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Time-trends in secondary sex ratio of firstborns and polychlorinated biphenyls in Norway for the time period 1967 - 2018

Ingrid Gabrielsen, class of 2015

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Preface

This master thesis is written as a mandatory requirement for the subject MED-3950 within the fifth year of medical studies at the Faculty of Health Sciences at The University of Tromsø – The Arctic University of Norway. The aim of the requirement is to initiate and conduct scientific work within a topic of choice, and ultimately compose a paper in medical science.
From an early age I have had an interest in environmental contaminants. This is much due to the fact that my father has been doing research on contaminants in the Arctic, which has given me the opportunity to work as a field assistant multiple summers in Kongsfjord, Svalbard. During this field work I have been involved in collecting and analyzing samples for various research projects, both monitoring and effect studies, of environmental contaminants in several Arctic seabird species. Being introduced to the concern of anthropogenic emissions possibly having an effect on wildlife in remote areas like Svalbard, further sparked my interest into how environmental contaminants could affect humans.
I got in contact with Torkjel Sandanger at the Department of Community Medicine, and he presented me with this topic they had wanted to explore for some time. It immediately caught my attention because it involved human reproductive health and the effects of environmental contaminants. Diving into the world of persistent organic pollutants has genuinely intrigued me by its complexity, and I will definitely take what I have learned with me as I soon begin my career in the medical profession.
Thank you to Therese Nøst for being my main supervisor, for great input, helping me out with everything statistics related and scientific writing. Your help has been unmeasurable. Thank you to Torkjel Sandanger for great discussions and guidance along the way, and to Erik Anda for providing the dataset from the MBRN. I would also like to use the opportunity to show gratitude towards my family and friends for always supporting me. A special thanks to Øyvind for being there for me, and providing me with coffee throughout this process.

Ingrid Gabrielsen,
Tromsø, august 2020

Table of contents

Abstract

Background: The secondary sex ratio (SSR), the ratio of males to females at birth, has been of much interest in epidemiologic research, as the ratio may be affected by endogenous and exogenous factors. There have been several claims of a declining SSR from the second half of the 20th century, and environmental contaminants, especially persistent organic pollutants (POPs) including polychlorinated biphenyls (PCBs) have been suspected as a cause due to the chemical's endocrine disrupting abilities.

Objectives: The overall aim is to explore if there are any similarities in time trends of SSR in firstborns in Norway and predicted maternal PCB serum concentrations in the time period 1967-2018. Specifically, we used population-level data for SSR, and predicted exposure trends for PCB-153 as a proxy for PCBs during this time period that included the period when PCB exposure was at their highest levels in humans in Norway.

Materials and method: The data used in the thesis is gathered from the Medical Birth Registry of Norway (MBRN) for the time period 1967-2018. We included firstborn singletons after week 21 of gestation and had a total study population of 1,233,767. We stratified our data by maternal age by years 20-24, 25-29, 30-35 and 35-39. Maternal serum concentrations of PCB-153 was predicted using the CoZMoMAN model.

Results: The results demonstrated no declining trend in SSR throughout the time period 1967 to 2018, as linear regression lines were flat and coefficients for change per birth year was close to zero for all maternal age groups. SSR did not appear to deviate from the overall SSR around the time period of peak PCB concentrations, more than yearly fluctuations in any other periods. Although the total number of births remained relatively unchanged, the maternal age composition has changed drastically throughout the study period.

Conclusion: Our findings indicate no change in SSR for firstborns in Norway in the study period 1967-2018. Further, there was no indication of any alterations in SSR on a population level in Norway in the years around the peak exposures of PCBs.

List of abbreviations

DDT	1,1-trichloro-2,2-bis(p-chlorophenyl)ethane
MBRN	Medical Birth Registry of Norway
ODS	Ovarian dysgenic syndrome
PCB	Polychlorinated biphenyl
PCB-153	2,2' ,4,4' ,5,5'-hexachlorobiphenyl
PCDDs	Polychlorinated dibenzo-p-dioxins
PCDFs	Polychlorinated dibenzofurans
POPs	Persistant organic pollutants
PSR	Primary sex ratio
REC	Regional Committees for Medical and Health Research Ethics
SSR	Secondary sex ratio
TCDD	2,3,7,8-Tetrachlorodibenzodioxin
TDS	Testicular dysgenesis syndrome
UNEP	United Nations Environment Programme

1 Introduction

Every day we are exposed to a mixture of thousands of different chemicals, through the air we breathe, the food we eat, in our homes and at work. In fact, the chemical industry is one of the fastest growing industries in the world, and over 140 000 new chemicals have been synthesized since 1950. There is growing evidence that chemical pollutants have the capacity to harm human health and the environment (1). Nearly 800 chemicals are known or suspected to have abilities that interfere with human and wildlife hormonal systems, known as endocrine disruptors (2). Chemicals classified as persistent organic pollutants (POPs) have been demonstrated to have endocrine disrupting abilities. The United Nations Environment Programme (UNEP) consider POPs to be "a serious, global threat to human health and to ecosystems " (3).

Global initiatives have attempted to terminate and regulate the use of POPs, leading to varying concentration loads of chemicals exposing several human generations at different magnitudes (4). One group of POPs that is subject to a great deal of research are polychlorinated biphenyls (PCBs), as PCBs have been associated with adverse health effects on humans and wildlife, as carcinogens, immunotoxins and interfering with reproductive organs (5). The human secondary sex ratio (SSR), the ratio of males to females at birth, may also be affected by increased exposure to PCBs (6, 7). Throughout history, social, medical, environmental and biological factors have been associated with SSR. Since the middle of the 20th century there have been numerous reports suggesting a decreasing trend of SSR in industrialized countries (8, 9). Studies have linked the decreasing trend in SSR to high exposure of PCBs, as PCBs have been heavily produced around the same time as decreasing trends in SSR is observed (10).

In public health epidemiology, causality between exposure and effect is one of the most central issues (11). However, correlation and trend studies on a population level have often led to a great deal of uncertainty when interpreting effect data. Little is known regarding maternal PCB exposure association to SSR in firstborns in Norway. Therefore, we wish to explore if there has indeed been a decreasing SSR and explore potential corresponding trends in predicted serum concentrations of PCB-153, as an indicator of PCB exposure on the population. Further, we wish to assess if PCB exposure affect SSR in firstborns in Norway in the time period from 1967 to 2018.

1.1 Sex ratio

1.1.1 Reporting of the sex ratio

There are several different ways of reporting sex ratio. Sex ratio is commonly defined as the number of male newborns per 100 female newborns. This means that a ratio > 1.00 demonstrate male excess, and a ratio < 1.00 demonstrate female excess (12). However, the relationship is also often reported as the male proportion of the total number of births, which gives a percentage of males born out of total number of births. For instance, if there are 106 males born, divided by 206 total number of births, the male proportion equals 0.515, or a percentage of 51,5% (13).

1.1.2 Sexual determination

Reproduction is a complex process involving several vulnerable developmental stages (13), and is regulated by the endocrine system (2). Sexual determination and development can be divided into four levels: chromosomal, gonadal, phenotypic and psychological (12). The chromosomal sex of the embryo is determined at fertilization, but it is first at 6 weeks of gestation that genital sex differentiation takes place through several sequential stages. In short, the chromosomal composition of genes on the Y chromosome drive the primitive gonad to differentiate into gonads: testis or ovaries. Testis produce androgens and anti-Müllerian hormone leading to the formation of male internal and external genitalia. In the absence of specific testicular hormones, the female genitalia develop (14).

The primary sex ratio (PSR) is the number of male-to-female at conception, while secondary sex ratio (SSR) is an endpoint defined as the number of male-to-female live births. The PSR is the number of ova successfully fertilized by a sperm bearing a Y-chromosome, compared to number of ova fertilized by a sperm containing an X-chromosome. The true PSR is relatively unknown, given the difficulty to measure all conceptions (15), but recently, the largest ever study of embryos, induced abortions, chorionic villus sampling, amniocentesis and fetal death and live births, estimated a PSR of 1. The same study indicated that there was a male-bias in abnormal embryos, an increase in SSR in week 10-15, and a decline in SSR from week 28-35 due to an excess late-pregnancy male mortality. The increased female mortality early in the pregnancy outnumbered the total male mortality during pregnancy (16), explaining a male biased SSR.

Maternal and paternal exposure of foreign substances prior to conception and birth has been linked to interfere with their ability to conceive, and to affect the health of their offspring (17). During embryogenesis, the timing of exposure and dosage, by hormones or other toxic

substances that affect hormonal regulation, make for several outcomes in gonadal development. As the fetal gonadal tissue is among the most rapidly dividing tissues during embryogenesis, these cells may be especially prone to abnormalities caused by external influences (13).

1.1.3 Global trends in SSR

SSR in human populations is often approximated to be between 1.02 and 1.06 (18), and is said to be remarkably constant (19), but through history there have been a few regional and temporal variations. A trend observed in several industrial countries up to the first half of the 20th century indicated an increasing trend in SSR, but during the second half of the century, there have been reports of SSR demonstrating more of a decreasing pattern (18). Reports from Norway (20), the Netherlands (9), Denmark (8), Canada and the United States (13) have indicated a significant decline in SSR, although the extent of the decline varies. For example, in Denmark the proportion of males steadily increased between 1851 and the 1960s, before making a decline from 0.515 (SSR 1.062) in 1950 to 0.513 (SSR 1.053) in 1994 (8). Studies of sex ratio in the Netherlands demonstrated similar trends in the time periods they studied, with a male proportion of 0.516 (SSR 1.066) in 1950 to 0.513 (SSR 1.053) in 1994 (9).

1.1.4 Possible determinants of SSR

Sex ratio has been extensively researched throughout history, and several variables have been suggested to explain temporal variations, since SSR may be influenced by both endogenous and exogenous factors (21). Some researchers have stated that the SSR sensitivity for exogenous factors is a useful public health measure in relation to environmental factors, as it may reflect embryonic mortality early in the development (13, 22). However, others have been more skeptical to the use of SSR as such, because population sex ratios change so gradually that it is difficult to make conclusions (23).

In 1959 it was suggested that a higher SSR of 1.05-1.06 could be associated with better living standards and medical care in the Western World, resulting in fewer stillborn babies, as late-pregnancy losses are significantly represented by males (24). Areas with lower living standards would have SSR around 1.02 and occupational exposures are often linked to effects on SSR (24, 25). Since then, a common opinion is that an increased SSR is connected to higher socioeconomic conditions (18).

Stress is another important variable that is often mentioned in literature. Apart from the trend of increasing SSR until the 1960s, and decline until current date, few significant peaks have been observed. The variations have notably been connected to major wars, especially in

Europe and the U.S. during and after World War I and World War II (26). Stress increases pituitary secretion of the peptide hormone corticotropin. In men, increased corticotropin levels result in higher levels of estrogen, possibly leading to more female offspring, while in females corticotropin leads to higher levels of testosterone, yielding for increased numbers of male offspring (27).

Climate change could be another external stress factor. Researchers in Japan have linked temperature fluctuations in the period 1968 to 2012 to increased fetal death, resulting a lower SSR (28). A study on temperature related to SSR has also been performed on populations in Denmark, Finland, Norway and Sweden between 1878 and 1914, and results suggested that in years of low temperatures the SSR declined (29).

Another hormonal cause is the maternal level of gonadotropin at the time of conception, with high levels associated in favor of female offspring (30). The time of insemination in relation to ovulation may also be of significance, as the state of the cervical mucus is related to levels of gonadotropins. A higher coital rate increases the chance of successful insemination early or late in the menstrual cycle, possibly indicating that lower levels of gonadotropins at implantation is associated with an increased SSR (31). Older maternal age (32), as well as older paternal age (33) has indicated a lower SSR. Birth order of the child may also be of significance, as a higher parity number has been linked to a trend in lowering SSR (34).

Several medical factors and conditions are suggested to decrease SSR, like multiple sclerosis, non-Hodgins lymphoma and hepatitis (13). Studies in Denmark on IVF and the use of fertility drugs has indicated decreased SSR (18).

Ethnicity has been mentioned as possible variables to SSR, following a study in the United States, demonstrating a SSR of around 1.06 for white populations, and between 1.02-1.04 for black populations (25). Cultural factors like sex-selective abortions have resulted in a notably higher SSR and population sex ratio in countries like China and India, where there is a strong tradition of preference of a male offspring (35).

Among the many explanatory mechanisms mentioned, the downward trend in sex ratio during the last half of the 20th century has many times been suggested attributed to new reproductive hazards in human exposure to endocrine disrupting compounds (13). Endocrine disruptors are chemical pollutants that mimic, block or alter the actions of the body's normal hormones (36). There are epidemiological studies suggesting that exposure to POPs prior to, and during, conception and pregnancy may adversely affect several physiological features involved in human reproduction, influencing reproductive hormones, menstrual cycles, semen quality, couple fecundity and decreasing SSR (17, 21).

1.2 POPs and PCBs

1.2.1 Properties of POPs and PCBs

POPs are a group of toxic organic chemical substances. POPs and POP-like chemicals can be grouped into those that are intentionally produced, and those that are accidental by-products of combustion processes (37). POPs are found all over the world, and have a particular set of properties once released into the environment; they are persistent, bioaccumulative, and have the potential for long-range transport (38). High persistency means that the substances remain longer in the environment before they are degraded into less hazardous compounds, as they have long half-life (39). They remain in water, soils and sediments for decades to centuries (40).

POPs are semi-volatile, meaning the substance can enter the atmosphere, which facilitates for long-range transport and global distribution. POPs have a tendency to volatize in hot regions, before condensing and thereby remaining in colder regions. This means that they can be transported far away, even into areas where they have never been in use (39), like remote areas in the Arctic (41).

As POPs have lipophilic abilities, POPs concentrate in the fatty tissues of an organism and bioaccumulate (39). Further, as they bioaccumulate, there is a biomagnification upwards in the food chain (38). This means that organisms at the top of the food chain, i.e. humans, are prone to high risk of bioaccumulation. Humans are primarily exposed through dietary intake, like dairy products, meat and fish (42, 43).

Organochlorides, their metabolites and dioxins have been characterized as endocrine disrupting compounds. For instance, PCBs and dioxins have structural similarities to thyroxine, which is an important hormone involved in fetal neurological development (44). Furthermore, several POPs, like PCBs and DDT has been linked to have estrogenic, anti-estrogenic or antiandrogenic potential (45).

1.2.2 PCBs

PCBs are a group of POPs that have been of much concern, thus a lot of research has been focused around this group of chemicals (41). Being both thermally and chemically stable, and have no to low flammability (46), PCBs have been widely used in industry as transformer and capacitor oils, hydraulic and heat exchange fluids, as lubricants and cutting oils (47). They were greatly produced in industrialized countries from the early 1930s until 1994, with a peak production around 1970. During the time of use, from 1930 to 1993, over 1,3 million ton of PCBs were produced globally (37).

PCBs consist of 209 congeners, where each congener has a different configuration based on the position of their chlorine atoms (48). One example of a PCB congener is PCB-153, which is one of the most studied PCB congeners. PCB-153 is considered to be among the estrogenic PCBs, and are more long-lived than other PCBs (49). Several PCB congeners follow the same time-trends in emission, and because there is much information on PCB-153 emission and expose, PCB-153 concentrations can be extended to act as a depiction of exposure for PCBs (50, 51).

1.2.3 Regulations of POPs

Gradually researchers have become aware of POPs' potential threat to the environment and human health. Several international agreements have been initiated to legislatively restrict and ban the manufacture and use of POPs (52). The Stockholm Convention is a global agreement of high importance in regulation of POPs. It was initiated in 1995 when the Governing Council of UNEP called for global action to protect human health and the environment against the use of POPs. An initial 12 POPs that had been much used in the 20th century were thoroughly assessed, known as "The Dirty Dozen". To mention a few, these were insecticides and pesticides like aldrin, organochlorides like dichlorodiphenyltrichlorethane (DDT) and PCBs, and high-temperature by-products like dioxins, i.e. 2,3,7,8-tetrachlorodibenzodioxin (TCDD). The agreement was signed in 2001 and entered into force in 2004. Since then, the list of POPs has expanded to 26 chemicals, and today 184 parties have pledged to work towards eliminating the use of the POPs included in the convention (38).

The production and use of PCBs was banned in most western countries from the late 1970s (53), and in Norway in 1980 (54). Despite the phasing out of production and open use of PCBs, PCBs are still present in our environment. Study samples collected in 2013 assessing waste handling facilities in Norway demonstrated that PCBs still enter the waste stream in Norway (55). None the less, initiatives to discontinue the use of PCBs have been considered to be successful, as the global emission of PCB-153 was in 2016 about 3% of what it was in 1970 (56).

1.2.4 PCB concentration trends in humans

Due to the regulations and bans, PCB emissions dropped rapidly following peak emissions in the 1970s. Human exposures and body burdens have varied accordingly, which has resulted in time-variant, age-dependent concentrations in human tissues. Exposure starts prior to conception, and continues through gestation and breastfeeding (6). *In utero* PCBs are passively transferred from maternal to fetal blood through the placenta. The levels of maternal

and fetal PCBs are thought to be close to 1:1, so fetal exposure is dependent on maternal PCB concentrations (43). Breast milk is lipid rich, and through breast feeding, much of lipid-stored maternal PCBs are transferred to the infant. Depending on the time of birth, PCB lifetime exposure through diet and environment will vary (4, 57).

The time-variant exposure trend in PCB concentration is demonstrated in a Northern Norwegian longitudinal study, which indicated a decreased PCB serum concentration in the time period from 1979 to 2007 (52). A similar longitudinal study in Sweden for the time period 1993-2007, demonstrated the same trend, with a statistically significant decrease of 7,2 % in sum PCB concentration for the study period. The same study indicated that breast-feeding > 8 months and parity >2 children yielded for a statistically significantly lower serum concentration of PCBs (58).

1.2.5 Effects of PCBs on human health

Several effects have been attributed to exposure of PCBs. Elevated serum concentrations of PCBs has been linked to several adverse effects, from affecting the male and female reproduction, to immunotoxic effects in children, as well as carcinogenic effects (5). One of the first events to indicate the toxic effects of PCB exposure took place in Japan in 1968, where more than 2000 people were poisoned by PCB contaminated rice oil (59). Following this incident, exposed adults demonstrated an array of symptoms, like dermal and ocular lesions, in addition to endocrine disruptive processes, such as irregularities in menstrual cycles and an altered immune response. Infants exposed *in utero* demonstrated symptoms of growth impairment, decreased motor skills and reduced IQ (60). A similar event involving PCB-contaminated rice oil, took place in Taiwan in 1979 (61). In these separate incidences, involving both adult and *in utero* exposure, later exposed reduced fecundity and abnormal reproductive development (62, 63). Studies following males exposed in the Taiwan incident of 1979 had abnormal morphology and reduced motility of their sperm (62). In addition, a cohort study following the same event demonstrated that paternal exposure before the age of 20 years had a lower SSR than same-aged neighborhood-matched controls (64). Exposure to PCBs have since been suggested many times as a cause of decreased SSR based on associations in observational studies (7, 10). We thus hypothesize that if there has been a change in SSR, and if SSR is affected by exposure to PCBs, we would observe similarities in any changes, or time trends, of SSR consistent with changes in exposures to PCBs.

1.3 Objective of the thesis

The overall aim is to explore similarities in time trends of SSR in firstborns in Norway and predicted maternal PCB serum concentrations in the time period 1967-2018. Specifically, we will use population-level data for SSR, and predicted exposure trends for PCB-153 as a proxy for PCB exposure. The examined time period included the period when PCB exposure was at their highest levels in humans in Norway. SSR during peak exposures were compared to periods with lower exposures. Potential maternal age differences were also evaluated with regards to time-trends in SSR.

2 Material and methods

2.1 Medical birth registry of Norway

2.1.1 About the MBRN

The Medical Birth Registry of Norway (MBRN) is an ongoing national health registry, containing medical information regarding pregnancies and births since January 1st, 1967 (65). The National Institute of Public Health is the register-responsible institution, and is owned by the State Health Inspectorate (66, 67). The aim of the registry was epidemiological surveillance of birth defects and other perinatal health problems, in order to detect future rate increase (66). The MBRN aim to facilitate easily accessible data for research and health management (65), within limits on data confidentiality and research ethics (66). Research in MBRN is related to clarifying causes of health issues in mother and child, and to illustrate consequences of risk factors (65).

The MBRN is based on compulsory notification of every birth or late abortion (66). All pregnancies, regardless of the outcome, including terminations, after week 12 and onwards are notified to MBRN (65). Health care workers, midwives, doctors or others who provide healthcare to any woman during pregnancy and/or labor, shall, without regard to duty of confidentiality, provide data to the MBRN (68). The notification form for the registry includes information with regards to maternal health pre- and post-pregnancy, including chronic illnesses and use of medicines. It registers any complications during pregnancy, in labor or after birth (65). Determination of sex is performed objectively by the health care provider registering the report, as chromosomal sex determination is not routinely performed.

2.1.2 Collecting data from the MBRN

The data provided from the MBRN in this thesis is accessed by request to the Health Data and Digitalization Department within the Public Health Institute of Norway. The dataset is based

on a population level of all live and still-births in Norway, from the commencing of the MBRN in 1967, until 2018. The dataset provided includes information about birth year, quantity of total births, maternal age group, mean maternal age within the age group, as well as quantity of annual male sex, females sex and unknown sex,

The inclusion criteria were all primiparous deliveries of singletons, with a gestational age over 21 weeks, birthweight 500 grams and up, and sex being either male or female. Exclusion criteria were multiple births, gestational age lower than 22 weeks and unknown sex. We based our data on the maternal age at the time of birth, and stratified the data into five maternal age groups by year: 20-24, 25-29, 30-34, 35-39, and all age groups (20-39 years). We only included first time mothers because hypothesized serum concentration of PCB decrease with every birth and lactation. By grouping the women into age intervals, the results would to a lesser extent be confounded by their concentrations of PCB related to their specific age.

2.2 CoZMoMAN

The knowledge of emission, bans, and environmental fate of PCBs has made it possible to model predicted values of PCBs in the environment and in humans. Lifetime exposure in humans is the cumulative exposure to a given contaminant over the lifetime of an individual resulting from prenatal, postnatal, childhood and adult exposure (69). The CoZMoMAN is a time-shift mechanistic model created with the aim to dynamically predict the human exposure to non-polar organic chemicals. It was created by combining two already existing models (70). The first model predicts organic chemical fate in the environment, the COZMo-POP 2 (71), and the second model, ACC-HUMAN, describes organic chemical bioaccumulation in the human food chain (72). The combined model, CoZMoMAN, therefore gives a non-steady-state model that describes mechanistically the entire sequence of events linking environmental emissions, or physical fate, and human food-chain bioaccumulation (70).

Evaluation of the long-term physical fate processes, both regionally (73) and on a global scale (74), plus, the bioaccumulation module (72) has suggested credibility to the algorithms included in the CoZMoMAN model (70). The long-term human bioaccumulation module is calculated through a marine and an agricultural food chain, as well as drinking water and inhalation (70). The user defines birth year, age, diet, sex, localization and parity, and through these variables the CoZMoMAN-model calculates the total human body burden and lipid normalized tissue concentration of PCB-153 (75).

In this thesis we use the model to predict levels of PCB-153 in primiparous, pregnant women and newborns in the time period 1930 to 2020, representing concentration trends in PCBs.

The predictions were performed separately for newborns and the following age groups: 20-year-olds, 30-year-olds and 40-year-olds. Model parameters were set as outlined by Breivik et al. (2010), and further described in Nøst et al. (2013, 2019).

2.3 Data treatment and statistical analysis

The literature used as background for this thesis is gathered through articles suggested by supervisors, in addition to database searches. IBM SPSS, version 26.0.0.1, has been used for data treatment and statistical analysis. Tables and figures 2 and 9 were created with Microsoft Excel version 16.40. Statistical significance has been defined as $p<0.05$ with a confidence interval (CI) of 95%. Illustrations for numbers of firstborns and the percentage of age group representation was created. Regression analysis and linear regression modulation was performed on SSR by year for the respective age groups. As the numbers could not be expected to have a normal distribution, and due to the low number of observations in subgroups, nonparametric tests were performed. Kruskal-Wallis one-way analysis of variance by ranks was performed to compare the SSR between the age groups for the total time period. Mann-Whitney U test was performed to compare the SSR for three different time period within the total time period 1967-2018. Confounding for age was dealt with by dividing into maternal age groups, 20-24 years, 25-29 years, 30-34 years and 35-39 years. As a result, within the age groups the ages were similar and confounding was minimized.

2.4 Ethical considerations

Norwegian law requires an application to Regional Committees for Medical and Health Research Ethics (REC) to get an authorization of ethics for medical and health research projects where person sensitive data is used. We did not apply to the REC for this thesis, and acquired our data from the MBRN without an application to REC. The reason for this is that the dataset is population based and we have no individual information about the specific participants. The information is therefore completely anonymous, and there would be no way of linking information to separate participants.

3 Results

3.1 Total firstborns per year per age group

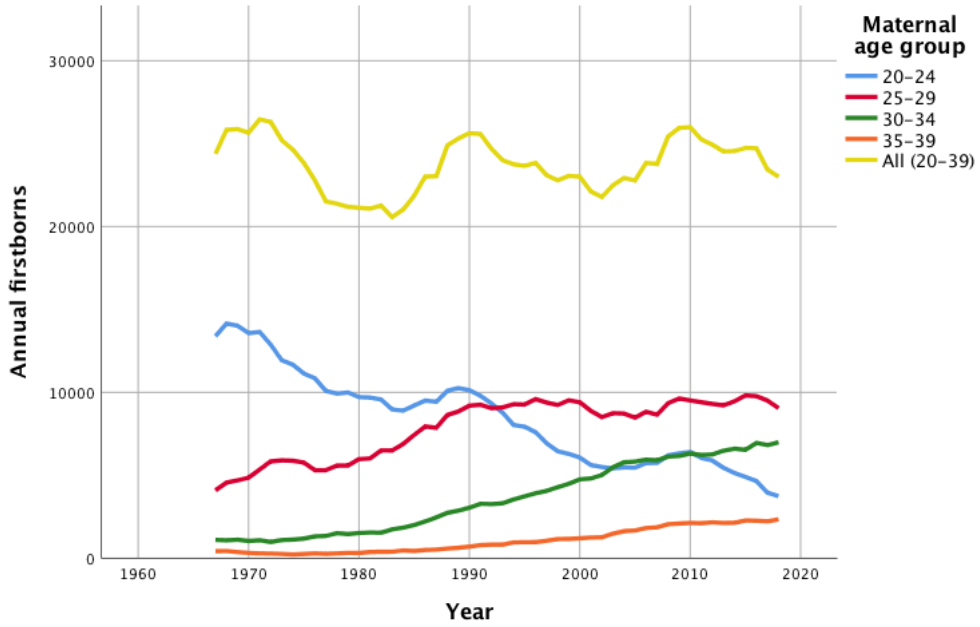

Figure 1: *Number of firstborns for the time period 1967-2018 per year displayed by maternal age groups; 20-24 years (blue line), 25-29 years (red line), 30-34 years (green line), 35-39 years (orange) and all years (20-39) (yellow line).*

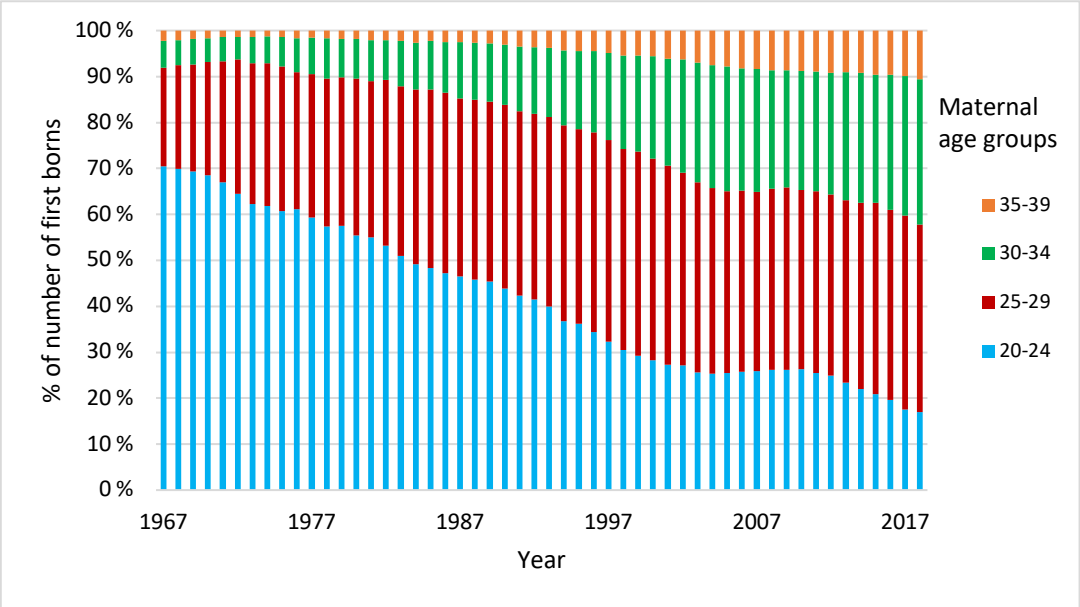

Figure 2: *Percentage of all firstborns per year by maternal age groups; 20-24 years (blue), 25-29 years (red), 30-34 years (green), 35-39 years (orange).*

Figure 1 displays the number (N) of firstborns per year within the maternal age groups. The quantity of annual births of firstborns to mothers ages 20-39 years within the time period 1967-2018 ranged between 20,573-25,992. There was a total of 1,233,767 firstborns for the whole study period.

The percentages of total firstborns to each maternal age group is displayed in Figure 2. Comparing Figure 1 and 2 reveals that the age composition has changed from 1967 to today. The total number annual firstborns in 2018 is similar to what it was in 1967. In 1967, the largest proportion, about 70% of all primiparous women were 20-24 years, while 20% were in the age group 25-29 years. At the same time, about 7% was 30-35, and 3% was 35-39 years. Figure 1 demonstrate that in 1993 the 20-24 year olds was bypassed by the 25-29 year olds, and again in about 2010 by the 30-34-year olds, making 75% of primiparous within the age groups 25-34-year olds in 2018. In 2018, the age group 20-24 years only represented 15% of all firstborn deliveries, and 35-39 was still the smallest age group at about 10%.

3.2 SSR

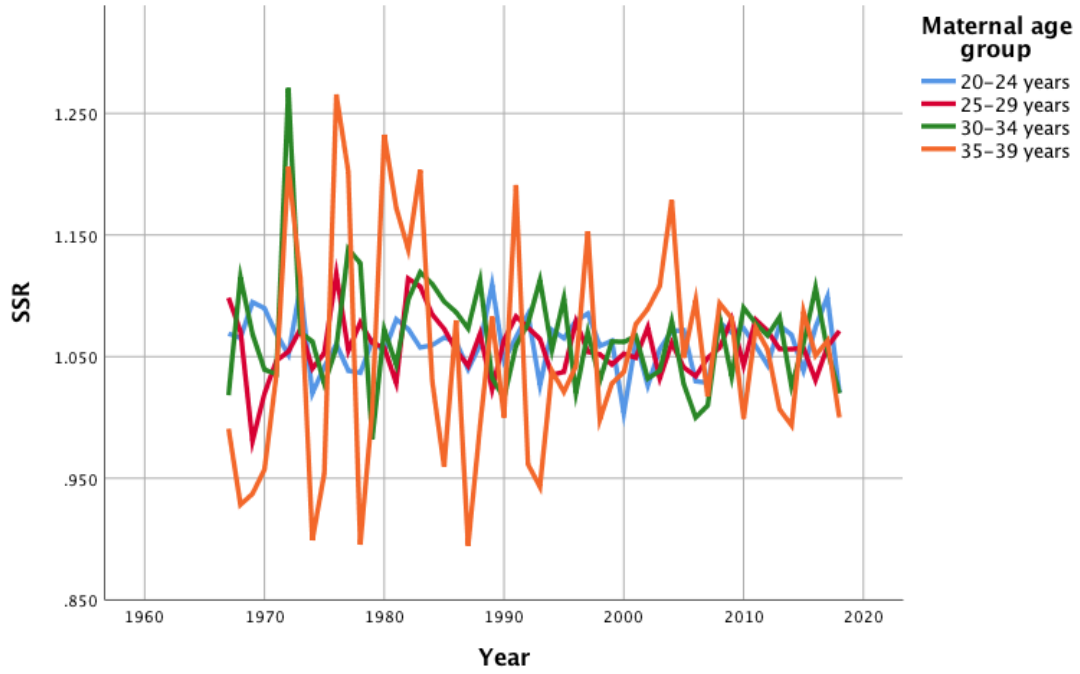

Figure 3a: *SSR by birth years from 1967 to 2018, displayed by multiple lines by maternal age groups; 20-24 years (blue line), 25-29 years (red line), 30-34 years (green line), 35-39 years (orange line).*

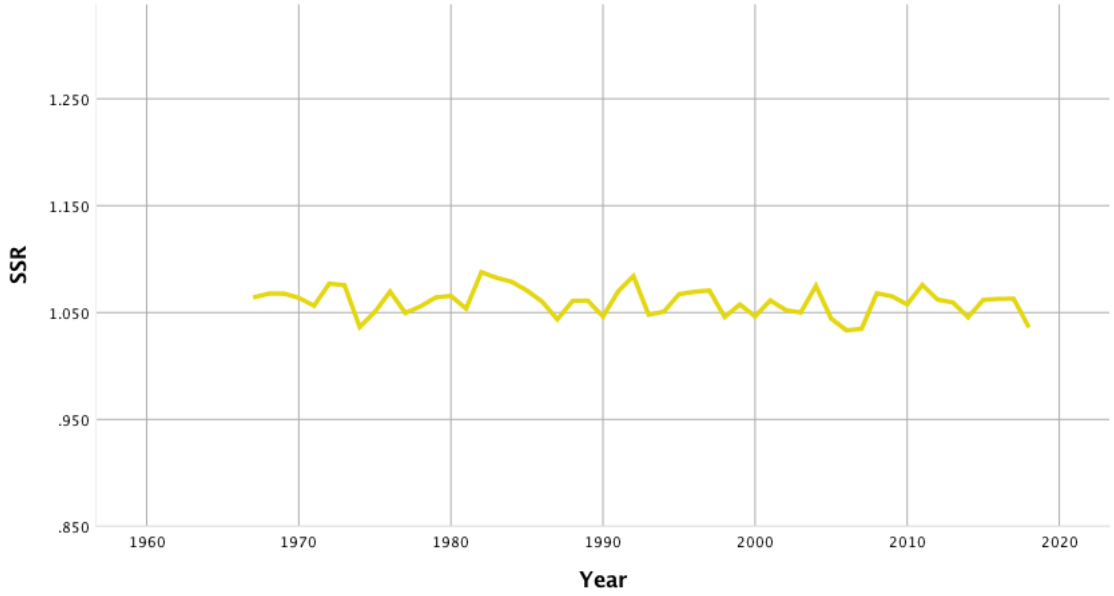

Figure 3b: *SSR displayed by simple line by year for all maternal age groups (20-39 years).*

Table 1: *SSR displayed as number of samples within each age group (N), mean SSR, standard deviation, minimum and maximum SSR, and for the time period 1967 to 2018, by maternal age groups by year 20-24, 25-29, 30-34, 35-39 and all (20-39).*

Maternal age group [years]	N	Mean	Standard deviation	Minimum	Maximum
20-24	52	1.061	0.022	1.004	1.109
25-29	52	1.059	0.024	0.981	1.118
30-34	52	1.067	0.045	0.982	1.271
35-39	52	1.052	0.090	0.895	1.266
All (20-39)	52	1.060	0.013	1.033	1.088

Figure 3a displays the SSR by year for the maternal age groups for the whole time period, 1967-2018, while Figure 3b display SSR for the maternal age group all years (20-39). The visual impression is that there is no general trend for SSR over time, as there is a constant line throughout the period, with small yearly variations. This is underlined in Table 1, displayed by numeric SSR for all maternal age groups by year for the whole time period. The overall mean SSR for the time period was 1.060, with a standard deviation of 0.013.

As demonstrated in Figure 3b, the lowest SSR within the total age group was in the year 2005, with a minimum SSR of 1.033, and the maximum was in 1982 with SSR 1.088. Table 1 demonstrate that the maternal age group with the highest mean SSR for the whole time period was the maternal age group 30-34 years, with a mean SSR of 1.067, while the age group 35-39 years had the lowest mean SSR of 1.052.

In Figure 3a, the age group 35-39 years stand out from the other groups, as the SSR appears to have larger year to year variation in this group, with peaks approximately every 10 years from 1967 to about 2005. Thereafter, the yearly variations are more similar to the other groups. This is also demonstrated in Table 1, where the maternal age group 35-39 years had a minimum SSR 0.895 in 1987, and maximum SSR of 1.266 in 1976, with a remarkably higher standard deviation (0.090) out of all groups. Table 1 and Figure 3a demonstrate that the maternal age group 20-24 years had the most stable SSR out of all age groups, ranging from 1.004-1.109, with a standard deviation of 0.022.

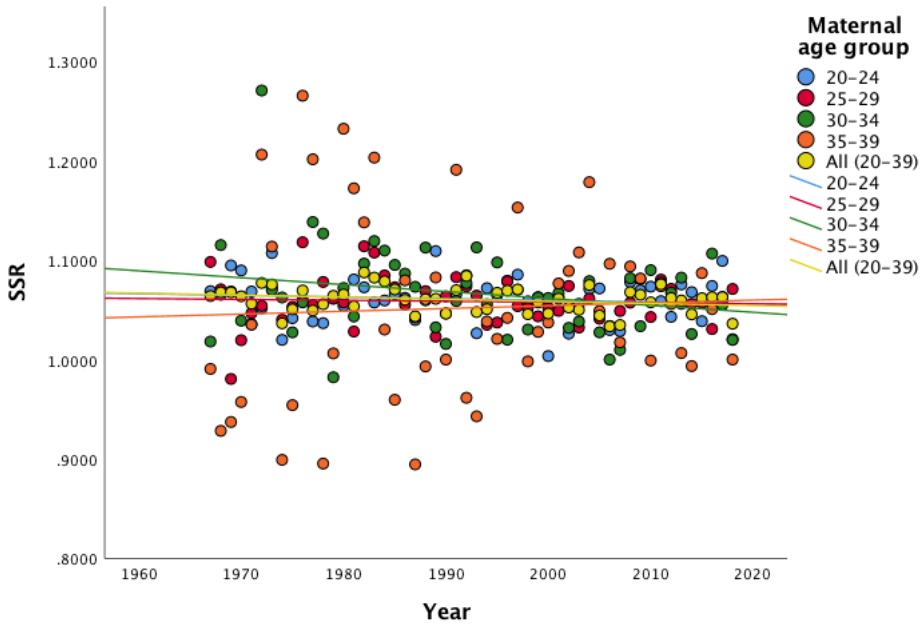

Figure 4: *Grouped scatter plot and with regression lines obtained from linear regression models based on SSR for the years 1967 to 2018 by maternal age groups 20-24 years (blue dots and line), 25-29 years (red dots and line), 30-34 years (green dots and line), 35-39 years (orange dots and line) and all (20-39 years) (yellow dots and line).*

Table 2: *Regression analysis of SSR for the maternal age groups by year 20-24, 25-29, 30-34, 35-39 and all (20-39) for the time period 1967-2018. Displaying the coefficient for change per one year in birth year, p-value and R^2 for the respective age groups.*

Maternal age group [years]	**Birth year**	**P-value**	**R^2**
20-24	.000	.419	.013
25-29	-9.407E-5	.690	.003
30-34	-.001	.098	.054
35-39	.001	.739	.002
All (20-39)	.000	.079	.060

Figure 4 displays modeled linear regressions for SSR by year, for the different age groups, for the total time period 1967-2018, and Table 2 displays values obtained from linear regression analysis for the coefficient for change in birth year, p-value and R^2. For more detailed modeled linear regression, the respective age groups are displayed in appendix 1 as Figures 10a-d. For the maternal age group 20-24 years, there was an almost flat line (R^2= 0.013, p=.419) which was supported by a coefficient for change per birth year of .000. For maternal age group 25-29, the regression line demonstrated a nearly flat line (R^2=0.003, p=.690), but the coefficient for change per birth year indicated a slightly decreasing trend by -9.407E-5, though not significant as p=.690. There was also a negative coefficient for change of -0.001 for maternal age group 30-34 years, and a decreasing trend line was observed for this age group (R^2=0.054, p=0.98) and for the total age group (R^2=0.060, p=0.79). Maternal age group 35-39 years demonstrated a slightly increasing trend line (R^2=0.002, p=0.739), but has a coefficient of change of 0.000.

When observing regression lines in Figure 4, the ages 25-29 years and 30-34 years indicated a slightly decreasing trend in SSR. Notably, all linear regression lines had small R^2, meaning only a few of the marks fit the line, and all p-values were non-significant as p>.05 with 95% CI.

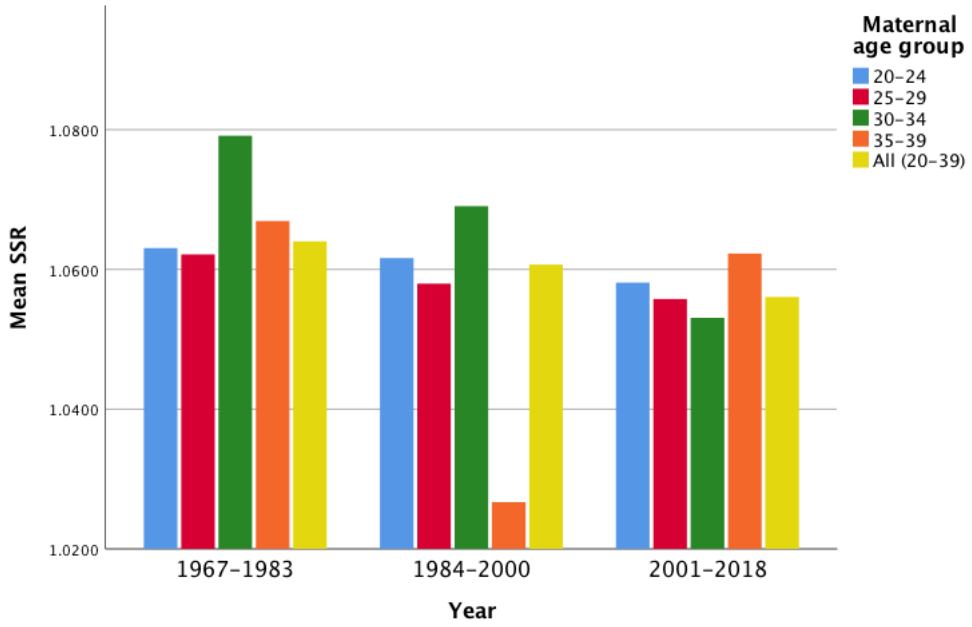

Figure 5: *Clustered multiple bars displaying mean SSR for sub time periods 1967-1983, 1984-2000 and 2001-2018 for maternal age groups 20-24 years (blue bars), 25-29 years (red bars), 30-34 years (green bars), 35-39 years (orange bars) and all age groups 20-39 years (yellow bars). Mean SSR for all ages in 1967-1983 is 1.064, in 1984-2000 mean SSR is 1.061 and mean SSR in 2001-2018 is 1.056.*

Figure 5 display the SSR of the three sub-groups of time periods (1967-1983, 1984-2000, 2001-2018) as histograms for the maternal age groups. The maternal age groups 20-24 years, 25-29 years, 30-34 years and the total age group (20-39 years) all had slightly decreased SSR from the first to the third time period. The age group 35-39 years had drastically decreased SSR from the first to the second time period, and then increased SSR in the time period 2001-2018.

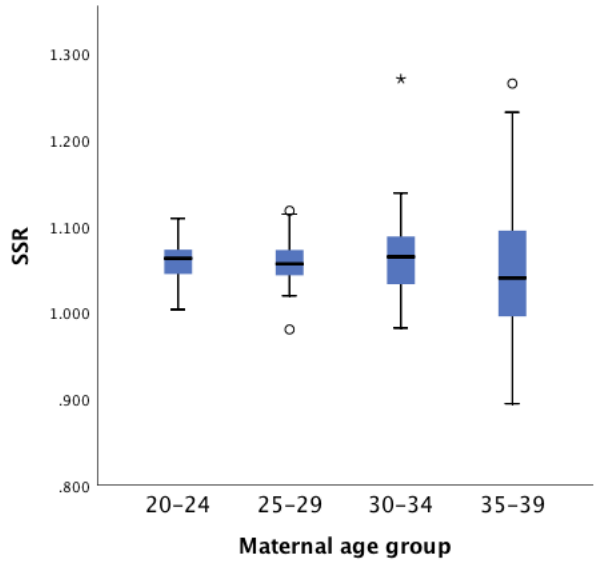

Figure 6: *Independent-samples Kruskal Wallis test for SSR by maternal age groups 20-24 years, 25-29 years, 30-34 years, 35-39 years, displaying variance of SSR within the respective age groups, for the time period 1967 - 2018.*

Table 3: *Nonparametric test, independent samples Kruskal-Wallis Test summary. Based on testing differences in SSR for the maternal age groups 20-24 years, 25-29 years, 30-34 years and 35-39 years, across the entire time period 1967 - 2018.*

Total N	**208**
Test Statistic	3.290a
Degree Of Freedom	3
Asymptotic Sig.(2-sided test)	.349
a. The test statistic is adjusted for ties.	

Table 3 displays results from independent-samples Kruskal-Wallis tests on SSR between the maternal age groups by years 20-24, 25-29, 30-34 and 35-39, for the whole time period 1967-2018, with a total N=208. The test indicated that the largest variance was observed in maternal age group 35-39 years, and the smallest variance was among the maternal age group 20-24 (Figure 6). No groups were significantly different from one another with regards to SSR as p=0.349 with 95% CI.

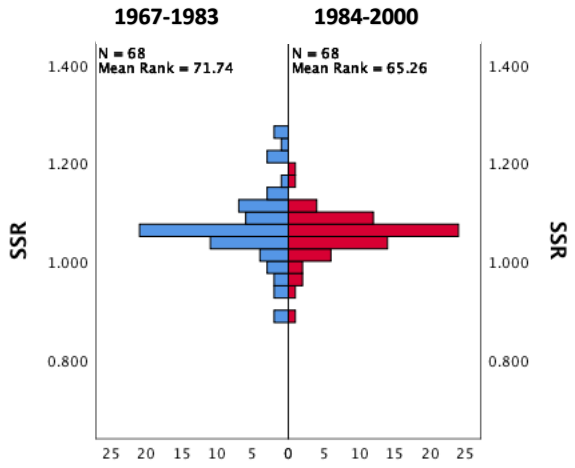

Figure 7a: *Independent-samples Mann-Whitney U test, displaying SSR for all maternal age groups (20-39 years) for the time period 1967-1983 (blue bars) and the time period 1984-2000 (red bars). Mann-Whitney U test statistic value was 2092, p-value 0.338 with significance level p>.05 and 95% CI.*

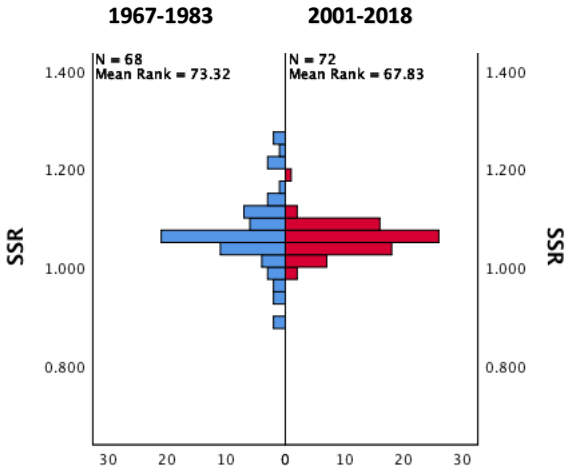

Figure 7b: *Independent-samples Mann-Whitney U, here displaying SSR for all maternal age groups (20-39 years) for the time period 1967-1983 (blue bars) and the time period 2001-2018 (red bars). Mann-Whitney U test statistic value was 2256, p-value 0.423 with significance level p>.05 and 95% CI.*

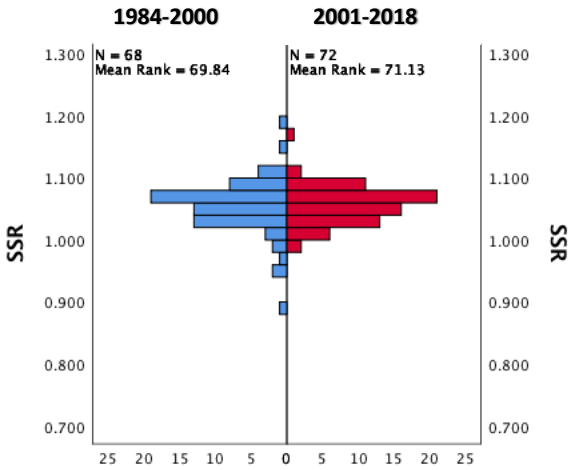

Figure 7c: *Independent-samples Mann-Whitney U, here displaying SSR for all maternal age groups (20-39 years) for the time period 1984-2000 (blue bars) and the time period 2001-2018 (red bars). Mann-Whitney U test statistic value was 2493, p-value 0.851 with significance level p>.05 and 95% CI.*

Figures 11-13 display Mann-Whitney U-tests for all maternal ages when dividing the time period 1967-2018 into three sub-groups; 1967-1983, 1984-2000 and 2001-2018. The test indicated no statistical significant differences between the time periods with 95% CI, when testing time period 1967-1983 and 1984-2000 (p=0.338), 1967-1983 and 2001-2018 (p=0.423), and 1984-2000 and 2001-2018 (p=0.851). Although these tests were performed across all age groups, divided the time period into intervals, there was no significant differences in SSR across the age groups for the three time periods.

3.3 Predicted PCB-153 concentrations (CoZMoMAN)

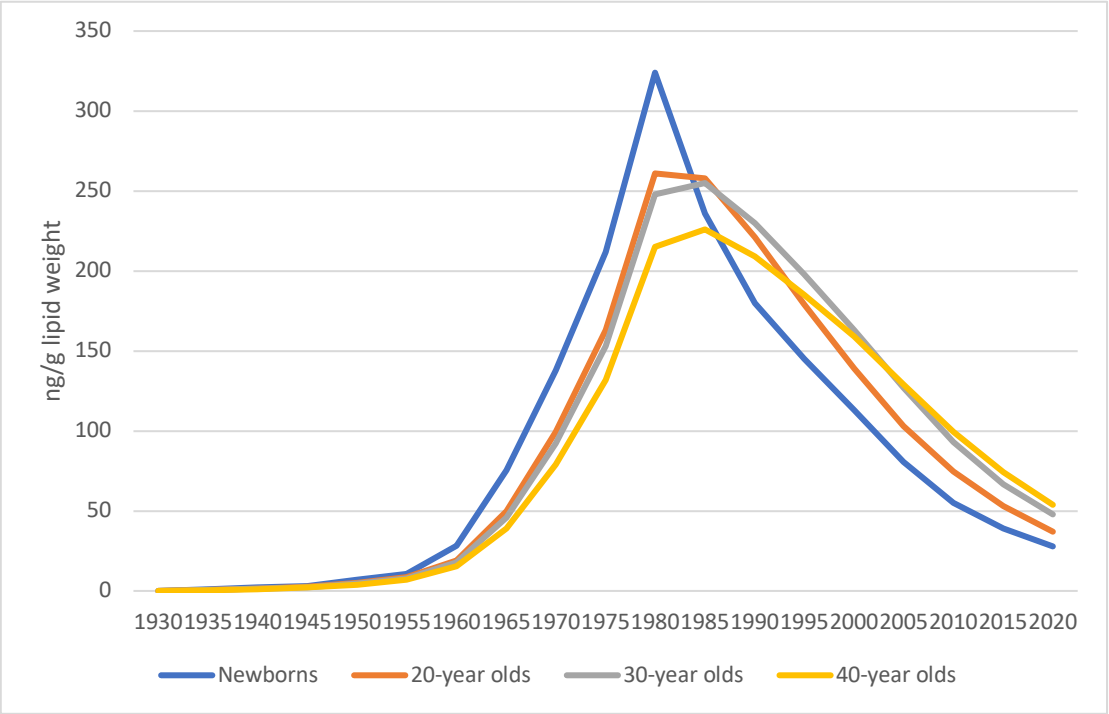

Figure 9: The *CoZMoMAN model with predicted concentrations of PCB-153 for the time period 1930-2020, for age groups; newborns (blue line), 20-year-olds (orange line), 30-year-olds (grey line) and 40-year-olds (yellow line).*

The predicted PCB-153 serum concentrations obtained from the CoZMoMAN model is displayed in Figure 9, with ng/g lipid weight by year for age groups; newborns, 20-year-olds, 30-year-olds and 40-year-olds. The modeled results were available from 1930, from the beginning of PCB emissions, where predicted PCB-153 in serum was close to zero. The model predictions indicated slowly increasing serum concentrations for all age groups from about 1940 to 1960. From this point the curve drastically increased to peak concentrations in 1980, the same year PCBs were banned in Norway. Newborns had the highest predicted serum concentrations at approximately 324 ng/g lipid weight in 1980, before declining to 38.9 ng/g in 2015. For 20- to 40-year-olds, serum concentrations peaked in 1980-1985 at 261 ng/g in 20-year-olds, 255 ng/g in 30-year-olds and 226 ng/g in 40-year-olds, before steadily declining to around 50 ng/g lipid weight in 2015.

4 Discussion

In the data we have studied, for 1,233,767 firstborns in Norway, over a time span of 51 years, there were no detectable changes in SSR during the time period 1967 to 2018, not even in the

shorter time intervals during peak exposures of PCBs. Overall, SSR of firstborns in Norway has been rather stable. There are no similarities in time trends between SSR and PCB exposures, which indicates changes in PCB exposure in this time period has influenced SSR. The data demonstrated that the mean SSR for the total time period was 1.060, with a span 1.033-1.088. The regression analysis of SSR displayed no significant changes in SSR for any of the age groups for the time period 1967-2018. No maternal age group was significantly different from the others with regards to SSR throughout the study period.

The peak serum concentrations of PCBs were predicted to be around the time period 1980-1985 for all age groups. We divided the time period 1967-2018 into three intervals; prior to peak PCB exposure (1967-1983), during and after peak exposure (1984-2000), and after peak exposure (2001-2018). SSR prior to peak PCB exposure was 1.064, SSR during peak exposure was 1.061, and SSR after peak exposure was 1.056. When comparing the SSR in these time intervals, we found no significant difference between the groups. When comparing the SSR prior to and after peak exposure of PCBs, prior to and during peak exposure, and during and after peak exposure, we found no significant differences. The Mann-Whitney U test, displayed in Figure 7c, comparing SSR in the peak exposure period 1984-2000 to SSR in the post exposure period 2001-2018, demonstrated no significant difference between the groups as $p=0.851$ ($p>0.05$, 95% CI). If PCBs influenced SSR, we would have observed a significant decreasing SSR around the time of peak exposure, which we did not.

There has been a shift in maternal primiparous age, which has led to the quantity of births within each age group to change drastically over the studied time period. The oldest maternal age group that we studied, 35-39 years, had the highest year to year variation in SSR. This could be explained by a low number of yearly deliveries in this age group throughout the time period. However, the proportion of deliveries in this age group has increased considerably in this time period.

In epidemiology, one critical and important challenge is distinguishing causal from non-causal explanations, especially when evaluating potentially hazardous agents (76) like PCBs. Among several aspects of associations to be considered in Hill's criteria of causality, dose-response is one of them (11). Although we observe monotonic trends in our results with the increasing levels of exposure, the associations are not necessarily causal, as confounding factors may have inherent biological gradient in its own, like increasing age may have on SSR. Another factor to consider, is temporality, which refers to the inarguable necessity for a cause to precede an effect in time (11). Implementing this to the hypotheses that increased PCB exposure leads to a decrease in SSR, we would expect to observe corresponding changes

in our data after the exposure, if there was a causal relationship. Therefore we suggest that there is no causality between increased PCB exposure and SSR in the period we studied of 1967-2018.

Bjerregaard et al (2012) investigated the claim of decreasing SSR in the Arctic caused by anthropogenic contaminants in the environment (77). The data from 27 circumpolar jurisdictions in Alaska, Canada, Greenland, Nordic countries and Russia, from early 1977 to 2007, was assessed. Although they had a larger study population than what is used in this thesis, with a total of 3,059,252 births, their results were similar to what we observed, as SSR was found to be approximately 1.05, and time trends close to zero in all jurisdictions. They found no deviation in SSR using linear regression models for each jurisdiction, similar to what we observed for the Norwegian population data in 1967-2018. Although they did not measure or predict concentration levels of POPs or PCBs in their study, they concluded that the absence of deviation in SSR indicated that there were no disrupting compounds affecting endocrine systems in the extent of having an effect on SSR, or that the contamination could not be widespread enough to have an effect on population level.

4.1 Associations between PCBs and SSR in previous studies

Several studies have used individual data and observed the effect as a declining SSR. Because we in this study are using group data, we would expect to observe the effect as a decline in SSR if the magnitude of exposure is big enough. To get an understanding of magnitude, Nøst et al. (2019) studied POP serum concentrations for 30-year-old women from Tromsø, Norway. Peak concentrations were measured in 1986, and the 31 women that were sampled the same year, had a median PCB-153 concentration of 165 ng/g lipid, with a range 30-425 ng/g lipid (78). CoZMoMAN predicted peak PCB-153 concentrations for 30-year-olds in 1980-1985, with concentrations of 248-255 ng/g lipid weight.

A cohort study on serum samples of 399 woman/child-pairs performed in the San Francisco Bay Area between 1964 and 1967, at production peak of PCBs, to examine its relation to SSR. This study found that the relative risk of a male birth decreased by 33% when women at the 90th percentile of total PCBs were compared to women in the 10th percentile. The population in this study had a mean PCB concentration 5.4 □g/L, and a median PCB-153 concentration 1.1 □g/L (79). 1.1 □g/L serum equals to 110 ng/g, and if assuming that there is 0.7 grams lipid in 100 grams plasma (80), we can approximate that the women in the San Francisco Bay Area had a median PCB-153 concentration of 157 ng/g lipid when the decreased SSR was described in 1964-1967. Hence, the population in this current study that

showed no change in SSR, experienced a similar magnitude of PCB exposure as women in a cohort where SSR was associated to a decreased SSR.

Another cohort study based on participants in counties along Lakes Erie and Ontario in New York State, studied 50 live births and maternal serum samples with mean PCB levels 5.61 ng/g serum, found no significant alteration in SSR in relation to PCB concentrations (10). This study did however link decreased SSR to a higher maternal age and higher parity, the same variables we considered initially when designing the selection criteria for this study.

4.2 Intergenerational effects

Quinn et al. (2011) studied the intergenerational differences in exposure and total body burden using the CoZMoMAN model. They suggested that the highest accumulated exposure occurred at reproductive ages for women born in the 1960s, and that children born in 1980 would be exposed to the highest prenatal levels of PCBs, making these children at risk of congenital reproduction disorders. They suggested that health repercussions of PCB exposures could affect several generations (4).

This means that the children being born from around 2000-2018 are the offspring of those that experienced the highest exposure *in utero*, and possibly had the highest risk of congenital reproductive disorders. If there was to be an intergenerational effect of PCB exposure, there should be a decrease in SSR from about 2000 or later. Neither the visual inspection of SSR in Figure 3a or Mann-Whitney testing 1967-1983 to 2001-2018 indicated that there was a significant change in SSR. This weakens the hypothesis of an intergenerational effect of PCB exposure that affects reproductive systems to the extent that one could observe changes in SSR.

4.3 Generalizing from PCBs to other POPs

Several POPs are considered to have endocrine disrupting properties (45), and have been linked to decrease SSR. For example, polychlorinated dibenzo-p-dioxins (PCDDs) have been suggested to lower SSR several years after parental exposure (81) along with polychlorinated dibenzofurans (PCDFs). Following an explosion of an herbicide plant in Seveso, Italy, fathers with high serum concentrations of TCDD had a lower SSR in the 20-year period after exposure, especially if they were exposed when they were younger than 19 years of age (81). Following the bans and regulations on POPs, for example the Stockholm Convention, legacy POPs follow the same time trends in global emission (41). For instance, assessment of DDT global emission for the time period 1940 to 2005, indicated that DDT followed the same decreasing scale trend as PCBs after production regulations were initiated (82). As other

POPs follow the same pattern in emission and exposure as PCBs, one can possibly expand the findings in this study on SSR to fathom other legacy POPs. This means that if the population has been exposed to an array of POPs in the same time period as PCBs, with peak exposure around 1980-1985, POPs would not have an effect on SSR, as SSR is not effected during or after this time period.

4.4 SSR and maternal age

Because SSR is thought to be confounded by maternal age groups (32), and because PCB concentrations vary by maternal age (4), we stratified our data into age groups. As displayed in Figure 1 and 2, it is evident that the age composition of primiparous women has changed dramatically throughout the study period. In 1967, 70% of primiparous women were in the maternal age group 20-24 years, while in 2018 75% of primiparous women were in the age group 25-34 years. In addition, the oldest maternal age group 34-39 years increased from about 3% to 10% of the age composition. The total number of births remain stable throughout the studied time period.

The change in age composition that happened 1967-2018 may have consequences as we assess our results, as some of the results may become biased due to the change in composition. Figure 5 demonstrated that the SSR for the maternal age group 35-39 years drastically decreased from the time period prior to peak PCB exposure, 1967-1983, to the period during peak exposure, 1984-2000. This was followed by an increase in SSR from peak exposure, in 1984-2000, to after peak exposure, 2001-2018. The decrease in SSR following peak exposure would be the pattern we would expect to observe if higher concentrations of PCBs were to have an influence on SSR. But for the same maternal age group, 35-39 years, as displayed in Figure 3a, there are high yearly variations in SSR compared to the other age groups until about 2005. This was confirmed in Figure 6, demonstrating the highest variance in SSR within the 35-39 age group.

Keeping in mind that in the year 1967 there were a total of 426 first-borns born to women in the maternal age group 35-39 years, and in 2018 there were 2345, it is likely that the large variability and the decrease in SSR in Figure 5 is likely attributed to the change in age composition throughout the study period. The linear regression lines for SSR by year by maternal age group, in Figure 4, displayed an almost flat line for 35-39 ($p=.739$, 95% CI). The linear regression model in Figure 4 implies that the maternal age group 30-34 years started with the highest SSR and ended with the lowest SSR out of all age groups ($p=.098$, 95% CI).

Again, in this age group, Figure 2 displays that the total number of firstborns within this age group was 20% in 1967 to 40% in 2018.

4.5 Other factors affecting SSR

As we have observed in the results, SSR has been stable throughout the studied period. However, we cannot exclude other variables confounding SSR in a way that masks possible decreasing effects on SSR caused by PCBs. Because we have no individual data, we can speculate around trends in other factors that could have influenced SSR in the population as a whole in the studied time period. Since 1967, much has happened in Norway as a nation. It has gradually become a wealthy nation due to its growing role as an exporting nation of oil and fish. Norway's social democracy politics has led to a population with a relatively equal distribution of wealth, thus having a larger population with a higher socioeconomic status, a factor that several times has been linked to higher SSR (18). The initiation of the National Health Insurance in 1967 (83) has also played an important part in improving peoples living standards by securing economic stability for Norway's inhabitants. The economic situation has facilitated equal and high-quality health care for the whole population, something that has led to less fetal loss late in the pregnancy, also contributing to a higher SSR. Because the population's economy has been rising in the same time of peak exposure of PCBs, this could possibly confound decreasing effects of PCBs on SSR.

In Norway there is supposedly little cultural bias in SSR, as there is assumed no selection of sex through elective abortion that could lead to a change in SSR (35). The stress of a population throughout the study period of 1967-2018 can be hard to assess. We do know that there have been no major wars affecting the population in this time, as wars are thought to demonstrate peaks in SSR (26, 84). Living standards have been comfortable and food has been accessible for the majority of the population. The suggested effect of temperature fluctuations in the environment causing maternal or paternal stress that was seen in the beginning of the last century (28, 29) may therefore not be comparable to the studied time period.

4.6 Other suspected effects following prenatal POP exposures

This thesis has focused on SSR as an endpoint potentially affected by the effects of PCB exposures on the endocrine system. However, there is growing concern due to increased incidents in several western countries over the second half of the 20th century, in events concerning the endocrine system. There are medical reports indicating several trends in male reproductive health (45). Some studies have indicated that there is an increased incidence of

testicular abnormalities, lowered sperm count and subfertility in several industrial countries (85). There are also reports of increased rates in congenital malformations of the male genitourinary organs, like cryptorchidism and hypospadias, and has been synthesized in the paradigms of the male testicular dysgenesis syndrome (TDS) (86). There is a similar hypothesis involving females, ovarian dysgenesis syndrome (ODS), with manifestations like fecundity impairments, gynecologic disorders, pregnancy related diseases and complications (87). Little is known of the cause of these, or if the findings are connected, but exposure to POPs have been suggested as a common prenatal etiological factor that disrupt the normal hormonal balance in the developing embryo (86, 88). This could be possible to investigate in future studies based on individual data from the MBRN.

4.7 Strengths & weaknesses

We acknowledge the information given by the MBRN to be high quality data, as it is a comprehensive national registry. Our conclusions are only valid for group level observations, meaning that our results indicate that population-level SSR have not been largely influenced by exposure to PCBs. We cannot exclude the presence of other confounding time trends on SSR at the same time as the data we have examined, thereby counterbalancing the possible decreasing effect of PCBs on SSR.

In order to study trends in SSR, it is crucial to have a dataset with a large number of participants. A limitation in many previous long-term effect studies of PCBs is that the population sizes have been small due to the difficulties of longitudinal study designs. With small samples sizes, there are always the risk that the observations of associations are chance findings, and with public health measures like trend studies of SSR, it is inherent that large datasets are required to reduce chance bias. In this thesis we had a large dataset for SSR. However, having such a large dataset, more subtle trends may not become apparent if they happen over a small time frame, and are confounded by time trends in confounding variables that cancel out the population-level trends.

Further, we lack individual data, making our observations valid only for a population level. If we had individual and more detailed information about the births, we could have adjusted further, for example regarding illnesses of the mother. More maternal information could have made it possible to further specify the CoZMoMAN-calculations into person-specific predictions. Even doing so, to be completely sure of concentrations, there would be need for individual data for all the births, including maternal, paternal and infant information and their serum concentrations.

The CoZMoMAN predictions have been evaluated several times with regards to concentrations in environmental compartments, organisms and humans, and has indicated predictions within the range of what has been measured in Scandinavia (57, 70). The model has successfully reproduced PCB contamination time trends, but the model consequently predicted slightly higher concentrations than measured (78). For example, Nøst et al. (2015) compared serum concentrations of PCB congeners to person-specific CoZMoMAN predictions for 310 pregnant women in Norway 2007-2009. They found that the median predicted PCB-153 concentrations were within 20% of measured values, indicating that there was consistency between the CoZMoMAN predicted concentrations and the actual measurements in pregnant women (75). Although studies have found the CoZMoMAN-model to have a high predictive value for pregnant women, there will always be uncertainties in use of models with multiple dynamic variables. Despite this, we believe the model's ability to demonstrate time-trends was satisfactory for the objectives of this thesis, as the aim of this thesis was to assess time-trends.

4.8 Future research

Studies using measured serum concentrations of PCBs and other POPs in individual-level data in a large dataset would bring valuable information on the topic. It would also be interesting to further investigate the possible carcinogenic and neurobehavioral effects PCBs may have, both in primary and intergenerational exposure. Further research on TDS and ODS in children born to mothers with high concentrations of PCBs and POPs is needed, as little is still known about the these effects and the strength of such associations.

5 Conclusion

In conclusion, there is no evidence indicating change in SSR for firstborns in Norway in the time period 1967–2018. During the same time period there has been a substantial change in PCB exposure in humans, but there was no indication of corresponding changes in SSR on a population level in Norway. Still, we cannot dismiss the possibility that PCBs may have other endocrine disrupting properties, and more research on this topic is needed.

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7 Appendix

7.1 Additional figures

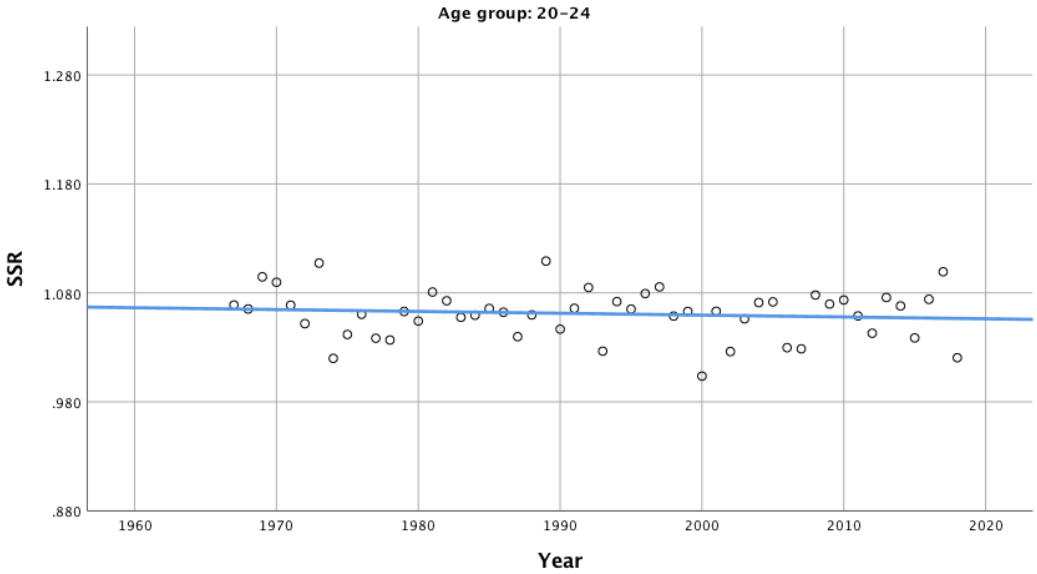

Figure 10a: *Trend line of SSR for maternal age group 20-24 years, based on linear regression for SSR for the time period 1967 to 2018.*

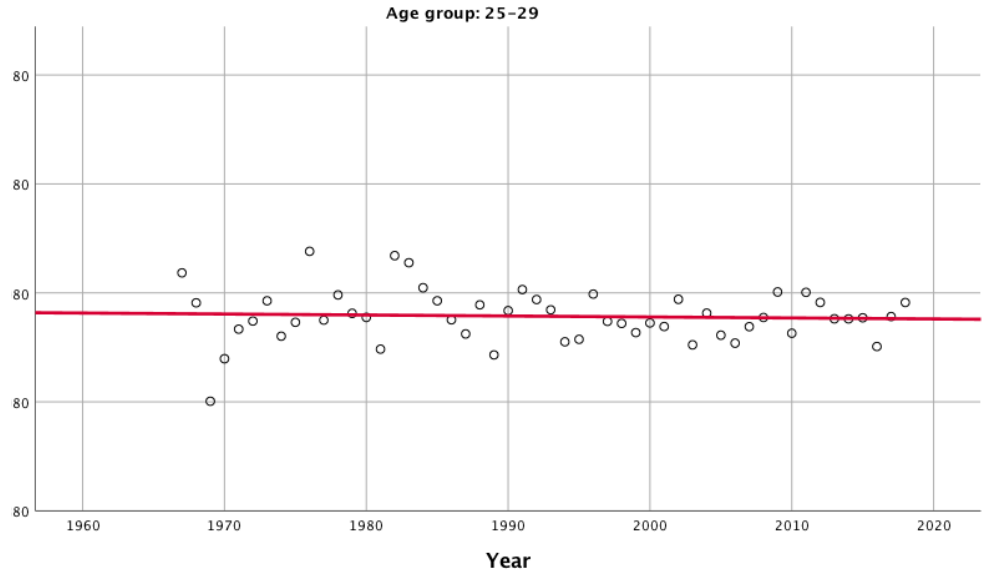

Figure 10b: *Trend line of SSR for maternal age group 25-29 years, based on linear regression for SSR for the time period 1967 to 2018.*

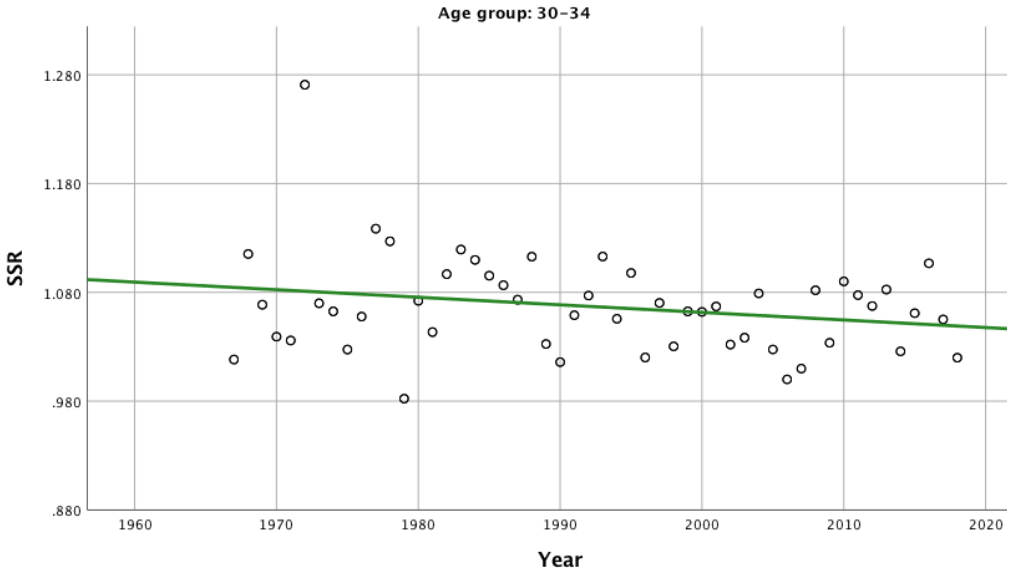

Figure 10c: *Trend line of SSR for maternal age group 30-34 years, based on linear regression for SSR for the time period 1967 to 2018.*

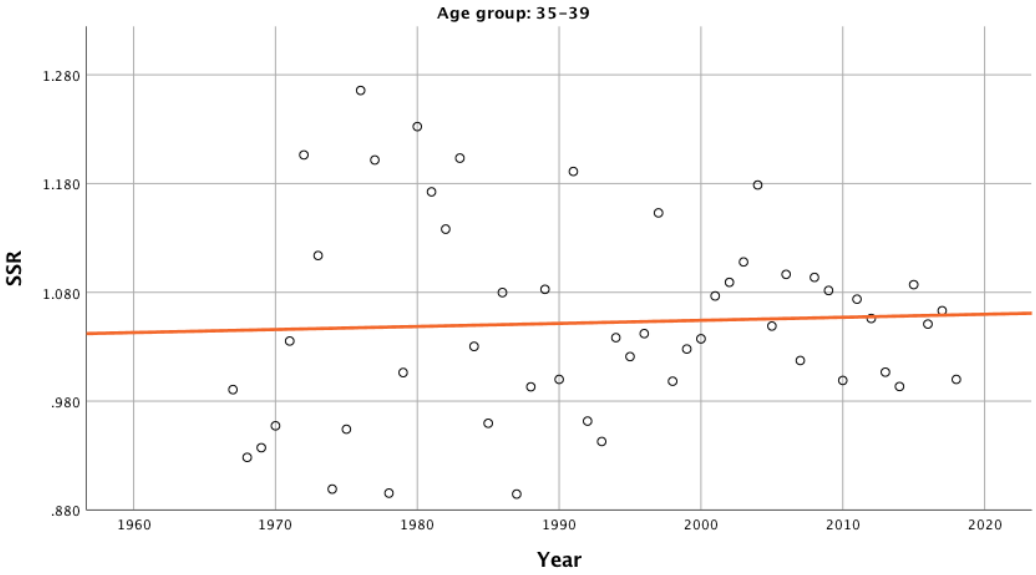

Figure 10d: *Trend line of SSR for maternal age group 35-39 years, based on linear regression for SSR for the time period 1967 to 2018.*

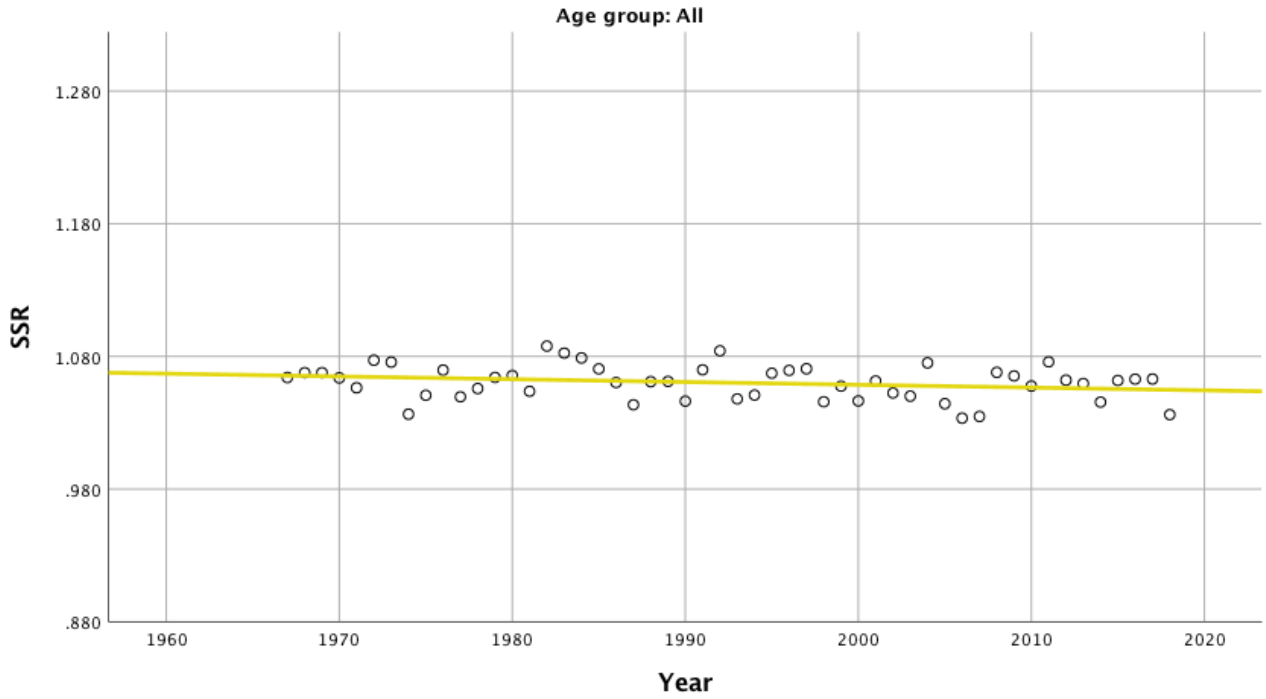

Figure 10e: *Trend line of SSR for all maternal age groups (20-39 years), based on linear regression for SSR for the time period 1967 to 2018.*

7.2 GRADE evaluations

Referanse: Taylor KC, Jackson LW, Lynch CD, Kostyniak PJ, Buck Louis GM. Preconception maternal polychlorinated biphenyl concentrations and the secondary sex ratio. *Environ Res*. 2007;103(1):99-105. doi:10.1016/j.envres.2006.04.009			**Studiedesign: Kohortestudie** Grade - kvalitet 2b, D
Formål	Materiale og metode	**Resultater**	**Diskusjon/kommentarer/sjekkliste**
Evaluate the relation between maternal serum PCB concentrations and the 2° sex ratio with particular attention to the purported hormonal activity of PCB congeners **Konklusjon** Absence of a relation between PCB concentrations and the SSR **Land** USA **År data innsamling** 1996-1997	**Populasjon:** 50 women that participated in the New York State Angler Prospective Pregnancy Cohort Study, 18-34 years and pregnant with a live birth **Hovedutfall:** Secondary sex ratio, male or female **Viktige konfunderende faktorer**: PCB congeners in serum concentrations: total PCBs (sum of 76 congeners), total estrogenic PCBs (congeners #4, 8, 15, 18, 31, 44, 47, 48, 52, 70, 77, 99, 101, 126, 136, 153, 188) and anti-estrogenic PCBs (# 77, 105, 114, 126, 156, 169) **Statistiske metoder:** Fisher's Exact Test was used to assess statistical significance ($p<0.5$) of categorical variables, and *t*-tests were used to compare means of continuous variables. Logistic regression to ralate PCB exposure to the odds of male birth.	**Hovedfunn** After adjusting for age and body mass index, odds of a male birth were elevated among women in the second (OR=1.29) and third (OR=1.48) tertiles of estrogenic PCBs; odds (OR=0.70) were reduced among women in the highest tertile of anti-estrogenic PCBs. All confidence intervals included one. The direction of the odds ratios in this preliminary study varied by PCB groupings, supporting the need to study specific PCB patterns when assessing environmental influences on the secondary sex ratio. **Bifunn:** Results corroborate earlier studies linking advancing maternal age and higher gravidity and parity as favoring a female excess, and observed maternal BMI to be significantly associated with male birth. No associations to maternal sigarette smoking and lower SSR.	**Sjekkliste:** • Formålet klart formulert? Ja • Er gruppene rekruttert fra samme populasjon/befolkningsgruppe? Kun en gruppe • Var gruppene sammenliknbare i forhold til viktige bakgrunnsfaktorer? Kun en gruppe. Populasjonen var for det meste hvite, gifte kvinner • Var de eksponerte individene representative for en definert befolkningsgruppe/populasjon? Kvinner som bodde i området rundt Lake Erie og Ontario i New York State • Ble eksposisjon og utfall målt likt og pålitelig (validert) i de to gruppene? Kun en gruppe. Målt likt for alle • Er den som vurderte resultatene (endepunkt- ene) blindet for gruppetilhørighet? Nei • Var studien prospektiv? Ja • Ble mange nok personer i kohorten fulgt opp? Ingen oppfølging ble gjort • Er det utført frafallsanalyser? Ja • Var oppfølgingstiden lang nok til å påvise positive og/eller negative utfall? Ja, utfallet var bare å vurdere sekundær sexratio • Er det tatt hensyn til viktige konfunderende faktorer i design/ gjennomføring/analyser? Ja • Tror du på resultatene? Ja, men alt for lite utvalg til å kunne overføre resultater til en populasjon • Kan resultatene overføres til den generelle befolkningen? Nei **Hva diskuterer forfatterne som:** • **Styrke:** Having preconception measurement of serum PCBs for all participationg women regardless of pregnancy outcome • **Svakhet:** Half of target population refused participation.

Referanse: Hertz-Picciotto I, Jusko TA, Willman EJ, et al. A cohort study of in utero polychlorinated biphenyl (PCB) exposures in relation to secondary sex ratio. *Environ Health*. 2008;7:37. Published 2008 Jul 15. doi:10.1186/1476-069X-7-37			**Studiedesign: Kohortestudie** Grade - kvalitet 2b, D
Formål	Materiale og metode	**Resultater**	**Diskusjon/kommentarer/sjekkliste**
Examine sex ratio in births occurring between 1964 and 1967 in the San Francisco Bay Area in relation to concentrations of serum polychlorinated biphenyls in archived serum specimens that were collected during the pregnancies **Konklusjon** Maternal exposure to PCBs may be detrimental to the success of male sperm or to the survival of male embryos. Findings could be due to contaminants, metabolites or PCBs themselves. **Land** USA **År data innsamling** 1964-1967	**Populasjon:** pregnant women participating in the Child Health and Development Study in the San Francisco Bay Area, who had completed an enrollment interview before delivery and donated a second or third trimester blood specimen, for which a sufficient volume still remained. Serum concentrations were determined for 399 women in the final sample. **Hovedutfall:** Sex ratio at birth, male or female **Viktige konfunderende faktorer:** Excluding factors were non-singleton deliveries, children with severe anomalities or who did not complete two cognitive exams and a hearing screening; mothers who were deaf, had rubella during pregnancy, were taking thyroid medication in the 60 days prior to blood draw, or took iodine-containing medications during pregnancy or in the six months prior to conception and infants born at gestational ages that were unknown, less than 35, or greater than 45 completed weeks. **Statistiske metoder:** RR, OR of male birth as a function of maternal PCB concentrations. Regression analysis.	**Hovedfunn** **Betweem exposes/unexposed:** The relative risk of a male birth decreased by 33% comparing women at the 90th percentile of total PCBs with women at the 10th percentile (RR = 0.67; 95% CI, 0.48–0.94; p = 0.02), or by approximately 7% for each 1 µg/L increase in total PCB concentration.	**Sjekkliste:** • Formålet klart formulert? Ja • Er gruppene rekruttert fra samme populasjon/befolkningsgruppe? Kun en gruppe, rekrutert fra CHDS • Var de eksponerte individene representative for en definert befolkningsgruppe/populasjon? Ja, en populasjon hvor store deler hadde stabil jobb, men ingen svært rike eller fattige. Øvrig god representasjon av utvalg fra San Fransisco Bay Area. • Er den som vurderte resultatene (endepunkt- ene) blindet for gruppetilhørighet? Nei • Var studien prospektiv? Ja • Ble mange nok personer i kohorten fulgt opp? (Attrition bias/follow-up-bias) Ja • Er det utført frafallsanalyser? (Eval. attrition bias) Nei • Var oppfølgingstiden lang nok til å påvise positive og/eller negative utfall? • Er det tatt hensyn til viktige konfunderende faktorer i design/ gjennomføring/analyser? Nei. Kan være mange potensielle konfunderende faktorer, feks. ikke tatt hensyn til mors alder. • Tror du på resultatene? Ja • Kan resultatene overføres til den generelle befolkningen? Det trengs større datasett for å si en klar årsak til kausalitet, men studiet gir en innsikt i PCB-konsentrasjoner hos gravide kvinner 1964-1967 og dets mulige effekt på SSR. **Hva diskuterer forfatterne som:** • **Styrke:** Prospektiv, tar stilling til mors PCB-eksponering, benytter seg av adekvat data, har individdata • **Svakhet:** Flere prøver som ikke lot seg analysere grunnet for lite prøvemateriale

Referanse: Weisskopf MG, Anderson HA, Hanrahan LP. Decreased sex ratio following maternal exposure to polychlorinated biphenyls from contaminated Great Lakes sport-caught fish: a retrospective cohort study. Environ Health. 2003;2(1):2.			**Studiedesign: Kohortestudie** **Grade - kvalitet** 2b, D
Formål	**Materiale og metode**	**Resultater**	**Diskusjon/kommentarer/sjekkliste**
Examine parental serum PCB concentration in relation to SSR **Konklusjon** Findings suggest that maternal exposure to polychlorinated biphenyls may decrease the sex ratio of offspring. These data add to the growing body of evidence that exposure to particular chemicals can alter the sex ratio at birth. **Land** USA **År data innsamling** 1970-1995	**Populasjon** **Kohorte:** Frequent Great Lakes Sports-Caught Fish consumers (GLSCF): charter boat captains from consumption states that were active in 1992 and their spouses.People in the general population that had not had more than five meals GLSCF since 1970 was also included to have more participants with hypothozised lower PCB concentrations. A total of 381 were included. **Inklusjons-/eksklusjonskrit:** Participants that donated blood for analyzing after being interviewed, and had children. **Hovedutfall:** sex ratio of last born child. **Hovedeksponering:** Analysis of their PCB and DDT concentration **Viktige konfunderende faktorer:** Parity of the mother at the child's birth, birth year of the child, maternal and paternal age, whether the child had an older brother. **Statistiske metoder:** Odds ratio fir having a male child was calculated using logistic regression and generalized estimating equations	**Hovedfunn** The adjusted odds ratio for having a male child among mothers in the highest quintile of serum polychlorinated biphenyl concentration was 0.18 (95% CI: 0.06–0.59) compared to mothers in the lowest quintile. Treating exposure as a continuous variable, the adjusted odds ratio for having a male child was 0.54 per unit increase in the natural log of maternal serum polychlorinated biphenyl concentration (95% CI: 0.33–0.89). There was little evidence of an association with paternal exposure. We found no association between either maternal or paternal serum dichlorodiphenyl-dichloroethene concentration and the sex ratio.	**Sjekkliste:** • Formålet klart formulert? Ja • Er gruppene rekruttert fra samme populasjon/befolkningsgruppe? Nei • Var gruppene sammenliknbare i forhold til viktige bakgrunnsfaktorer? Nei • Var de eksponerte individene representative for en definert befolkningsgruppe/populasjon? Ja • Ble eksposisjon og utfall målt likt og pålitelig (validert) i de to gruppene? Ja • Er den som vurderte resultatene (endepunkt- ene) blindet for gruppetilhørighet? Nei • Var studien prospektiv? Nei • Ble mange nok personer i kohorten fulgt opp? Ingen • Er det utført frafallsanalyser? Nei • Var oppfølgingstiden lang nok til å påvise positive og/eller negative utfall? Ja • Er det tatt hensyn til viktige konfunderende faktorer i design/ gjennomføring/analyser? Ja • Tror du på resultatene? Ja • Kan resultatene overføres til den generelle befolkningen? PCB eksponeringen til de med høyest serumkonsentrasjon kan ikke sammenlignes med den generelle befolkningen ettersom de vil ha lavere nivå. Studien gir verdifull informasjon når det gjelder dose av eksponering, men gir usikker tilknytning til utfall når det gjelder SSR **Hva diskuterer forfatterne som** **Styrke:** Rubust results for change of parameters for maternal exposure **Svakhet:** The serum organochloride measurements were made several years after the birth considered for this analysis. Not having measurements of other possible contaminants, as other chemicals than fish could be related to changes in SSR. Results could be confunfef by fish consumption in itself, if fish consumption affects SSR.

Referanse: McDonald E, Watterson A, Tyler AN, McArthur J, Scott EM. Multi-factorial influences on sex ratio: a spatio-temporal investigation of endocrine disruptor pollution and neighborhood stress. Int J Occup Environ Health. 2014;20(3):235-46.			**Studiedesign: Kasus-kontroll** Grade - kvalitet 3 C
Formål	Materiale og metode	**Resultater**	**Diskusjon/kommentarer/sjekkliste**
To test the hypothesis that dual factors of pollution and population stress affects sex proportion at birth through geographical analysis of Central Scotland. **Konklusjon** There was no overall concentration in Central Scotland of low sex ratio neighborhoods with areas where endocrine disruptor air pollution and deprivation or economic stress were high. Use of small area data sets and pollution inventories is a potential new method of inquiry for reproductive environmental and health protection monitoring and has produced interesting findings. **Land** Scotland **År data innsamling** SSR was sampled in redions 1973-2010. Cumulative Endocrine Disruptor Pollutin was sampled 2002, 2004-2010	**Populasjon** **Kasus:** Regions in Central Scotland with high **Kontroll:** Yorkshire and Humber **Metode:** The study incorporates the use of Geographical Information Systems (GIS) tools to overlay modeled point source endocrine disruptor air emissions with ''small-area'' data on multiple deprivation (a proxy measurement of stress) and birth sex. Historical review of regional sex ratio trends presents additional data on sex ratio in Scotland to consider. **Hoved utfall:** SSR male or female. Areas of high air pollution. **Viktige konfunderende faktorer** **Statistiske metoder:** Z-test for testing SSR withing regions of Scotland to SSR to other regional SSR in the UK. Spatial analysis for cential regions of Scotland in regards of pollution.	**Hovedfunn** Results show an upwards skewing in the sex ratio among the least deprived and wealthiest populations. No combined effect for pollution and socio-economic status was found, as there was not a consistent concentration of low sex ratio neighborhoods with endocrine disruptor air pollution and deprivation.	**Sjekkliste:** • **Er formålet klart formulert?** Ja • **Er kasus-kontroll design egnet for formålet?** Ja • **Er kasus rekruttert på en «god» måte? (Alle i en tidspieriode/grader av sykd.)** Ja, tror det • **Var kasus-kontrollgruppene hentet fra sammenlignbare befolkningsgrupper?*** Ja • **Er gruppene sammenlignbare i forhold til viktige bakgrunnsfaktorer?*** Ja • **Er main exposure validert?** Ja • **Er gruppene «behandlet» likt – kan påvirke «exposure»?** (deteksjonsbias?) Ja • **Har forfatterne tatt hensyn til viktige konfunderende faktorer i design/analyse?** Ja • **Var den som målte eksponering/samlet inn data blinda mht hvem som var kasus/kontroll?** Nei • **Tror du på resultatene?** Ja • **Kan resultatene overføres til praksis?** Ja • **Støtter litteratruen resultatene?** Ja **Hva diskuterer forfatterne som** **Styrke:** Large dataset comparing regions in Central Scotland to other regions in the UK **Svakhet**: Area-based measurement can also be imprecise as not all persons residing in an area display the characteristics shown by the indicator, i.e. individuals experiencing deprivation can live in non-deprived areas. There is also a difficulty in temporally aligning all the indicators from the data sets.

Referanse: Møller H, Skakkebaek NE. Risk of testicular cancer in subfertile men: case-control study. BMJ. 1999;318(7183):559-62.			**Studiedesign: Kasus-kontroll** **Grade - kvalitet** 2a, B
Formål	**Materiale og metode**	**Resultater**	**Diskusjon/kommentarer/sjekkliste**
To evaluate the association between subfertility in men and the subsequent risk of testicular cancer. **Konklusjon** These data are consistent with the hypothesis that male subfertility and testicular cancer share important aetiological factors. **Land** Danmark **År data innsamling** 1989-1990	**Participants:** Cases were identified in the Danish Cancer Registry; controls were randomly selected from the Danish population with the computerised Danish Central Population Register. Men were interviewed by telephone; 514 men with cancer and 720 controls participated. **Outcome measure:** Occurrence of testicular cancer. **Hovedeksponering:** Testicular cancer **Viktige konfunderende faktorer:** cryptorchidism or testicular atrophy **Statistiske metoder:** logistic regression analysis. Two sided statistical tests for trend. Regression analysis.	**Hovedfunn** A reduced risk of testicular cancer was associated with paternity (relative risk 0.63; 95% confidence interval 0.47 to 0.85). In men who before the diagnosis of testicular cancer had a lower number of children than expected on the basis of their age, the relative risk was 1.98 (1.43 to 2.75). There was no corresponding protective effect associated with a higher number of children than expected. The associations were similar for seminoma and non-seminoma and were not influenced by adjustment for potential confounding factors.	**Sjekkliste:** • **Er formålet klart formulert?** Ja • **Er kasus-kontroll design egnet for formålet?** Ja • **Er kasus rekruttert på en «god» måte?** Ja • **Diangosen validert?** Ja • **Er kontrollene rekrutterte på en «god» måte?** Ja, samme måte som kasus • **Kan det utelukkes at kontrollgr. fri for aktuelle sykdom?** Nei, men mest sannsynlig ikke • **Var kasus-kontrollgruppene hentet fra sammenlignbare befolkningsgrupper?** Ja • **Non-responders/nekter å delta – frafalls analyser? Forskjeller kasus/kontroll-gruppe?** Nei • **Er gruppene sammenlignbare i forhold til viktige bakgrunnsfaktorer?** Ja • **Tror du på resultatene?** Ja • **Kan resultatene overføres til praksis?** Ja • **Støtter litteratruen resultatene?** Ja **Hva diskuterer forfatterne som:** **Styrke:** Used population based sampling of a large number of cases. **Svakhet**: Relative fertility is an imperfect measure of subfertility. The information provided by the men in regards of number of children they have fathered, as it may be a question up for interpretation. Har resultatene plausible biologiske forklaringer?