid levels. This does not support the hypothesis that having additional pregnancies reduces

Table 3 OR with 95% CI for no and one lifetime pregnancy (reference: women with ≥2 pregnancies) by prepregnant lipid quintiles in 4322 women in the Cohort of Norway (1994–2003), analyses stratified by BMI

	Women with prepregnant BMI<25							Women with prepregnant BMI≥25						
Lipid quintiles* in mmol/L	n	n[a]	n[b]	N	Nulliparous OR (95% CI)	One-child mothers OR (95% CI)	P for trend	n	n[a]	n[b]	N	Nulliparous OR (95% CI)	One-child mothers OR (95% CI)	P for trend
LDL cholesterol†							0.84							0.44
<2.42	377	219	69	665	1.0 (reference)	1.0 (reference)		72	41	11	124	1.0 (reference)	1.0 (reference)	
2.43–2.84	343	240	64	647	1.1 (0.9 to 1.5)	0.9 (0.6 to 1.4)		90	68	20	178	1.1 (0.6 to 2.1)	1.4 (0.6 to 3.3)	
2.85–3.24	341	216	67	624	1.0 (0.7 to 1.3)	0.9 (0.6 to 1.3)		113	109	23	245	1.5 (0.8 to 2.6)	1.4 (0.6 to 3.3)	
3.25–3.76	315	198	69	582	1.0 (0.8 to 1.4)	1.1 (0.7 to 1.6)		111	152	39	302	1.7 (1.0 to 3.0)	1.8 (0.8 to 4.0)	
>3.77	230	187	64	481	1.2 (0.9 to 1.6)	1.2 (0.8 to 1.8)		158	233	57	448	1.7 (1.0 to 3.0)	1.8 (0.8 to 3.8)	
Total cholesterol							0.13							0.04
<4.19	359	198	75	632	1.0 (reference)	1.0 (reference)		73	60	19	152	1.0 (reference)	1.0 (reference)	
4.20–4.61	341	231	57	629	1.4 (1.1 to 1.9)	0.8 (0.5 to 1.2)		115	73	17	205	0.6 (0.3 to 1.0)	0.7 (0.3 to 1.5)	
4.62–5.0	332	191	64	587	1.0 (0.7 to 1.3)	0.8 (0.5 to 1.2)		102	114	27	243	1.3 (0.8 to 2.2)	1.2 (0.6 to 2.5)	
5.1–5.63	327	244	72	643	1.3 (0.9 to 1.7)	0.9 (0.6 to 1.4)		114	167	37	318	1.5 (0.9 to 2.5)	1.2 (0.6 to 2.4)	
>5.64	248	196	66	510	1.3 (0.9 to 1.7)	1.0 (0.7 to 1.5)		143	197	53	393	1.2 (0.7 to 2.0)	1.2 (0.6 to 2.4)	
TG							0.04							<0.001
<0.66	363	311	69	743	1.0 (reference)	1.0 (reference)		66	59	12	137	1.0 (reference)	1.0 (reference)	
0.67–0.86	364	248	82	694	0.8 (0.6 to 1.0)	1.2 (0.5 to 2.8)		83	102	18	203	1.7 (1.0 to 3.0)	1.2 (0.5 to 2.8)	
0.87–1.09	339	208	65	612	0.9 (0.7 to 1.2)	1.3 (0.7 to 3.8)		115	85	23	223	1.6 (0.9 to 2.8)	1.7 (0.7 to 3.8)	
1.10–1.45	319	156	58	533	0.8 (0.6 to 1.1)	1.3 (0.9 to 4.8)		133	146	30	309	2.5 (1.5 to 4.4)	2.1 (0.9 to 4.8)	
>1.46	222	137	59	418	1.0 (0.7 to 1.4)	2.0 (1.7 to 7.4)		149	219	70	438	2.8 (1.7 to 4.7)	3.5 (1.6 to 7.4)	
HDL cholesterol							0.05							0.17
<1.20	198	70	47	315	0.9 (0.7 to 1.2)	1.7 (1.1 to 2.6)		127	191	50	368	1.9 (1.2 to 3.0)	2.6 (1.3 to 5.3)	
1.21–1.40	189	138	43	370	0.9 (0.7 to 1.1)	1.0 (0.6 to 1.5)		81	122	31	234	1.7 (1.0 to 2.8)	2.3 (1.1 to 4.9)	
1.41–1.60	475	287	93	855	0.8 (0.6 to 1.1)	1.1 (0.8 to 1.5)		158	143	37	338	1.4 (0.9 to 2.2)	1.8 (0.9 to 3.6)	
1.61–1.84	358	272	74	704	0.7 (0.5 to 1.0)	1.0 (0.7 to 1.4)		85	82	18	185	1.4 (0.8 to 2.4)	1.8 (0.8 to 4.0)	
>1.85	387	293	77	757	1.0 (reference)	1.0 (reference)		96	73	17	186	1.0 (reference)	1.0 (reference)	
TG:HDL-c ratio							0.04							<0.001
<0.39	374	321	70	765	1.0 (reference)	1.0 (reference)		67	42	10	119	1.0 (reference)	1.0 (reference)	
0.40–0.54	365	245	75	685	0.8 (0.6 to 1.0)	1.2 (0.8 to 1.7)		85	99	17	201	2.2 (1.2 to 4.0)	1.9 (0.7 to 4.9)	
0.55–0.73	365	213	71	649	0.8 (0.6 to 1.1)	1.2 (0.8 to 1.8)		101	91	22	214	2.4 (1.3 to 4.3)	2.2 (0.9 to 5.5)	
0.74–1.04	281	172	64	517	0.9 (0.7 to 1.2)	1.6 (1.1 to 2.4)		138	131	33	302	3.3 (1.9 to 5.9)	3.1 (1.3 to 7.4)	
>1.05	222	109	53	384	0.8 (0.6 to 1.0)	1.8 (1.2 to 2.8)		155	248	71	474	3.6 (2.1 to 6.1)	4.3 (1.9 to 10.0)	

The estimates were obtained by multinomial logistic regression, stratified by BMI and adjusted for age at examination, educational level, smoking, time since last meal and oral contraceptive use.
Number of women: with ≥2 children (n, reference group), nulliparous women (n[a]), one-child mothers (n[b]) and total number of women within the category (N).
*Quintiles calculated on a total sample prior to pregnancy.
†Missing data within lipids on 16 cases of LDL, 2 cases of TG and 2 cases of TG:HDL-c ratio.
BMI, body mass index; LDL, low-density lipoprotein; TG, triglyceride; TG:HDL, TG to high-density lipoprotein ratio.

CVD risk.[3] Rather, unfavourable lipid profiles may be related to both subfertility and later CVD.

There is a lack of studies evaluating the relation between preconception lipid levels and fertility in women. The LIFE (Longitudinal Investigation of Fertility and the Environment) study found concentrations of free cholesterol to be associated with fecundity in both sexes.[9] In contrast to our study, TGs and total cholesterol were not found to be significant in individual and couple-based adjusted models (as well as two other measured lipid components: phospholipids and total lipids); however, the authors used a different study design and lipid measurement methods. In accordance with our findings is the Framingham Heart Study, which detected a trend towards TG elevation and lower HDL serum levels among women with self-reported infertility (as not achieving pregnancy for ≥1 year).[15] The presence of HDL cholesterol and ApoB in follicular fluid from human oocytes suggests that these lipids play a direct role in reproduction.[7 8 16] Previous animal studies have reported an association between dyslipidaemia and infertility.[17] Posed explanations have been that abnormalities in HDL metabolism including change in structure, concentration or function compromise female fertility.[7 8 16] It has been suggested that genetic polymorphisms that alter function in proteins engaged in cholesterol metabolism may affect human fertility.[18 19] One of the possible molecular mechanisms could be through a mediating role of HDL on paraoxonase 1 activity. Paraoxonase is an HDL-associated enzyme that inhibits LDL oxidation, and thus protects cells from oxidative stress.[20] Its stability and binding affinity are strongly influenced by changes in shape and size of HDL particles.[21] These changes may lead to decreased antioxidative capacity and consecutively oxidative stress. Oxidative stress is associated with adverse cardiovascular and fertility outcomes, including atherosclerosis, PCOS, pre-eclampsia, endometriosis and infertility.[19 22] A recent study in women of reproductive age with upper normal ranges of thyroid-stimulating hormone has suggested a direct link between unfavourable lipid profile and increased oxidative membrane damage.[23]

Recent insights suggest TG:HDL-c ratio to be a reliable marker of insulin resistance and atherogenicity,[24] highlighting its ability to identify insulin resistance in apparently healthy individuals.[25] Observed higher levels of TG:HDL-c ratio in our study are indicative of possible pre-existing metabolic risk factors among women with one lifetime pregnancy, as well as a subpopulation of nulliparous women (overweight, obese and with reported partners—as a proxy for exposed to pregnancy). This is also consistent with increasing rates of infertility in both sexes among population with metabolic syndrome.[9] The higher proportion of diabetes in this group of women further supports this notion. In agreement, the Japan Nurses' Health Study reported a significant increase in the risk of diabetes in young nulliparous women (<45 years of age) with ovarian infertility.[26] Accordingly, the Framingham Heart Study found infertile premenopausal women to have increased odds of diabetes and obesity.[15] Given the accompanying metabolic irregularities among major causes of female infertility,[15 27] substantially higher proportion of IVF treatment among one-child mothers indirectly supports metabolic implications. The latter finding remains after exclusion of women older than 34 years at the time of first delivery.

Dyslipidaemia is associated with PCOS.[28–30] However, we only identified three women with PCOS in our study sample. Thus, the presence of subclinical forms or under-reporting may be present.

In accordance with the literature,[27 31] the risk of having no and only one child showed strong effects in overweight and obese women (BMI ≥25) in stratified analyses (table 3). Nevertheless, the higher risk of having only one child remained in normal-weight women (BMI<25) with the lowest HDL quintile and the highest TG and TG:HDL-c ratio quintiles. These findings mirror observations from the literature of metabolic irregularities among normal-weight women as an independent risk factor for future fertility impairments.[32 33] The LIFE study reported both female and male lipid concentrations to affect fecundity, irrespective of their BMI.[9]

Compared with women with two or more pregnancies, total cholesterol levels above clinically recommended range were associated with risk of having no children, irrespective of BMI. The LIFE study reported a higher percentage of women with a history of irregular menstrual cycles in the highest quartile of free cholesterol.[9] The Japan Nurses' Health Study found women with ovarian infertility to be at high risk of hypercholesterolaemia.[26] In our study, total cholesterol levels were not associated with the risk of having one lifetime pregnancy, except among overweight and obese women. This could suggest that total cholesterol levels play varied roles in different subfecundity or infertility subtypes. In addition, nulliparous women in our study were older at examination and had higher BMI. Both age and obesity are associated with systemic oxidative stress.[19 22] It is possible that in such physiological environment, clinically abnormal levels of certain lipids might activate additional pathological processes that adversely affect reproductive function.[28]

In our study, women with one lifetime pregnancy had poorer lifestyle factors (BMI, smoking), were older and less educated. The lower mean education among one-child mothers is in agreement with a Nordic demographic study,[34] which shows that later onset of childbearing is related to a lower number of children finally born in women with low education. Given that educational level and occupation are key indicators of socioeconomic status,[35] observed lower parity among women with low education could also reflect unfavourable socioeconomic position as a limiting factor to further pregnancies. However, a study exploring age at first birth, parity and postreproductive mortality suggests that late childbearing in itself may be a signal of pre-existing poor health of a woman.[36]

The observed risk differences between nulliparous women and one-child mothers in our main results

(figure 2, table 2) could be explained by the heterogeneity of causes for childlessness among nulliparous women in this cohort. The risk may, therefore, be diluted by low-risk groups of women who are voluntarily childless[37] or have not been exposed to pregnancy (ever). Given the lack of information on women's reproductive planning in our data, we tried to address this in a subanalysis including only women with reported partner (ever) as a proxy for being exposed to pregnancy. Here we found that the results for nulliparous women were similar to our main results for one-child mothers. Women with reported partner had higher risk of having no children (compared with partnered women with ≥2 births) if their TG and TG:HDL-c ratio levels were in the highest quintiles and HDL in the lowest quintile (OR 1.9, 95% CI 0.9 to 4.2; OR 2.0, 95% CI 1.0 to 4.1; and OR 1.6, 95% CI 0.7 to 3.6, respectively). These findings support the role of serum lipids in lifetime nulliparity among women with partners.

Strengths and limitations

Our subset of women was from a large population-based health study with prepregnant health data. Linked data from the MBRN provided complete registration of total reproduction. The prospective design minimised the potential for bias. A weakness is that blood sampling was performed in a non-fasting state. Studies show that TG levels are sensitive to recent food intake, while cholesterol levels seem to be less affected.[38] We addressed this by adjusting our analyses for time since last meal and the main results were unchanged, suggesting that non-fasting lipids are not likely to introduce a systematic bias. Non-fasting lipids have successfully been used in lipid and CVD research.[9 39 40]

The study lacked data on apolipoprotein E genotype, CRP (C-reactive protein) and thyroid tests/thyroid antibodies, factors that all are found to affect female fertility.[23 41] No assessments of duration or temporal proximity of OC use, dietary intake or stress were available; therefore, unmeasured confounding cannot be ruled out. Smoking adversely influences female fertility,[42] with most of its effects attributed to HDL cholesterol decrease.[43] We accounted for this in our analyses; however, smoking status of participants was only available at enrolment. The ethnic homogeneity of the included women may reduce the generalisability of our findings.

Unfavourable prepregnant lipid levels were associated with having no and one lifetime pregnancy. Women's metabolic homeostasis is important for reproduction and also has cardiometabolic implications.[32 44] Pre-existing poor lipid and metabolic profiles could represent one of the possible linkages between previously observed reduced fertility and later CVD.

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Contributors All authors had full access to the data and are responsible for the integrity of the data. AP, RS, LAD and N-HM designed the study. AP and N-HM conducted the analyses. N-HM created the figure and AP created the tables and the flow chart. AP drafted the manuscript. N-HM, RS and LAD reviewed the preliminary analyses and initial draft and provided critical comments.

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Competing interests None declared.

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Data sharing statement This data set contains personal data and cannot be made public due to confidentiality requirements according to Norwegian legislations. Researchers who are interested in analysing data from CONOR or the MBRN may apply to the appropriate organisations after having obtained all necessary approvals according to Norwegian legislations.

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III

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ORIGINAL RESEARCH ARTICLE

Risk of having one lifetime pregnancy and modification by outcome of pregnancy and perinatal loss

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Abstract

Introduction: With increasing cesarean section rates, adverse pregnancy outcomes such as preterm delivery and small-for-gestational-age continue to be public health challenges. Besides having high co-occurrence and interrelation, it is suggested that these outcomes, along with preeclampsia, are associated with reduced subsequent fertility. On the other hand, the loss of a child during the perinatal period is associated with increased reproduction. Failure to consider this factor when estimating the effects of pregnancy outcomes on future reproduction may lead to erroneous conclusions. However, few studies have explored to what degree a perinatal loss contributes to having a next pregnancy in various adverse pregnancy outcomes.

Material and methods: This was a population-based study of mothers giving birth to their first singleton infant (≥22 gestational weeks) during 1967-2007 who were followed for the occurrence of a second birth in the Medical Birth Registry of Norway until 2014. Relative risks with 95% confidence intervals for having one lifetime pregnancy by preterm delivery, small-for-gestational-age, preeclampsia and cesarean section were obtained by generalized linear models for the binary family and adjusted for maternal age at first birth, education and year of first childbirth. Main outcome measure was having one lifetime pregnancy.

Results: Nearly 900 000 women gave birth to their first singleton infant in 1967-2007, of which 16% had only one lifetime pregnancy. These women were older at first delivery, had less education and there was a higher proportion of unmarried women than women with two or more births. In women with pregnancy complications where the infant survived the perinatal period, there were the following relative risks for one lifetime pregnancy: increased preterm delivery: 1.21 (1.19-1.22)], small-for-gestational-age: 1.13 (1.12-1.15), preeclampsia: 1.09 (1.07-1.11), cesarean section: 1.24 (1.23-1.25). The risk was significantly reduced if the child was lost (preterm delivery: 0.63 [0.59-0.68], small-for-gestational-age: 0.57 [0.51-0.63], preeclampsia: 0.69 [0.59-0.80], cesarean section: 0.67 [0.56-0.79]), compared with women with no perinatal loss and no adverse outcome.

Conclusions: The associations between adverse outcomes of pregnancy and the risk of having one lifetime pregnancy were strongly modified by child survival in the perinatal period.

Abbreviations: CI, confidence interval; CS, cesarean section; MBRN, Medical Birth Registry of Norway; PE, preeclampsia; PTD, preterm delivery; RR, relative risk ratio; SGA, small-for-gestational-age.

KEYWORDS
cesarean section, one lifetime pregnancy, perinatal loss, preeclampsia, preterm delivery, selective fertility, small-for-gestational-age

1 | INTRODUCTION

Fertility rates are declining worldwide, with various reasons for this.[1] Family planning decisions are multifactorial and include both biological and socioeconomic factors.[2,3] Preexisting poor health can shape women's decision for future pregnancies,[2,4,5] as can previous experience of adverse pregnancy outcomes.[2,4] With increasing cesarean section (CS) rates,[3,6] adverse pregnancy outcomes such as preterm delivery (PTD) and small-for-gestational-age (SGA) continue to be public health challenges, despite health system improvements.[6] Besides having high co-occurrence and interrelation,[6] it is suggested that these outcomes, along with preeclampsia (PE), are associated with reduced subsequent fertility.[2,7,8] On the other hand, the loss of a child during the perinatal period is associated with increased reproduction.[3,7,9] The tendency to replace losses in order to obtain a desired number of children has been described as "selective fertility,"[9] and failure to consider this factor when estimating the effects of pregnancy outcomes on future reproduction may lead to erroneous conclusions.[10] However, few studies have explored to what degree a perinatal loss contributes to having a next pregnancy in various adverse pregnancy outcomes.

The aim was to estimate risk of having one lifetime pregnancy by adverse pregnancy outcomes (PTD, PE, SGA and CS) and to assess the modifying effect of perinatal loss.

Key message

Perinatal loss of a first child is a strong modifier of the association between adverse outcomes of pregnancy and one lifetime pregnancy risk. Not considering perinatal loss when estimating the influence of pregnancy complications on future reproduction might be misleading.

2 | MATERIAL AND METHODS

We used population-based data from the Medical Birth Registry of Norway (MBRN) during 1967-2014.[11] Notification of births is compulsory (including stillbirths and abortions ≥16 weeks and >12 weeks since 2002). Records are matched with the files of the Central Person Register, to ensure notification of every newborn in Norway and to collect dates of deaths.[11] All births were linked to the mother (sibship files) using the personal identification number given to all permanent residents in Norway. Data on maternal education (in years) were obtained by linkage to the National Education Database.[4] Education in Norway is organized as follows: primary school (7 years), lower secondary school (3 years), upper secondary school (3 years) and higher education. The first 10 years are mandatory.

As part of the preventive health program of pregnancy in Norway, all women were examined and interviewed by a midwife and/or a general practitioner on a regular basis throughout pregnancy. Information on medical history, body measures, previous births, and previous and current complications were collected. The obtained information was regularly updated on the antenatal chart during health visits. At the time of delivery, the antenatal chart is brought to the delivery department and all compulsory data were collected by the attending physician or midwife and transferred to the MBRN.[11] From 1999 onwards, the MBRN has collected gestational age estimated by ultrasonography and date of the last menstrual period (the latter from 1967 onwards). Detailed data on in vitro fertilization (IVF) were available from 1988, and were reported from all fertility clinics in the country.

Perinatal loss (referred to hereafter as "loss") included stillbirths and neonatal deaths within 7 days after delivery. Stillbirth was defined as stillborn with gestational age ≥22 completed gestational weeks. PTD was defined as delivery occurring before 37 completed weeks. SGA was defined as infant birthweight below the 10th percentile by gestational age and sex, based on a previous standard using data from MBRN.[12] PE was defined as any recording of blood pressure of ≥140/90 mmHg after 20 weeks of gestation with proteinuria ≥0.3 g/L/24 hours, and included all forms of PE, eclampsia and syndrome of hemolysis, elevated liver enzymes and low platelet count (HELLP), in accordance with recommendations by the Norwegian Society of Gynecology and Obstetrics.[13] Previous validation studies of PE in the MBRN showed satisfactory positive predictive value, with increasing PPV over time.[14] CS included planned, emergency and unspecified CS. Second delivery, as all observed deliveries, was defined as delivery after ≥22 weeks of gestation.

Diabetes status was defined as occurrence of any type of registered diabetes prior to conception (pregestational diabetes type 1, pregestational diabetes type 2, pregestational diabetes unspecified/other). The overall recording of diabetes was of good quality, although may be underreported.[15] Hypertension was defined as blood pressure ≥140/90 mmHg diagnosed prior to pregnancy.

2.1 | Statistical analyses

For each of the adverse outcomes of pregnancy (PTD, SGA, PE and CS) a 4-category variable was created: (1) women with the outcome and a live infant, (2) women without the outcome and with loss, (3) women

TABLE 1 Characteristics of 882 803 Norwegian women giving birth to their first child during 1967-2007 and followed for the occurrence of a second birth until 2014 in the Medical Birth Registry of Norway

	One-child mothers, n (%)	2+ child mothers, n (%)
Total mothers	142 378 (16.13)	740 425 (83.87)
Maternal age		
<20	12 954 (9.10)	100 495 (13.57)
20-24	39 744 (27.91)	308 837 (41.71)
25-29	41 940 (29.46)	239 673 (32.37)
30-34	29 169 (20.48)	78 040 (10.54)
35-39	14 871 (10.44)	12 744 (1.72)
40+	3709 (2.60)	636 (0.08)
Education category		
Low	33 244 (23.35)	146 406 (19.77)
High	104 644 (73.50)	588 933 (79.54)
Missing	4490 (3.15)	5086 (0.68)
Marital/cohabiting status		
Married	70 411 (49.45)	440 709 (59.52)
Cohabitant/ registered partner[a]	35 973 (25.26)	189 482 (25.59)
Unmarried/single	33 804 (23.74)	105 056 (14.19)
Divorced/ separated[b]	1372 (0.96)	1748 (0.23)
Other/missing	818 (0.57)	3422 (0.46)

Values are numbers (percentages) unless stated otherwise.
[a]Registration of cohabitation introduced from 1977, registration of partners introduced from 2008.
[b]This category includes widowed women.

with the outcome and loss and (4) women with no adverse outcome and no loss (reference category). Each appropriate category of complication included only the complication in question (stated) and no other observed complications. We also constructed a composite variable that combined the effect of two, three and all the four outcomes (PTD, SGA, PE and CS) on risk of having one lifetime pregnancy, and stratified this analysis by perinatal loss.

2.2 | Characteristics of the included women are presented as numbers and proportions

Differences between one-child mothers and mothers with two or more children were analyzed by *t*-test and Chi-square tests. We used generalized linear models for the binary family to calculate crude and adjusted relative risks (RR) with 95% confidence interval (CI) for having one lifetime pregnancy in women experiencing the adverse pregnancy outcomes compared with women without these conditions. Analyses were adjusted for maternal age as a 6-category variable (years: <20, 20-24, 25-39, 30-34, 35-39 and >40), attained education as a 2-category variable (years: <11 and >11), and year of first childbirth (continuous). We explored possible interactions between the observed outcomes (PTD, SGA, PE and CS) and perinatal loss by examining statistical significance of interaction terms. Due to overall higher risk of pregnancy complications among women with multiple pregnancies,[3] we included only women with singleton first births in our analyses. In subanalyses, we excluded women with preexisting diabetes, previous hypertension, in vitro-fertilized pregnancies (from 1988) and women with chronic conditions (asthma, chronic renal disease, cardiovascular disease and rheumatoid arthritis), and performed stratified analyses on marital/cohabitant status (to examine the effect of having a partner). We truncated the data to allow all included women at least a 7-year timeframe to complete their birth record (ie, we included first births that occurred before 2007, and all women were followed until 2014 for the occurrence of a second pregnancy), as 95% of Norwegian women will have a second pregnancy within 7 years.[4] To account for time trends of PTD, SGA, PE and CS, we analyzed our data by the following time periods: 1967-1976, 1977-1986, 1987-1996 and 1997-2007. We additionally examined women's characteristics during the same time spans. Due to low number of missing cases (1.1% for education and 0.48% for marital status) these were handled by listwise deletion in the analyses. Additional exclusion of women with missing data on education and cohabitation showed no effect on our results. The proportion of missing data for other variables was low—birthweight (0.16%) and gestational age (5.05%)— and these were excluded from our calculations for the 4-level variable. All statistical analyses were performed using STATA Software, version 15 (StataCorp, College Station, TX, USA).

2.3 | Ethical approval

This study was approved by the internal review board of the MBRN and by the regional ethics committee, REK Vest, Norway (reference number: 2009/1868).

3 | RESULTS

We identified 882 803 women giving birth to their first singleton infant (≥22 weeks) between 1967 and 2007. Of all women, 16.1% had one lifetime pregnancy. Characteristics are shown in Table 1. Women with one lifetime pregnancy were older at first delivery, had less education and were more frequently unmarried or single compared with women with two or more births.

Numbers, proportions, crude and adjusted RR with 95% CI for one lifetime pregnancy by the 4-category variable (for: PTD, SGA, PE and CS) are presented in Table 2. The proportion of one-child mothers with no observed complications and no loss at first birth ranged from 15 to 16% for all outcomes. Women with surviving child and complications in the first pregnancy had a higher proportion of one lifetime pregnancy. For women who experienced loss along with the adverse outcome, a decrease in the proportion of one lifetime pregnancy was observed across all outcomes: PTD: 10.3%,

TABLE 2 Crude and adjusted relative risks (RR) with 95% confidence interval (CI) for having one lifetime pregnancy in 882 803 women by preterm delivery (PTD), small-for-gestational-age (SGA), preeclampsia (PE) and cesarean section (CS) with and without a perinatal loss in first pregnancy (Medical Birth Registry of Norway 1967-2007)

Outcomes	N (total)	One-child mothers, n (%)	Crude RR, 95% CI	Adjusted RR, 95% CI
PTD and no loss	47 984	10 326 (21.51)	1.35 (1.33-1.38)	1.21 (1.19-1.22)
No PTD and loss	3782	269 (7.11)	0.45 (0.40-0.50)	0.42 (0.37-0.47)
PTD and loss	6196	635 (10.29)	0.64 (0.60-0.69)	0.63 (0.59-0.68)
No PTD and no loss	824 841	131 148 (15.89)	1.00 (Reference)	1.00 (Reference)
SGA and no loss	103 714	19 320 (18.63)	1.17 (1.16-1.19)	1.13 (1.12-1.15)
No SGA and loss	6675	593 (8.90)	0.56 (0.52-0.60)	0.54 (0.50-0.58)
SGA and loss	3303	311 (9.41)	0.59 (0.53-0.66)	0.57 (0.51-0.63)
No SGA no loss	769 111	122 154 (15.90)	1.00 (Reference)	1.00 (Reference)
PE and no loss	36 825	7006 (19.02)	1.18 (1.16-1.20)	1.09 (1.07-1.11)
No PE and loss	9142	799 (8.74)	0.54 (0.50-0.58)	0.52 (0.49-0.56)
PE and loss	836	105 (12.56)	0.78 (0.66-0.91)	0.69 (0.59-0.80)
No PE no loss	836 000	134 468 (16.08)	1.00 (Reference)	1.00 (Reference)
CS and no loss	92 161	23 439 (25.43)	1.68 (1.66-1.70)	1.24 (1.23-1.25)
No CS and loss	9059	793 (8.75)	0.58 (0.54-0.62)	0.55 (0.52-0.59)
CS and loss	919	111 (12.08)	0.80 (0.67-0.95)	0.67 (0.56-0.79)
No CS no loss	780 664	118 035 (15.12)	1.00 (Reference)	1.00 (Reference)

Women were followed for the occurrence of a second pregnancy until 2014. Estimates were adjusted for maternal age at first birth, education, and year of childbirth.

SGA: 9.4%, PE: 12.6% and CS: 12.1%. The biggest reduction was found in women with SGA (compared with those with no SGA and no loss) (9.4 vs 15.9%, respectively), and the smallest reduction was seen in women with PE (12.6 vs 16.1%, respectively). RR for having one lifetime pregnancy when the child survived the perinatal period was significantly increased for all the examined outcomes: 1.21 (1.19-1.22); 1.13 (1.12-1.15); 1.09 (1.07-1.11) and 1.24 (1.23-1.25) for PTD, SGA, PE and CS, respectively. If the woman had any of the adverse outcomes and lost the child, RR for having one lifetime pregnancy was significantly reduced: PTD: 0.63 (0.59-0.68), SGA: 0.57 (0.51-0.63), PE: 0.69 (0.59-0.80) and CS: 0.67 (0.56-0.79).

The crude RR of having one lifetime pregnancy for the all the adverse outcomes remained fairly stable over the four time periods, apart from the first decade (Table 3). This corresponds to increased notification of milder forms of PE[14] and increasing rates of CS with broader indications over the decades.[3]

For women with complications and no loss, change of RR toward the null after adjustments suggests that observed associations between adverse outcomes and probability of one lifetime pregnancy were partially explained by adjusted factors, with maternal age at first birth changing the estimates the most. The associations, however, became stronger after the adjustments for women with complications and a loss.

Tests of the interaction between PTD, SGA and CS at first birth and loss showed significant effect on the risk of having one lifetime pregnancy ($P = 0.001$, $P = 0.005$ and $P < 0.001$, respectively). We found no significant interaction between PE in first birth and loss ($P = 0.47$).

Exclusion of women with preexisting diabetes, hypertension and in vitro-fertilized pregnancies (from 1988) did not substantially change the results (data not shown). Additional exclusion of asthma, chronic renal disease, cardiovascular disease and rheumatoid arthritis showed only a slight effect for women with the surviving child (PE: [crude] RR 1.15, 95% CI 1.13-1.17 and CS: [crude] RR 1.65 95% CI 1.63-1.68), whereas for women with no loss, results remained unaltered. Stratification by marital/cohabitating status showed a decrease in RR of having one lifetime pregnancy among unmarried and single women with observed complications, implicating an effect of having a long-term partner (Supporting Information Table S1). In our examination of changes in maternal characteristics throughout four decades of MBRN, we found an increase in the proportion of one-child mothers (first vs last decade: 14.4 vs 18.4%) with more than a doubled proportion of women aged ≥35 at first birth. In the same group, an almost one-third decrease in the number of single women was accompanied by a 7.6% increase in education level (Supporting Information Table S2).

The results of the combined effects of PTD, SGA, PE and CS (composite variable) and stratified by loss are presented in Figure 1. Similar to the results for the separate effects of the outcomes, the analyses for the combined variable showed that the increased risk of having one lifetime pregnancy consistently varied by the survival of the infant in the perinatal period.

4 | DISCUSSION

Women with PTD, SGA, PE or CS and one surviving child had a higher risk of having only one child compared with women without

TABLE 3 Crude relative risks (RR) with 95% confidence interval (CI) for having one lifetime pregnancy in 882 803 women by preterm delivery (PTD), small-for-gestational-age (SGA), preeclampsia (PE) and cesarean section (CS) with and without a perinatal loss in first pregnancy (Medical Birth Registry of Norway, 1967-2007) by 10-year periods

	1967-1976 RR (95% CI)	1977-1986 RR (95% CI)	1987-1996 RR (95% CI)	1997-2007 RR (95% CI)
PTD and no loss	1.35 (1.30-1.40)	1.39 (1.34-1.45)	1.31 (1.26-1.35)	1.34 (1.29-1.38)
No PTD and loss	0.48 (0.41-0.57)	0.37 (0.28-0.49)	0.49 (0.37-0.65)	0.54 (0.42-0.71)
PTD and loss	0.71 (0.63-0.79)	0.63 (0.54-0.74)	0.61 (0.50-0.73)	0.66 (0.54-0.81)
No PTD and no loss	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
SGA and no loss	1.17 (1.14-1.21)	1.21 (1.18-1.25)	1.20 (1.16-1.23)	1.22 (1.18-1.25)
No SGA and loss	0.62 (0.55-0.69)	0.52 (0.44-0.62)	0.52 (0.43-0.63)	0.63 (0.53-0.76)
SGA and loss	0.64 (0.55-0.75)	0.58 (0.47-0.73)	0.67 (0.53-0.86)	0.57 (0.41-0.77)
No SGA no loss	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
PE and no loss	1.27 (1.22-1.32)	1.20 (1.16-1.25)	1.12 (1.08-1.16)	1.10 (1.06-1.14)
No PE and loss	0.57 (0.52-0.63)	0.50 (0.43-0.59)	0.59 (0.51-0.69)	0.58 (0.49-0.69)
PE and loss	0.98 (0.79-1.21)	0.80 (0.51-1.11)	0.22 (0.09-0.52)	0.83 (0.54-1.27)
No PE no loss	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
CS and no loss	2.06 (1.98-2.13)	1.62 (1.58-1.66)	1.60 (1.56-1.63)	1.60 (1.55-1.62)
No CS and loss	0.61 (0.56-0.67)	0.54 (0.47-0.63)	0.57 (0.48-0.68)	0.65 (0.55-0.77)
CS and loss	1.05 (0.76-1.45)	0.72 (0.76-1.45)	0.74 (0.52-1.06)	0.72 (0.47-1.08)
No CS no loss	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)

Women were followed for the occurrence of a second pregnancy until 2014.

adverse outcome and no loss in the first pregnancy. However, having the same outcomes and losing the child significantly reduced the risk of having one lifetime pregnancy. In line with previous studies,[3,9,10] results potentiate "selective fertility" as an important force in reproduction.

Substantial decline in one lifetime pregnancy was found in women with PTD and loss. This contrasts with findings of PTD as a factor for reduced fertility.[16] Although recurrence risk of PTD proposes the importance of shared maternal-fetal genetic aspects,[5,6] a study on familial patterns of PTD found little evidence of fetal contribution.[5] Maternal health and intrauterine environment are likely determinants of PTD.[5,17] Nevertheless, increased reproduction following the loss of a preterm child suggests that the effects of biological aspects of PTD on future reproduction are likely smaller than the effects of other possible factors.

Similar results were found for women with SGA babies, supporting shared determinants and recurrence risk with PTD.[6] The larger effect with SGA may be related to stresses incurred during the often prolonged management of a recognized growth-restricted fetus, in comparison with PE and PTD, which are events more often resulting in delivery. Increase in one lifetime pregnancy risk when PTD was followed by SGA (Figure 1, PTD-SGA) is in line with reports of increased severity when these exposures are combined.[6]

The RR for having one lifetime pregnancy in women with PE (and a surviving child) was marginally increased, consistent with studies that have reported a lower birth rate after PE in first pregnancy.[9,18,19] A recent study suggested that women who developed PE are more likely to be older, have comorbidities or assisted reproductive treatment.[19] In our study, the risk of having one lifetime pregnancy after PE slightly decreased after accounting for maternal age (Table 2), while excluding cases of preexisting diabetes, hypertension and in vitro fertilization showed no substantial effect. A Norwegian study reported substantially increased risk of PE among the youngest mothers over time, emphasizing lifestyle factors over age.[20] Increased risk of having one lifetime pregnancy for women with three combined outcomes (PTD-PE-CS, Figure 1) mirrors the findings that distinguish two conditions of PE: term and preterm.[17,20] The increase could be due to more severe clinical presentations compared with term PE, resulting in preterm delivery and related fetal complications.[18,19] We observed low dependency of preterm PE on perinatal survival (further supported by nonsignificant interaction between PE in first birth and perinatal loss). Recent reports of a 3.6% recurrence of PTD in women with previous early onset PE (<34 weeks) suggest that these women have a predisposition to both PTD and PE from underlying conditions.[19] A lower likelihood of continuing reproduction after a loss in preterm PE could reflect a possibility of residual confounding by subclinical or underlying health factors other than clinical conditions evaluated in our study (ie, possibility of genetic and/or metabolic conditions[2]). Preterm PE has more familial aggregation, increases fetal and maternal morbidities, and is associated with chronic factors.[18] The psychosocial burden of preterm birth and maternal health problems after PE could also contribute to women's reluctance to conceive again.[19]

An increased risk of having one lifetime pregnancy in women with CS and a surviving child, and the markedly decreased risk

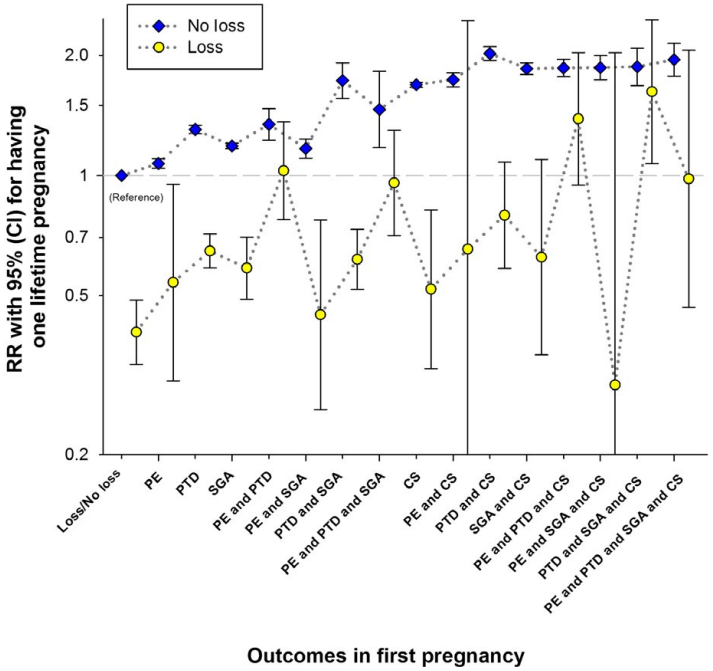

FIGURE 1 Crude relative risks (RR) with 95% confidence intervals (CI) for having one lifetime pregnancy by preeclampsia (PE), preterm delivery (PTD), small-for-gestational-age (SGA), cesarean section (CS) and combination of two, three or all four outcomes by loss or no loss of the first baby in 882 803 women (Medical Birth Registry of Norway, 1967 to 2007). Women were followed for the occurrence of a second birth until 2014 [Color figure can be viewed at wileyonlinelibrary.com]

if the child was lost, suggests that maternal choice plays an important role. This concurs with several previous studies that have suggested voluntary factors,[3,7,16,21] in contrast to the few studies that found no association with reduced fertility[22,23] or concluded that there were obstructive physical consequences of CS on fecundity.[1,24] It has been suggested that an inverse cesarean–parity association was not specific for CS, but rather for all operative births.[23] A reluctance to conceive due to previous surgical intervention[25] might explain the higher risk of having one child after CS in women with no loss (Figure 1). However, in a scenario with three combined outcomes (PE-PTD-CS and PTD-SGA-CS; Figure 1), increased risk remained irrespective of child loss, suggesting condition severity[26] and/or underlying health factors. Similarly, in a Danish study, increased birth rate after death of a child in maternal-requested CS but only a slight reduction for women with elective/emergency CS, led the authors to suggest residual confounding in the latter group.[27]

Although one-child mothers were older, less educated and had a higher proportion of single women, remaining risks after accounting for maternal age and education make these factors less likely as prevailing factors. Stratified subanalyses suggest the importance of a long-term partner (Table S1). However, fairly stable RR throughout decades (despite an increase in the proportion of one-child mothers and a decrease in single women among them) implicate the involvement of other factors (Table S2). Preexisting poor health might play a role, given its association with both later onset of first birth[28] and lower education.[29]

A major strength is the use of a large dataset, which enabled linkage of all births to the same mother and study of a rare perinatal event (perinatal loss). Limitations included lack of information on family decision planning, body mass index (BMI), smoking, fertility treatments other than in vitro fertilization, and limited registration of ultrasound dates (from 1999). The level of education may serve as a proxy for BMI (higher education correlates with lower BMI[30]) and adjustment for education may partially have accounted for BMI. A Danish study reported stronger effect of BMI on elective than on emergency CS.[27] Residual confounding by CS indication cannot be excluded. Exclusion of women with chronic conditions makes these less likely as indication. A Norwegian study found the results consistent in both high and low risk cesarean delivery, implicating cesarean delivery as a reason for reduced fertility, and not the indications that lead to it.[3] Additionally, possible errors in gestational age estimates (last menstrual period vs ultrasound-based) are not likely to be substantial in the MBRN.[5] Validation studies showed good ascertainment of pregestational diabetes and PE;[14,15] however, there might be the possibility of under-reporting of type 2 diabetes and

over-reporting of preexisting hypertension with spontaneous normalization. It is likely that industrialized countries with a similar level of obstetric interventions and facilities for treating preterm babies will show similar results.

5 | CONCLUSION

Our findings suggest that adverse pregnancy outcomes with a surviving child in the first pregnancy increases the likelihood of having only one child, but that a loss increases the probability of having another pregnancy, regardless of other observed complications. This reinforces selective fertility as an important factor in reproduction and suggests that not considering perinatal loss when estimating the effect of pregnancy complications on future reproduction might be misleading.

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CONFLICT OF INTEREST

None.

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SUPPORTING INFORMATION

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

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Supporting information

Table S1. Adjusted relative risks (RRs) with 95% confidence interval (CI) for one lifetime pregnancy in 882 803 women by preterm delivery (PTD), small for gestational age (SGA), preeclampsia (PE) and cesarean section (CS) with and without perinatal loss in first pregnancy, Medical Birth Registry of Norway 1967 to 2007. Women were followed for the occurrence of a second pregnancy until 2014. Estimates were adjusted for maternal age at first birth, education and year of childbirth and stratified on marital/cohabitating status.

Table S2. Characteristics of 882 803 Norwegian women giving birth to their first child during 1967-2007 by 10-year periods. Women were followed for the occurrence of a second pregnancy until 2014. Numbers are percentages unless stated otherwise.

Table S1. Adjusted relative risks (RRs) with 95% confidence interval (CI) for one lifetime pregnancy in 882 803 women by preterm delivery (PTD), small for gestational age (SGA), preeclampsia (PE) and cesarean section (CS) with and without perinatal loss in first pregnancy, Medical Birth Registry of Norway 1967 to 2007. Women were followed for the occurrence of a second pregnancy until 2014. Estimates were adjusted for maternal age at first birth, education and year of childbirth and stratified on marital/cohabitating status.

Outcomes	Married/Cohabitating	Unmarried/Single
PTD and no loss	1.22 (1.20-1.24)	1.11 (1.08-1.14)
No PTD and loss	0.37 (0.32-0.42)	0.52 (0.43-0.62)
PTD and loss	0.62 (0.57-0.68)	0.59 (0.52-0.66)
No PTD no loss	1.00 (Reference)	1.00 (Reference)
SGA and no loss	1.13 (1.11-1.15)	1.04 (1.02-1.06)
No SGA and loss	0.52 (0.47-0.57)	0.55 (0.48-0.62)
SGA and loss	0.53 (0.47-0.60)	0.58 (0.49-0.69)
No SGA no loss	1.00 (Reference)	1.00 (Reference)
PE and no loss	1.12 (1.10-1.14)	1.04 (1.01-1.07)
No PE and loss	0.49 (0.46-0.54)	0.55 (0.49-0.61)
PE and PD	0.69 (0.58-0.82)	0.66 (0.50-0.86)
No PE no loss	1.00 (Reference)	1.00 (Reference)
CS and no loss	1.29 (1.27-1.31)	1.07 (1.04-1.09)
No CS and loss	0.52 (0.48-0.56)	0.56 80.51-0.63)
CS and loss	0.70 (0.58-0.85)	0.54 (0.38-0.77)
No CS no loss	1.00 (Reference)	1.00 (Reference)

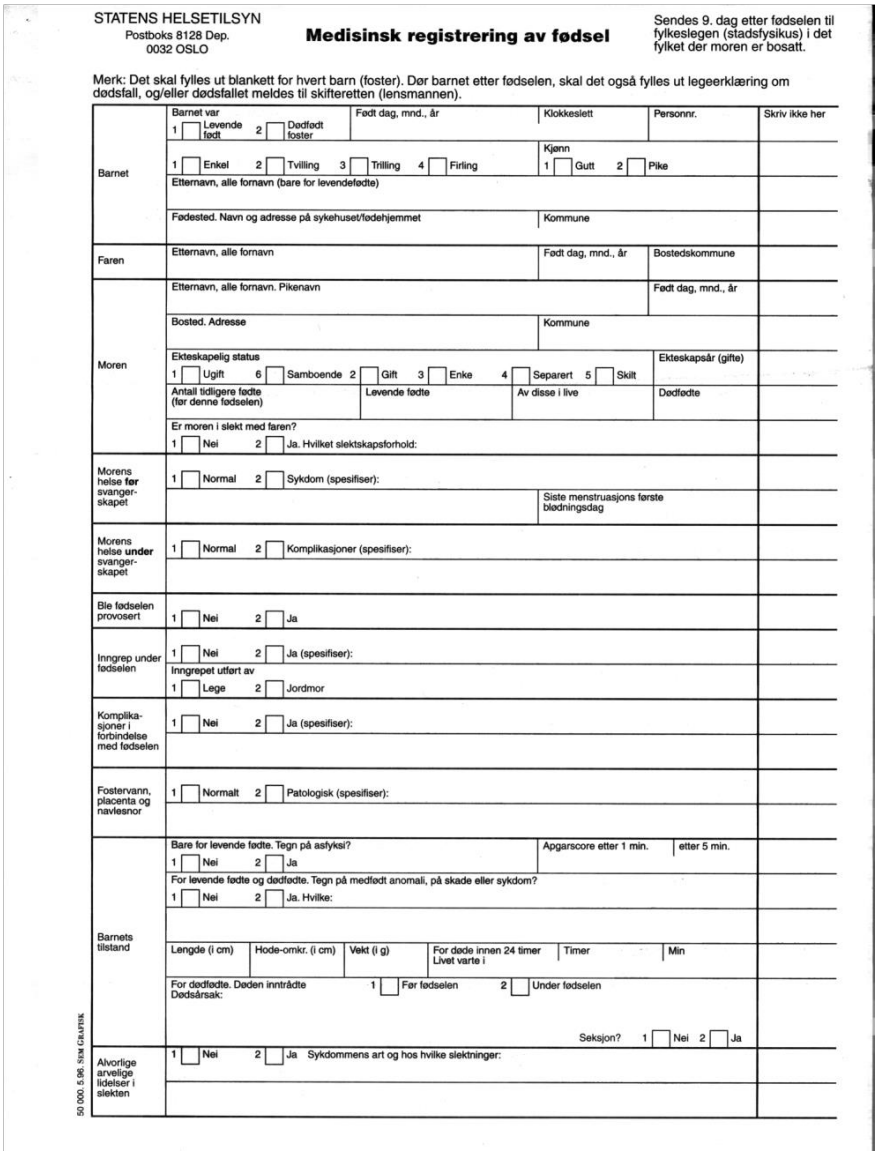
Table S2. Characteristics of 882 803 Norwegian women giving birth to their first child during 1967-2007 by10-year periods. Women were followed for the occurrence of a second pregnancy until 2014. Numbers are percentages unless stated otherwise

Maternal age at 1st birth	1967 - 1976 N =240770		1977 - 1986 N =197888		1987 - 1996 N =219753		1997 – 2007 N =224392	
	1 child mother	2+child. mother	1 child mother	2+child. mother	1 child mother	2+child. mother	1 child mother	2+child. mother
<20	5924 (17.00)	47093 (22.82)	3151 (10.19)	26641 (15.89)	1892 (5.36)	16588 (8.99)	1987 (4.79)	10173 (5.55)
20-24	13504 (38.80)	109003(52.90)	9689 (31.40)	78475 (47.01)	8721 (24.73)	72462 (39.28)	7830 (18.90)	48897 (26.72)
25-29	8943 (25.70)	41673 (20.18)	9872 (32.01)	50288 (30.11)	11585 (32.85)	70889 (38.42)	11520 (27.81)	76823 (41.98)
30-34	3861 (11.10)	6911 (3.39)	5532 (17.90)	10231 (6.09)	8281 (23.48)	21330 (11.56)	11486 (27.73)	39568 (21.62)
35-39	1866 (5.40)	1158 (0.61)	2160 (7.02)	1346 (0.79)	3971 (11.26)	3081 (1.67)	6874 (16.59)	7159 (3.91)
40+	735 (2.10)	99 (0.01)	436 (1.38)	67 (0.01)	813 (2.30)	140 (0.07)	1725 (4.16)	350 (0.19)
Education category								
low	9643 (27.68)	49385 (23.98)	7783 (25.24)	40661 (24.33)	7956 (21.70)	32849 (17.80)	7862 (18.98)	23511 (12.84)
high	24307 (69.78)	155916(75.71)	22128(71.75)	125606(75.20)	26292 (74.50)	150777(81.72)	31917 (77.05)	156634 (85.60)
missing	883 (2.64)	636 (0.39)	929 (3.01)	781 (0.47)	1045 (3.80)	834 (0.45)	1643 (3.97)	2825 (1.56)
Martial/cohabitating status								
married	25969 (74.55)	177216(86.05)	18610(60.34)	118614(71.00)	12532 (35.54)	77977(42.27)	13300 (32.10)	66902 (36.56)
cohabitant/registered[1]	/	/	1205 (3.90)	6475 (3.87)	14508 (41.14)	83096 (45.04)	20260 (48.91)	99919 (54.60)
unmarried/single	8574 (24.61)	28218 (13.75)	10439(33.84)	41079 (24.59)	7853 (22.27)	22672 (12.29)	6938 (16.74)	13087 (7.15)
divorced/separated [2]	264 (0.76)	340 (0.16)	552 (1.79)	687 (0.41)	300 (0.85)	385 (0.21)	257 (0.62)	336 (0.18)
other/missing	26 (0.07)	163 (0.01)	35 (0.11)	193 (0.11)	70 (0.20)	360 (0.19)	687 (1.65)	2706 (1.47)

[1] Registration of cohabitation introduced from 1977, registration of partners introduced from 2008; [2]Includes widowed.

10. Appendix

Appendix I. Notification form, the Medical Birth Registry of Norway, 1967-1998



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 svanger-skapet

1 ☐ Normal 2 ☐ Sykdom (spesifiser):

Siste menstruasjons første blødningsdag

Morens helse under svanger-skapet

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

Komplika-sjoner 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:

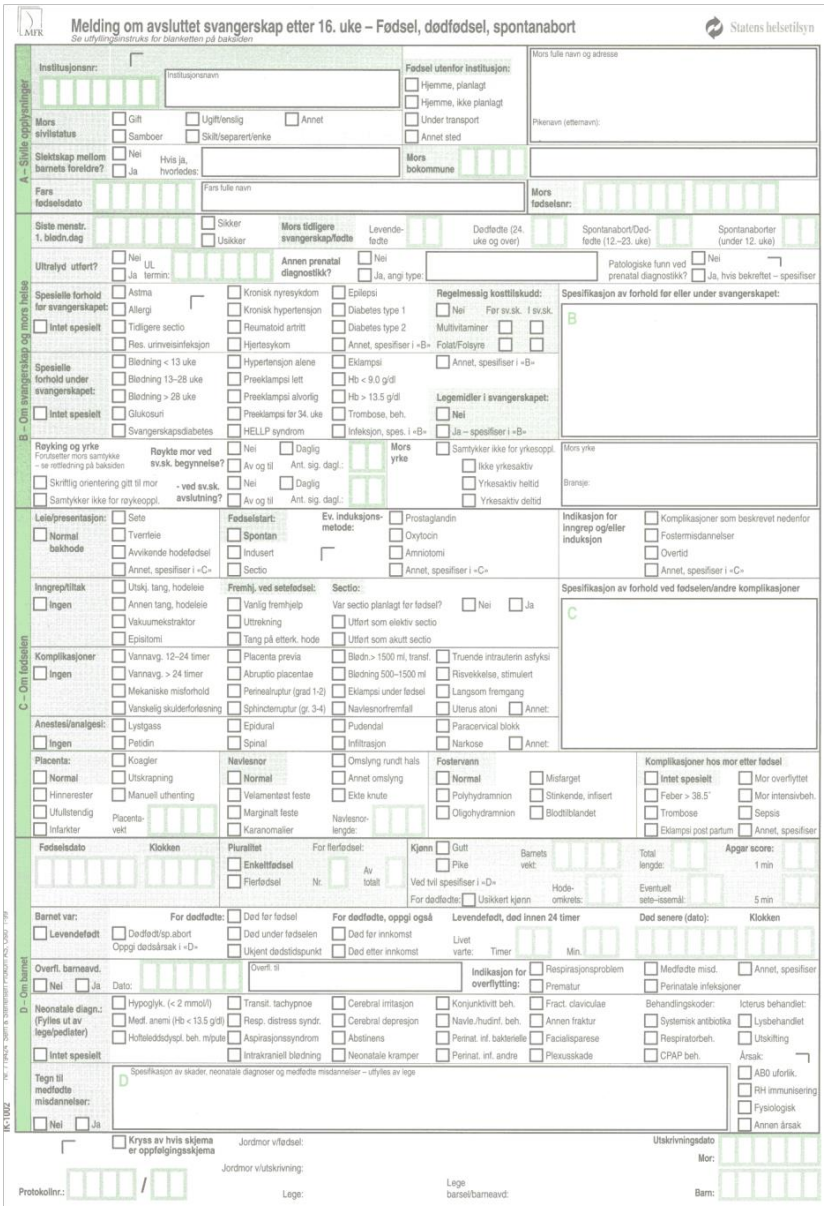
Seksjon? 1 ☐ Nei 2 ☐ Ja

Alvorlige arvelige lidelser i slekten

1 ☐ Nei 2 ☐ Ja Sykdommens art og hos hvilke slektninger:

50 000. 6.86. SEM GRAFISK

Appendix II. Notification form, the Medical Birth Registry of Norway, 1999-



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

Se utfyllingsinstruks for blanketten på baksiden

Statens helsetilsyn

A – Sivile opplysninger

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

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

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

Fars fødselsdato | Fars fulle navn | Mors fødselsnr:

B – Om svangerskap og tidlig 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 før svangerskapet: Astma; Allergi; Intet spesielt; Tidligere sectio; Res. urinveisinfeksjon; Kronisk nyresykdom; Kronisk hypertensjon; Reumatoid artritt; Hjertesykom; Epilepsi; Diabetes type 1; Diabetes type 2; Annet, spesifiser i «B»

Regelmessig kosttilskudd: Nei; Før sv.sk.; I sv.sk.; Multivitaminer; Folat/Folsyre

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»; Annet, spesifiser i «B»

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

Røyking og yrke (Forutsetter mors samtykke – se rettledning 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; Avvikende 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 previa; Abruptio placentae; Perinealruptur (grad 1-2); Sphincterruptur (gr. 3-4); Blødn.> 1500 ml, transf.; Blødning 500–1500 ml; Eklampsi under fødsel; Navlesnorfremfall; 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; Hinnerester; Utullstendig; Infarkter; Koagler; Utskrapning; Manuell uthenting; Placenta-vekt

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

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

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

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

Barnets vekt: | Hode-omkrets:

Total lengde: | Eventuelt sete-issemål:

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) Intet spesielt; 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:

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

ABO uforlik.; RH immunisering; Fysiologisk; Annen årsak

IK-1002

Kryss av hvis skjema er oppfølgingsskjema

Jordmor v/fødsel: | Jordmor v/utskrivning: | Lege: | Lege barsel/barneavd:

Utskrivningsdato: Mor: Barn:

Protokollnr.:

Errata for
Number of children: pre- and post-pregnancy lipids, effect of pregnancy outcome and modification by perinatal loss

Aleksandra Pirnat

Thesis for the degree philosophiae doctor (PhD)

at the University of Bergen

12.12.2019

(date and sign. of candidate)

12.12.2019

(date and sign. of faculty)

Errata

Page XI, under II): Missing words: “Lipid levels after childbirth and association with number of children” – corrected to “Lipid levels after childbirth and association with number of children: A population-based cohort study”.

Page 53 Misspelling: “Examinating” – corrected to “Examining“.

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