Threatened abortion and the long-term health of children and mothers

PhD dissertation

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Dedication

Моей семье и Доминику

***

To my family and Dominik, my soulmate and partner

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Elena Dudukina
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List of papers

The thesis is based on the following four original studies that are referred to by their Roman numerals (I–IV).

I. **Dudukina E**, Horváth-Puhó E, Sørensen HT, Ehrenstein V. Long-term risk of epilepsy, cerebral palsy and attention-deficit/hyperactivity disorder in children affected by a threatened abortion in utero. International Journal of Epidemiology. 2021 Apr 13. Available from: https://doi.org/10.1093/ije/dyab069

II. **Dudukina E**, Horváth-Puhó E, Sørensen HT, Ehrenstein V. Women's mortality following childbirths affected by vaginal bleeding due to threatened miscarriage: a Danish cohort study.
In preparation

III. Dudukina E, Horváth-Puhó E, Sørensen HT, Ehrenstein V. Risk of diabetes and cardiovascular diseases in women with vaginal bleeding before 20 gestational weeks: Danish population-based cohort study. medRxiv; 2022. p. 2022.03.18.22272466. Available from: https://www.medrxiv.org/content/10.1101/2022.03.18.22272466v2
In preparation (available as a preprint, Version 2)

IV. **Dudukina E**, Horváth-Puhó E, Sørensen HT, Ehrenstein V. Association between having a pregnancy complicated by vaginal bleeding and risk of cancer. medRxiv; 2022. p. 2021.12.29.21268235. Available from: https://www.medrxiv.org/content/10.1101/2021.12.29.21268235v2
In preparation (available as a preprint, Version 2)

Abbreviations

ADHD; Attention-deficit/hyperactivity disorder

BMI; Body mass index

CI; Confidence interval

CPR; Det Centrale Personregister

CRS; Civil Registration System

DAGs; Directed acyclic graphs

DCPR; Danish Cerebral Palsy Registry

DCR; Danish Cancer Registry

DNPR; Danish National Patient Registry

DRCD; Danish Register of Causes of Death

HR; Hazard ratio

ICD; International Classification of Diseases

IPT; inverse probability of treatment

MBR: Medical Birth Registry

NPR; National Prescription Registry

PCRR; Psychiatric Central Research Register

PPV; Positive predictive value

PYs; Person-years

RR; Risk ratio

TAB; Threatened abortion

TNM; Tumour, node, metastasis

VB; Vaginal bleeding

Abbreviations used in tables/figures are defined in a footnote below each table/figure.

Contents

List of Figures:

List of Tables:

1. Introduction

Threatened abortion (TAB) is vaginal bleeding (VB) before 20 weeks of gestation during a viable intrauterine pregnancy in the absence of cervical dilation. TAB affects up to 30% of clinically recognised pregnancies, and at 6-8 gestational weeks, it is associated with a 12% increased absolute risk of miscarriage compared with unaffected pregnancies. The underlying biological mechanisms leading to VB in pregnancy are not fully understood; they may be related to placental dysfunction associated with decreased progesterone levels and to a pro-inflammatory immune state. TAB is associated with adverse short-term outcomes in the infant (e.g. congenital anomalies) and the mother (e.g. placenta praevia, placental abruption, preterm prelabour rupture of membranes). Less is known regarding the long-term sequelae on maternal and offspring health. Moreover, despite an established association of miscarriage with maternal mortality and morbidity (cardiovascular diseases, venous thrombosis, posttraumatic stress disorder, suicide), there is little data on long-term maternal and child health following pregnancy affected by TAB. In the first study of this thesis, we investigated the association between *in utero* exposure to TAB and the risk of neurological and neurodevelopmental disorders in children using conventional cohort design and sibling comparison design to address time-invariant confounding. In the remaining three studies, we examined the association between VB in pregnancy and subsequent health of women themselves, as measured by risk of all-cause and cause-specific mortality (study II), risk of diabetes and cardiovascular morbidity (study III), and risk of cancer (study IV). In the studies of women's outcomes, three comparators were used: 1) women with a VB-unaffected pregnancy ending in a delivery, 2) women with a pregnancy ending in a termination, and 3) women with a pregnancy ending in a miscarriage.

2. Background

TAB is a common pregnancy complication, clinically manifested as VB before 20 weeks of a viable intrauterine gestation without signs of cervical dilation.[1–3] In the first 12 weeks of pregnancy, VB occurs in 20-30% of identifiable pregnancies (Table 1).[4–17] Although terms like "threatened abortion" and "threatened miscarriage" are used in the literature, "vaginal bleeding in pregnancy" is currently considered as the most patient-centred term.[1,18] In this thesis, we use the term "children TAB-affected *in utero*" to refer to the exposure in the children and the term "VB-affected pregnancy" or "VB-unaffected pregnancy" to refer to the exposure in women. We acknowledge, support, and implement the use of patient-centred medical terminology to the best of our ability.

Table 1. Summary of the literature: the prevalence of vaginal bleeding in pregnancy

Author, year	Setting, data sources, period	Study population, N	Timing of vaginal bleeding	Prevalence of vaginal bleeding
Wilkerson et al., 1966[6]	- USA - Data from hospital records, physician's prenatal records, and in part from an interview with the mother - 1954-1964	61,137	Not reported	1.3%
Strobino & Pantel-Silverman, 1989[5]	- USA - Women seeking prenatal care in New York City - 1975-1985	3531	First trimester	22.0%
Sipilä et al., 1992[7]	- Finland - Northern part of Finland in the administrative provinces of Oulu and Lapland - Women with singleton pregnancies - 1985-1986	8718	First and second trimesters	9.3%
Axelsen et al., 1995[8]	- Denmark - Women receiving routine antenatal care at Aarhus University Hospital -1989-1991	5868	First and second trimesters First trimester Second trimester	19.0% 16.0% 5.0%
Everett, 1997[9]	- UK - General practice - Women with a positive pregnancy test result + ongoing pregnancy - 1989-1990	550	< 20 weeks of gestation	21.0%
Harville et al., 2003[10]	- USA	151	First 8 gestational weeks	9.3%

Author, year	Setting, data sources, period	Study population, N	Timing of vaginal bleeding	Prevalence of vaginal bleeding
	- Women >18 years planning to become pregnant			
Weiss et al., 2004[11]	- USA - The First and Second Trimester Evaluation of Risk (FASTER) trial database - Women enrolled at 10 to 14 weeks of gestation	16,506	First trimester	- Light vaginal bleeding: 13.0% - Heavy vaginal bleeding: 1.0%
Mulik et al., 2004[12]	- UK - Cardiff Births Survey (a computerised maternity database logging all deliveries in South Glamorgan since 1965) - Primigravid women with a singleton pregnancy - 1995-1999	6903	Early pregnancy (no indication of gestational age was given)	7.0%
Wijesiriwardana et al., 2006[13]	- UK - Aberdeen Maternity and Neonatal Databank - Women in their first pregnancy - 1976-2004	39,260	First trimester	19.4%
Hasan et al., 2010[14]	-USA, North Carolina -Women 18-45 years enrolled in the Right From the Start pregnancy study - On average, enrolled at 53 days of gestation - 2000-2008	4539	Any vaginal bleeding in pregnancy; data began at gestational week 4	26.7%
Smits et al., 2012[15]	- New Zealand, Australia, UK, Ireland - Screening for Pregnancy Endpoints (SCOPE) - Nulliparous women enrolled at 14-16 gestational weeks - 2004-2008	3431	1-20 weeks of gestation 1-12 weeks of gestation 13-20 weeks of gestation	22.7% 18.9% 6.4%
Sun et al., 2012[16]	- China - C-ABC cohort study (China Anhui Birth Defects and Child Development) - Women enrolled at the first prenatal visit - 2008-2010	4342	First trimester	24.0%
DeVilbiss et al., 2020[17]	- USA - Effects of Aspirin in Gestation and Reproduction (EAGeR) trial - Women 18-40 years actively trying to conceive with a history of regular menstrual cycle and 1-2 pregnancy losses - 2006-2012	701	2-8 weeks of gestation 2 and <4 weeks of gestation 4 and <6 weeks of gestation 6-8 weeks of gestation	30.1% 5.9% 14.6% 20.8%

Risk factors for VB in pregnancy include age ≥35 years, obesity, lack of physical exercise, stress, cigarette smoking, alcohol abuse, inflammation, and low plasma levels of progesterone.[7,19–26] The risk of miscarriage for pregnancies irrespective of VB is 12-31%.[14,27] In the Danish population, 14% of clinically recognised pregnancies end in a miscarriage.[28] The risk of miscarriage following VB is challenging to measure. An American study showed that VB between 6-8 gestational weeks was associated with 11.7% (95% confidence interval [CI]: 4.0-19.3) elevated absolute risk of clinically recognised pregnancy loss (25.3% vs 13.7% risk of clinical pregnancy loss for pregnancies with vs without bleeding).[17] Several studies reported that 12-15% of pregnancies with VB occurring in the first trimester ended in a miscarriage.[10,14,29,30] One study showed a risk of miscarriage in women having VB in pregnancy of 31.1% (95% CI: 23.3-33.0).[31] Several small studies showed that up to 50% of pregnancies with VB in the first or second trimester ended in a miscarriage.[3,9,29,32] The sensitivity of any VB for the diagnosis of miscarriage is 43.2% and the specificity is 83.1%.[31] A potential mechanism of early VB progressing to an early pregnancy loss might be increased oxygenation of the tissues surrounding an embryo and free radical formation resulting in impaired placentation due to oxidative stress.[2,33–35]

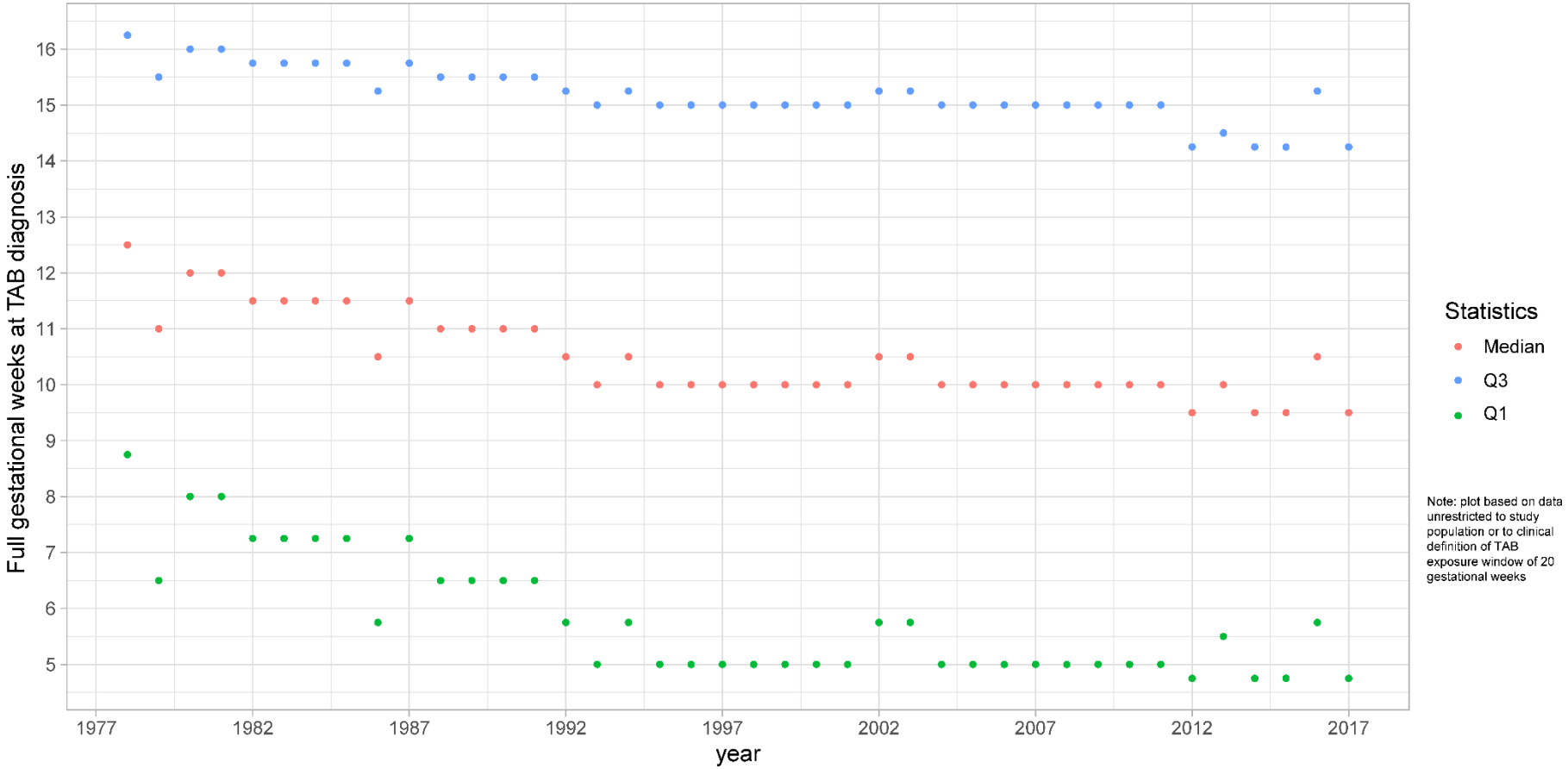
Several studies described patterns of VB in pregnancy.[8,10,14,17] Most bleeding episodes occurred in the first trimester (72-88%),[8,15] and the median gestational week of the first recognised bleeding episode in pregnancy was 8th (1st-3rd quartiles: 4th-20th).[8,14,17] An early timing of VB at about 5-8 gestational weeks is likely associated with a reduction of progesterone production due to the shift of production site from the corpus luteum to the placenta ("luteo-placental shift").[14,17,36,37]

A Danish study based on questionnaire data reported a median duration of the VB episode of 2 (1st-3rd quartiles: 1-12) days.[8] Most women (54.0-73.0%)[15,17] experienced one bleeding episode per pregnancy, and 55.0-66.8%[8,15,17] described the bleeding intensity as very light or spotting. Of women with VB in pregnancy, approximately 36% reported abdominal pain coinciding with the

haemorrhage (31% reported light pain and 5% moderate to severe pain).[8] In Denmark, 14% of women with VB in the first trimester were admitted to the hospital, while 28% of women were hospitalised when the bleeding occurred in the second trimester.[8] In this study, 98% of women with VB in pregnancy received an ultrasound examination.[8]

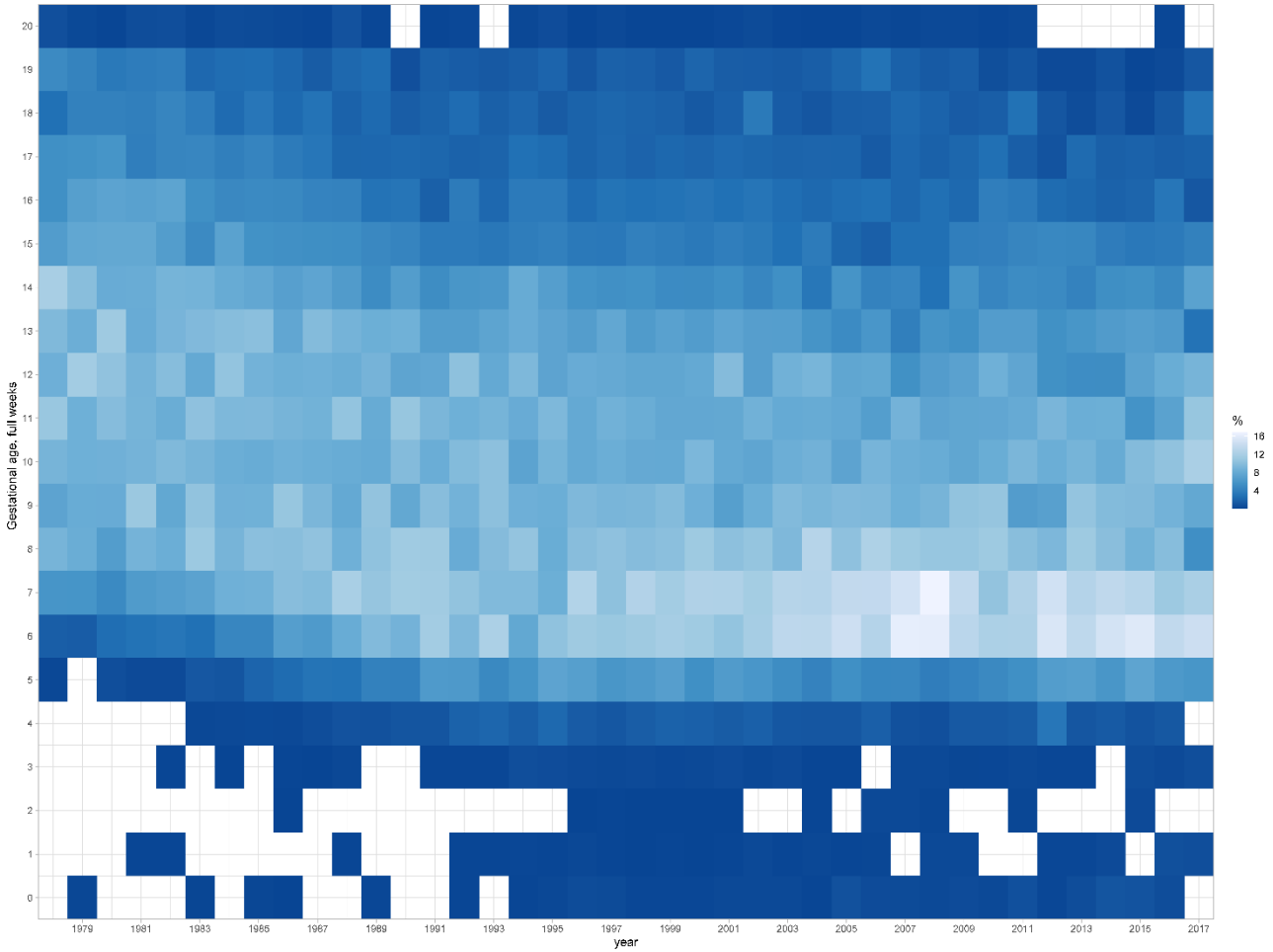
In the data used for this thesis, the median gestational age at TAB irrespective of a later childbirth was similar to that based on questionnaire data (Figure 1).[8] When restricted to TAB records within 20 weeks of gestation of a pregnancy ending in a childbirth, the highest frequency of a hospital-based TAB diagnosis was observed between 6 and 12 gestational weeks (Figure 2).[8] The distribution of gestational age at receiving TAB diagnosis was similar across women's age categories (Figure 3). Although it is challenging to accurately establish the number of TAB episodes in the same pregnancy using health registries, our data was broadly in line with questionnaire-based data from Denmark[8] and showed that most women (60-70%) received one diagnostic record of TAB within 20 gestational weeks of pregnancy (Figure 4).

Figure 1. Distribution of gestational age at TAB by year, Denmark, 1979-2017



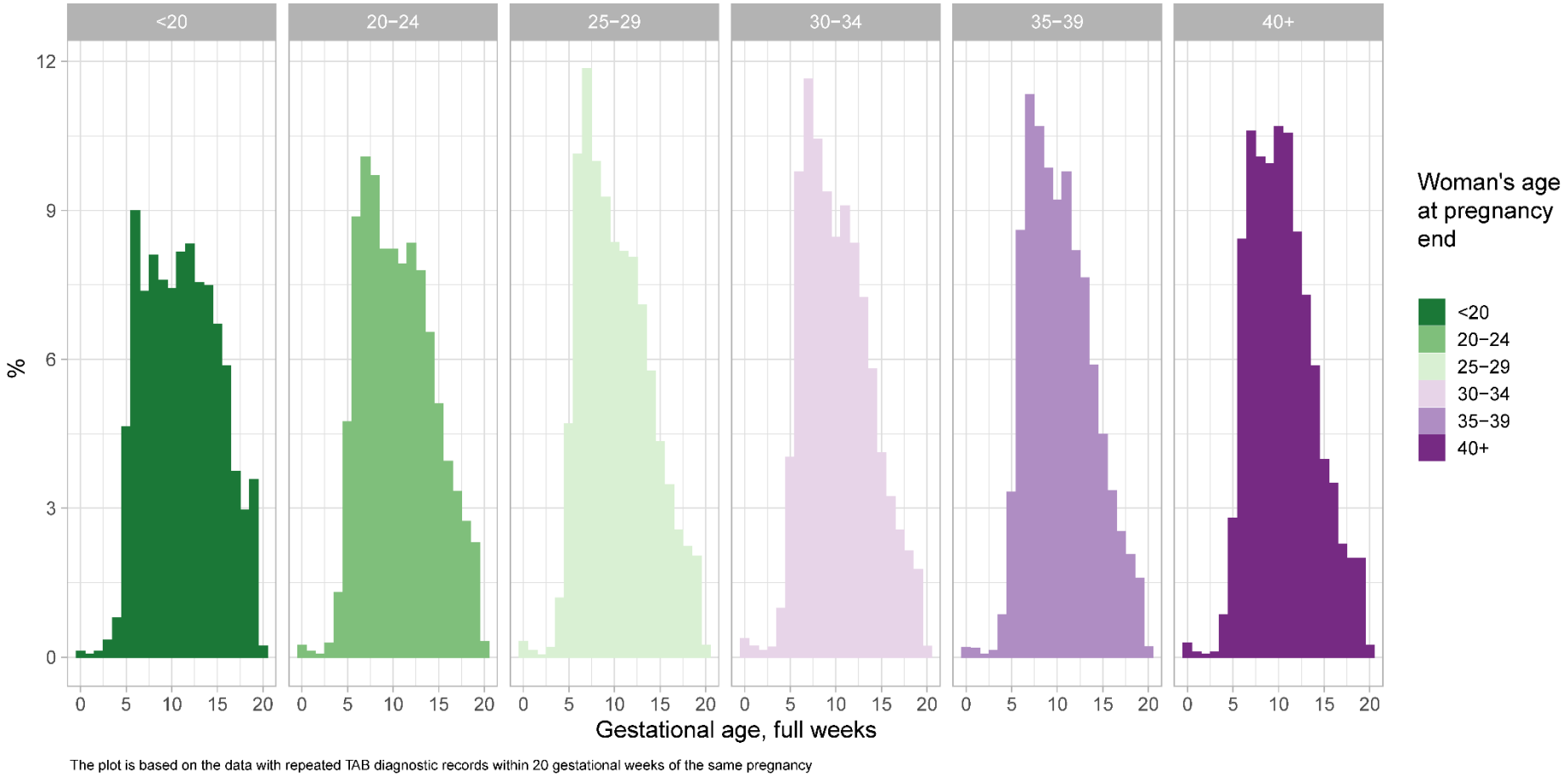
Notes: The plot is based on data unrestricted to final study period and study population. Multiple diagnostic records of TAB per pregnancy were included.

Figure 2. Frequency (%) of TAB diagnostic records by gestational age and year, Denmark, 1979-2017



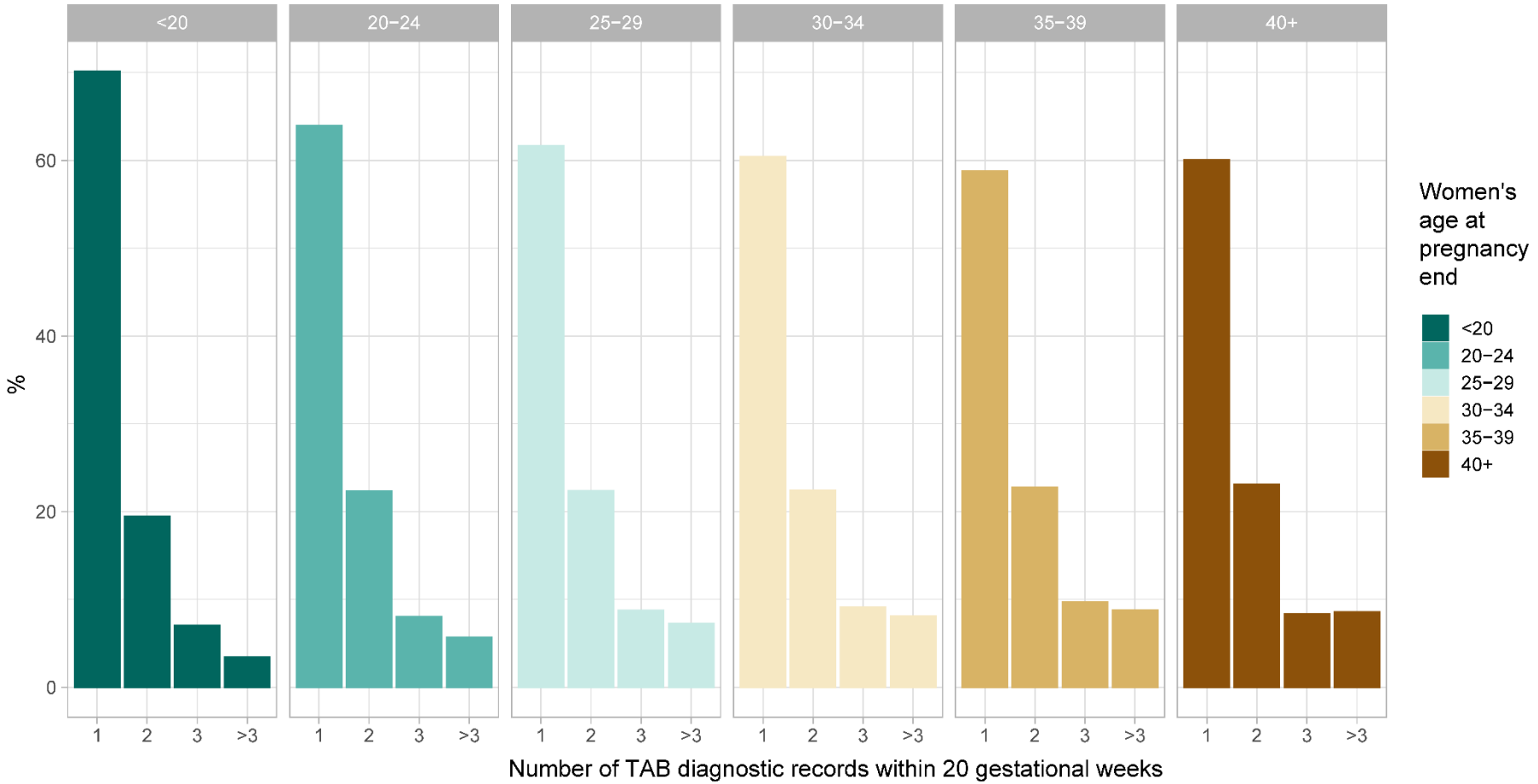
Notes: Heatmap is based on data unrestricted to final study population; multiple diagnostic records of TAB in the same woman during the same pregnancy were not excluded. Percentages within each year across all gestational weeks add up to 100%.

Figure 3. Frequency (%) of TAB by gestational age and women's age category at the pregnancy end, Denmark, 1979-2017



Notes: The plot is based on data unrestricted to final study population. Multiple diagnostic records of TAB per pregnancy were included. Percentages within each age category add up to 100%.

Figure 4. Frequency (%) of TAB (1, 2, 3, >3 diagnostic records) within 20 gestational weeks of a pregnancy ending in childbirth by women's age category at the pregnancy end, Denmark, 1979-2017



Notes: The plot is based on data unrestricted to final study population. Percentages within each age category add up to 100%.

In the short term, children TAB-affected *in utero* are at greater risk of perinatal death, congenital malformations, preterm birth, low birth weight, being born small for gestational age, low 5-minute Apgar score, and admission to the neonatal intensive care unit.[5,7,6,11,13,38–44] For the women, VB in pregnancy, in the short term, is a risk factor for preterm prelabour rupture of membranes, Caesarean section, antepartum and postpartum haemorrhages, placenta praevia, placental abruption, and manual removal of placenta.[5,11–13,38,41,42,45] However, multiple of previous studies were based on selective populations. Of note, limited knowledge exists on the association between *in utero* exposure to TAB and long-term health outcomes in children[46,47] and between pregnancy with VB in women and their later life health outcomes.[48]

This thesis investigated the association of *in utero* exposure to TAB with neurodevelopmental and neurological outcomes in children and of exposure to VB in pregnancy with mortality and morbidity later in women's life. This work includes four nationwide registry-based cohort studies. Study I investigated the risk of epilepsy, cerebral palsy, and attention-deficit/hyperactivity disorder (ADHD) in children. Study II evaluated the association between pregnancies with VB and all-cause and cause-specific mortality in women. Study III examined the association between pregnancies with VB and cardiovascular morbidity in women. Study IV investigated the risk of cancer in women following the pregnancy affected by VB.

2.1. Possible biological mechanisms underlying threatened abortion

Risk factors for VB are not completely understood. Some of the implicated risk factors are women's age at pregnancy ≥ 35 years, infections,[49–52] diabetes, obesity,[53,54,28] diseases of thyroid, stress, immunological dysregulation in pregnancy, and decreased progesterone levels.[18,55–58] The history of a previous pregnancy loss increases the risk of VB in an ongoing pregnancy nearly 2-fold.[14,22,55,56,59–62] Despite several shared risk factors, the aetiology of threatened abortion and miscarriage may overlap only partly. Available evidence shows that up to 70% of pregnancy losses before 20 gestational weeks were associated with chromosomal abnormalities in the embryo or foetal tissue.[57,63–67] A possible biologic pathway leading to a threatened

abortion is placental dysfunction accompanied by low levels of progesterone, a pregnancy-sustaining hormone.[21,58,68–70] The level of serum progesterone <35.0 nmol/L (11.0 ng/mL) among pregnancies with VB was predictive of a miscarriage by 16 weeks of gestation; however, the positive predictive value (PPV) of this cut-off was 67%.[69] In another study, a higher PPV of 76% was reported for a cut-off of <31.8 nmol/L (10.0 ng/mL) for serum progesterone.[31,71,72] Despite several established risk factors for threatened abortion, the aetiology of VB at presentation cannot easily be determined.

The PRISM randomised clinical trial, assessing the effects of progesterone to prevent miscarriage in women with early pregnancy bleeding, has shown a 3% greater proportion of live births at 34 gestational weeks or later among women with VB in the first 12 weeks of pregnancy who were randomised to receive vaginal micronised progesterone vs placebo.[24,31] The proportions of live births at ≥ 34 gestational weeks in the subgroup of women with recurrent miscarriages receiving progesterone vs placebo were 75% vs 70%, respectively, suggesting a modest beneficial effect.[23,24,73] The results of one systematic review found evidence of an effect of progestogens vs placebo on reducing the risk of miscarriage in pregnancies with VB (risk ratio [RR]=0.64, 95% CI: 0.47-0.87; data from seven trials conducted through 2018, N=696 women).[74] Another systematic review of nine randomised clinical trials (N=4907 women) showed a reduced rate of miscarriage in women with VB receiving progesterone vs placebo or no treatment (RR=0.70, 95% CI: 0.52-0.94).[75] Of note, no evidence of a reduced rate of miscarriage or increased rate of live births was found for the use of human chorionic gonadotropin in pregnancies with VB, suggesting a true role of low progesterone in the pathogenesis of VB.[31,76] Furthermore, no evidence of a miscarriage risk reduction exists for the use of bed rest[77] or uterine muscle relaxants[78] in pregnancies with VB.

Another potential biologic pathway leading to VB in pregnancy is inflammation. The Th-1 and Th-2 immunity balance is of paramount importance for sustaining a pregnancy.[79] The activation of interferon-gamma (IFNγ), tumour necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6)

production along with oxidative stress are possible mechanistic pathways of TAB[80–83] and also of miscarriage.[2,33–35,84,85]

2.2. Threatened abortion and outcomes in children and mothers

The association between TAB and adverse pregnancy and birth outcomes has been investigated since the 1970s (Table 2).[5–7,11,13,38–44] There is a body of evidence for an association between TAB-affected pregnancy and the adverse short-term outcomes including low birth weight, congenital malformations, preterm delivery, and perinatal mortality in newborns and placental abruption, preterm prelabour rupture of membranes, antepartum haemorrhage, and placenta praevia in mothers (Table 2).

Table 2. Summary of the literature: adverse pregnancy and neonatal outcomes after TAB-affected pregnancy

Author, year	Setting, data sources, period	Study population, exposure	Outcomes	Results and comments
Wilkerson et al., 1966[6]	- USA - Hospital records, the physician's prenatal records, and in part from an interview with the mother - 1954-1964	- N=61,137 deliveries in the participating hospitals; - TAB-affected pregnancies, n=790	- Perinatal death - Birth weight - Congenital malformations	TAB-affected *in utero* children who were carried to viability vs TAB-unaffected in-utero children had a 4-fold increase in perinatal mortality rates, 2-fold increased incidence of LBW, and 1.4-fold increased incidence of congenital malformations.
Hertz & Heisterberg, 1985[38]	- Denmark - Departments of Obstetrics and Gynecology at Gentofte and Herlev Hospitals - 1977-1978	- Pregnancies complicated by TAB and carried until at least 28 weeks of pregnancy, n=93; - Comparison group of TAB-unaffected pregnancies carried until at least 28 weeks of pregnancy, n=282	- Preterm birth - Retention of the placenta - LBW - SGA	*In utero* TAB exposure was associated with preterm birth, retention of placenta, birth weight below 2000 g, and SGA infants.
Strobino & Pantel-Silverman, 1989[5]	- USA, New York City - Women seeking prenatal care in New York City and interviewed in the second trimester or later - 1975-1985	- Women with light (n=619) or heavy (n=141) bleeding (investigated separately) in first trimester of pregnancy - Women without vaginal bleeding during pregnancy, n=2676	- LBW - Preterm delivery - SGA - Congenital malformations - Chromosomal anomalies - Stillbirth - Placenta praevia or placental abruption	Light bleeding vs no bleeding and heavy bleeding vs no bleeding, respectively, aOR (95% CI)*: - LBW: 1.1 (0.7-1.6) and 1.7 (0.9-3.3) - Preterm delivery: 1.3 (0.9-1.9) and 0.9 (0.4-2.2) - SGA: 0.8 (0.5-1.4) and 1.3 (0.5-3.1) *adjusted for maternal age, race, place of birth, pregnancy weight, phase of study entrance, sex, length of gestation at interview, smoking, parity, induced/spontaneous abortion history, working during pregnancy, gynaecological conditions. Any bleeding vs no bleeding in first trimester, OR (95% CI): - Congenital malformations: 1.7 (1.0-2.9) - Chromosomal anomaly: 2.4 (0.6-9.7) - Stillbirth: 1.0 (0.3-4.1) - Placenta praevia or placental abruption: 1.3 (0.6-2.6)
Williams et al., 1991[39]	- USA - Delivery Interview Program - 1977-1980	- Bleeding in first trimester of pregnancy, n=1174 - Bleeding in first trimester of pregnancy and later, n=215 - Comparison singleton pregnancies, n=10,055	- LBW (<2500 g) - VLBW (<1500g) - Preterm birth (<33 gestational weeks, <37 gestational weeks) - SGA - Stillbirth - Neonatal death	Bleeding in first trimester of pregnancy vs no bleeding in first trimester of pregnancy, RR (95% CI): - LBW: 2.3 (1.9-2.7) - VLBW: 2.2 (1.3-3.5) - Preterm birth <33 gestational weeks: 2.7 (1.8-39) - Preterm birth <37 gestational weeks: 2.0 (1.6-2.5) - SGA: 1. 6 (1.3-2.0) - Stillbirth: 1.1 (0.5-2.7) - Neonatal death: 2.5 (1.1-5.5)
Williams et al., 1992[40]	- USA - Prenatal diagnosis	-Uncomplicated pregnancies before second trimester ultrasound evaluation, n= 458	- LBW (<2500 g) - Preterm delivery (<37 week)	Pregnancies with early gestational bleeding and normal MSAFP vs pregnancies without early gestational bleeding, RR (95% CI):

Author, year	Setting, data sources, period	Study population, exposure	Outcomes	Results and comments
	- Clinic of Swedish Hospital Medical Center - 1989-1991	- Pregnancies with an early-gestation vaginal bleeding history and elevated MSAFP, n=48 - Vaginal bleeding history and normal MSAFP, n=39 - Never bled and elevated MSAFP, n=153 - Never bled and normal MSAFP, n=172	- SGA	-LBW: 2.1 (1.0-4.7) - Preterm delivery: 4.3 (1.5-12.2) - SGA: 1.3 (0.5-3.4)
Sipilä et al., 1992[7]	- Finland - Northern part of Finland in the administrative provinces of Oulu and Lapland - Women enrolled in second trimester before 25th week of gestation, giving birth in 1985-1986	- 8718 singleton pregnancies - First or second trimester bleeding, n=807 - First trimester bleeding, n=601 (light bleeding, n=470; heavy bleeding, n=131) - Second trimester bleeding, n=206 (light bleeding, n=125; heavy bleeding, n=85) - Comparison pregnancies without bleeding, n=7911	- LBW (<2500 g) - Preterm birth (< 37 weeks) - SGA (≤2 SD) - Congenital malformations (major and minor) - Perinatal mortality <7 days	Light first or second trimester bleeding vs no bleeding, aOR (95% CI)*: - LBW: 2.9 (2.0-4.0) - Preterm delivery: 2.1 (1.5-2.8) - SGA: 1.5 (0.9-2.5) - Congenital malformations: 1.9 (1.3-2.8) - Perinatal mortality: 1.4 (0.6-3.4) Heavy first or second trimester bleeding vs no bleeding, aOR (95% CI)*: - LBW: 2.6 (1.5-4.6) - Preterm delivery: 2.3 (1.4-3.8) - SGA: 1.9 (0.9-4.2) - Congenital malformations: 1.4 (0.7-2.7) - Perinatal mortality: 0.6 (0.1-4.5) First trimester bleeding vs no bleeding, aOR (95% CI)*: - LBW: 2.1 (1.5-3.1) - Preterm delivery: 1.8 (1.3-2.5) - SGA: 1.3 (0.8-2.2) - Congenital malformations: 1.4 (1.0-2.1) - Perinatal mortality: 1.1 (0.4-2.7) Second trimester bleeding vs no bleeding, aOR (95% CI):* - LBW: 4.1 (2.6-6.4) - Preterm delivery: 2.9 (1.9-4.6) - SGA: 2.5 (1.3-4.9) - Congenital malformations: 2.9 (1.7-4.7) - Perinatal mortality: 1.3 (0.3-5.4) *adjusted for: previous LBW; previous miscarriages; infertility examined and/or treated; contraception method; maternal age; parity; previous preterm birth; and previous stillbirths and/or perinatal mortality (<7 days)

Author, year	Setting, data sources, period	Study population, exposure	Outcomes	Results and comments
Johns et al., 2003[41]	- UK - University College London Hospitals - 2000-2001	- Pregnant women presenting with vaginal bleeding or lower abdominal pain at less than 12 completed weeks of pregnancy (cases), n=144 - Age-matched controls, n=144	- Pregnancy-induced hypertension - Foetal growth restriction - Placental abruption - PPROM - Preterm labour	Pregnancies with vaginal bleeding vs without vaginal bleeding, RR (95% CI): - Pregnancy-induced hypertension: 0.83 (0.19-3.64) - Foetal growth restriction: 1.66 (0.28-9.79) - PPROM: 3.55 (0.37-33.6) - Preterm labour: 3.05 (0.99-9.34) - Any adverse outcome: 2.22 (1.12-4.39)
Weiss et al., 2004[11]	- USA - The First and Second Trimester Evaluation of Risk (FASTER) trial database - Women enrolled at 10 to 14 weeks of gestation - 1999-2002	- 2094 patients with light vaginal bleeding in first trimester - 252 patients with heavy bleeding in first trimester - 14,160 patients without bleeding in first pregnancy trimester	- IUGR - Gestational hypertension - Preeclampsia - Preterm delivery - PPROM - Placental abruption - Placenta praevia - CS - SAB before 24 weeks of gestation	Light vaginal bleeding vs no bleeding in first trimester of pregnancy, OR (95% CI): - Preeclampsia: 1.4 (1.1-1.8) - Preterm delivery: 1.3 (1.1-1.7) - Placental abruption: 1.6 (1.1-2.6) - CS: 1.1 (1.0-1.3) - SAB: 2.5 (1.5-4.3) Heavy vaginal bleeding vs no bleeding in first trimester of pregnancy, OR (95% CI): - IUGR: 2.6 (1.2-5.6) - Preterm delivery: 3.0 (1.9-4.5) - PPROM: 3.2 (1.8-5.7) - Placental abruption: 3.6 (1.6-7.9) - CS: 1.4 (1.0-1.8) - SAB: 4.2 (1.6-10.9)
Johns & Jauniaux, 2006[42]	- UK - Referred to the Early Pregnancy Unit of a London teaching hospital by the general practitioner or from the Accident and Emergency departments - 2003-2004	- 214 women with bleeding in the first trimester of pregnancy - 214 age-matched comparisons without bleeding	- Preterm labour - Late miscarriage (14 to 22 + 6 days gestation) - PPROM	Pregnancies with vs without bleeding in the first trimester of pregnancy, RR (95%): - Preterm labour: 2.29 (1.4-4.6) - Late miscarriage: 6.9 (0.86-56) - PPROM: 3.72 (1.2-11.2)
Wijesiriwardana et al., 2006[13]	- UK - Aberdeen Maternity and Neonatal Databank - Women who sought hospital assessment for vaginal bleeding before 12 weeks of gestation - 1976-2004	- Women with a history of first trimester vaginal bleeding, n=7627 - All women delivering after 24 weeks of gestation (within the same period of time as women with a history of first trimester vaginal bleeding), n=31,633	- Placenta praevia - Other antepartum haemorrhage - Elective CS - Postpartum haemorrhage - Manual removal of placenta - Preterm delivery (<34 weeks; >34 weeks) - Malpresentation - Neonatal death - Birth weight <2500 g	Pregnancies with vs without bleeding in the first trimester of pregnancy, aOR (95%)*: - Placenta praevia: 1.77 (1.09-2.87) - Other antepartum haemorrhage: 1.83 (1.73-2.01) - Elective CS: 1.30 (1.14-1.48) - Postpartum haemorrhage: 1.08 (0.99-1.19) - Manual removal of placenta: 1.40 (1.21-1.62) - Preterm delivery: 1.56 (1.43-1.71) < 34 weeks: 1.89 (1.62-2.19) > 34 weeks: 1.38 (1.23-1.54) - Malpresentation: 1.26 (1.13-1.40) - Neonatal death: 1.27 (0.91-1.78) - Birth weight <2500 g: 1.22 (1.09-1.37) - Admission to neonatal unit: 0.94 (0.83-1.06)

Author, year	Setting, data sources, period	Study population, exposure	Outcomes	Results and comments
			- Admission to the neonatal unit	*adjusted for marital status, husband's/partner's social class, and smoking
Hossain et al., 2007[43]	- USA - Women participating in the study attended prenatal care clinics affiliated with Swedish Medical Center (Seattle) and Tacoma General Hospital (Tacoma) in Washington State - Women who initiated prenatal care prior to 20 weeks of gestation, were 18 years of age or older - 1996-2004	- Women without vaginal bleeding in pregnancy, n=1807 - Women with vaginal bleeding in pregnancy, n=622	- Preterm delivery	Pregnancies with vs without bleeding in pregnancy, aOR (95%)*: - Preterm delivery: 1.57 (1.16-2.11) *adjusted for maternal age (continuous), nulliparity (yes/no), ethnicity (white, African American, other), education, and smoking during pregnancy
De Sutter et al., 2006[44]	- Belgium - ART database - 1993-2002	- Singleton ongoing pregnancies with first trimester vaginal bleeding, n=253 - Singleton ongoing pregnancies without vaginal bleeding, n=1179	- Second trimester and third trimester bleeding - PPROM - Preterm contractions - IUGR - Intrauterine death - CS - Preterm birth - Very preterm birth - LBW - VLBW - NICU admission - Perinatal mortality	Pregnancies with vs without bleeding in pregnancy, aOR (95%)*: - Second trimester bleeding: 4.56 (2.76-7.56) - Third trimester bleeding: 2.85 (1.42-5.73) - PPROM: 2.44 (1.83-4.31) - Preterm contractions: 2.27 (1.48-3.47) - IUGR: 0.57 (0.27-1.21) - Intrauterine death: 0.78 (0.17-3.48) - CS: 0.98 (0.69-1.39) - Preterm birth: 1.64 (1.05-2.55) - Very preterm birth: 3.05 (1.12-8.31) - LBW: 1.24 (0.76-2.02) - VLBW: 3.56 (1.28-9.90) - NICU admission: 1.75 (1.21-.54) - Perinatal mortality: 0.87 (0.25-3.02)
Mulik et al., 2004[12]	- UK - Cardiff Births Survey (a computerised maternity database logging all deliveries in South Glamorgan since 1965) - Primigravid women with a single foetus pregnancy - 1995-1999	- Women with an early pregnancy bleeding, n=458 - Women without an early pregnancy bleeding, n=6445	- Placental abruption - Antepartum bleeding - Preterm delivery - LBW - Early neonatal death - Late neonatal death	Women having pregnancies with vs without vaginal bleeding, OR (95%): - Placental abruption: 2.8 (2.0-3.7) - Antepartum bleeding: 2.3 (1.1-5.1) - Preterm delivery: 2.0 (1.3-3.3) - LBW: 1.3 (0.8-1.9) - Early neonatal death: 5.7 (2.1-14.1) - Late neonatal death: 2.9 (0.6-12.9)
Dadkhah et al., 2010[86]	- Iran - Women referred to prenatal clinic for prenatal care - 2007-2008	- Women with vaginal bleeding in the first half of the pregnancy, n=500 - Women without vaginal bleeding in the first half of the pregnancy, n=500	- Preterm delivery - PPROM - Placental abruption	Women with vs without vaginal bleeding in the first half of the pregnancy, RR (95% CI): - Preterm delivery: 1.4 (1.2-1.5) - PPROM: 2.1 (1.2-2.3) - Placental abruption: 1.1 (1.01-1.2)

Author, year	Setting, data sources, period	Study population, exposure	Outcomes	Results and comments
Saraswat et al., 2010[4]	Systematic review and meta-analysis	- Women with vs without threatened abortion in the first trimester	- Preterm delivery - IUGR - LBW - Perinatal mortality - Perinatal morbidity (Apgar score, neonatal unit admission) - Congenital malformations	OR (95% CI): - Preterm delivery: 2.05 (1.76-2.40) - IUGR: 1.54 (1.16-2.00) - LBW: 1.43 (1.40-2.20) - Perinatal mortality: 2.16 (1.41-3.27) - Neonatal unit admission: 1.13 (1.03-1.23) - Congenital malformations: 1.26 (0.89-1.79)
Sun et al., 2012[16]	- China - C-ABC cohort study (China Anhui Birth Defects and Child Development) - Women enrolled at the first prenatal visit - 2008-2010	- Women with vaginal bleeding in the first trimester of pregnancy, n=1010 (visited a clinician due to vaginal bleeding, n=657; did not visit a clinician due to bleeding, n=393) - Women with bleeding in the first trimester of pregnancy, n=3292	- Preterm birth - LBW - SGA	Pregnancies with vs without bleeding in the first trimester, RR (95% CI): - Preterm birth: 1.59 (1.13-2.23) - LBW: 2.03 (1.25-3.29) - SGA: 1.34 (0.89-2.00)
Ozdemirci et al., 2015[87]	- Turkey, Research and Educational Hospital, Ankara - Hospital records of 12,050 singleton pregnant women in first trimester	- Women with threatened abortion in the first trimester of pregnancy, n=481 - Women without threatened abortion in the first trimester of pregnancy, n=486	- Spontaneous abortion - Preterm birth - Preeclampsia - Antenatal hematoma - LBW	Women with vs without threatened abortion in the first trimester of pregnancy, OR (95%CI): - Spontaneous abortion: 2.55 (1.62-3.91) - Preterm birth: 1.95 (1.15-3.24) - Preeclampsia: 0.76 (0.31-1.62) - Antenatal hematoma: 4.01 (0.45-36.52) - LBW: 2.33 (1.45-3.83)

Abbreviations: aOR, Adjusted odds ratio; ART, Assisted reproductive technique; CI, Confidence interval; CS, Caesarean section; IUGR, Intrauterine growth restriction; LBW, Low birth weight; MSAFP, Maternal serum alpha-fetoprotein; NICU, Neonatal intensive care unit; OR, Odds ratio; PPROM, Preterm prelabour rupture of membranes; RR, Risk ratio; SAB, Spontaneous abortion; SD, Standard deviation; SGA, Small for gestational age; TAB, Threatened abortion; VLBW, Very low birth weight

Few studies investigated the long-term health outcomes among children TAB-affected *in utero* and among women following pregnancy with vaginal bleeding (Table 3). An association between *in utero* exposure to TAB and increased hazard of developmental coordination disorder,[47] autism spectrum disorder,[46,88–90] and ADHD[90] later in children's life were reported (Table 3). However, at the time of writing, the literature is dominated by small case-control and cross-sectional studies.[47,88–91] Several small studies among *in utero* TAB-affected children investigated the autism spectrum disorder risk and a meta-analysis[46] showed a 2-fold increased risk.

Lifestyle factors and chronic conditions in mothers related to increased acute or chronic inflammation, e.g. smoking, obesity, autoimmune diseases, and infections, are associated with neurodevelopmental disorders in the offspring.[92,93] The potential mechanisms may include the influence of pro-inflammatory cytokines on the developing brain and immature blood-brain barrier, activation of microglia, or epigenetic alterations.[92–94] Although most of these inflammation pathways leading to adverse pregnancy outcomes or disruption of neurodevelopment in offspring were described based on animal models,[95–100] an increased level of pro-inflammatory cytokines in TAB-affected pregnancy may lead to disruption in the neurodevelopment of the foetus in humans.

Neurodevelopmental conditions have multifactorial aetiology with a prominent hereditary component.[101–107] Reports show that having a sibling with cerebral palsy is associated with a 9 to 11-fold elevated risk of this condition[103] and up to a 2-fold elevated risk of other neurodevelopmental and neurological disorders (e.g. epilepsy, schizophrenia, ADHD, ASD, intellectual disability).[102] Previous studies did not address family-shared confounding possibly explaining a large part of the observed associations between being TAB-affected *in utero* and subsequent risk of neurodevelopmental conditions.

Few studies have investigated the association between pregnancies with VB and the subsequent risk of dying, cardiovascular diseases, or cancer in women. One Danish study[48] reported a 20-

60% increased relative risk of cardiovascular morbidity in women having first-trimester bleeding without miscarriage; however, the hazard ratios (HRs) in that study were adjusted for multiple post-exposure characteristics of the mothers and newborns and did not control for pre-pregnancy morbidity and socioeconomic factors (Table 3).

A large body of evidence is available for miscarriage being a predictor of long-term health outcomes and premature death in women.[18,108–115] Miscarriages are associated with up to a 2-fold increased relative risk of cardiovascular disease in women.[18,116–118] In particular, miscarriages are a risk factor for hypertension,[108] hypercholesterolaemia,[108,118] ischaemic heart disease,[110] myocardial infarction, heart failure,[110] stroke,[110] venous thromboembolism,[18] and type 2 diabetes.[18,108,119] Results of one study using Danish data showed increased mortality in women with a history of miscarriages and stillbirths as well as pregnancy terminations, respectively, when compared with women having had live births only.[114] The same study showed that over the follow-up of 25 years (1980-2004), women whose reproductive history included solely pregnancy terminations and those with only early and late pregnancy losses (stillbirths and miscarriages) had a 4-fold increased mortality compared with women who had live births only.[114] Women with both pregnancy losses and live births were at 1.3-fold elevated mortality risk.[114] Another Danish study reported that compared with women whose first pregnancy ended in a childbirth, women whose first pregnancy ended in a miscarriage or termination had a higher mortality each year throughout the follow-up period of 10 years.[115] A study using data from The Nurses' Health Study II provided further evidence for an association between miscarriage and premature all-cause and cause-specific mortality in women.[109] Women with a history of miscarriages had a nearly 50% increased relative risk of death from cardiovascular disease than their counterparts who reported having no miscarriages.[109] A higher number of miscarriages was associated with a more pronounced increase in women's risk of dying prematurely. The risk of premature death was 60% higher for women reporting more than three miscarriages, 23% higher for women with a history of two miscarriages, and 16% higher for those reporting a history of one miscarriage.[109]

Pregnancies both “complete” (ending in childbirth)[120] and “incomplete”[121] (ending in miscarriage or termination[120]) are associated with a reduced risks of ovarian and endometrial cancer.[55,120,122–129] The underlying protective mechanisms of pregnancy may potentially be attributed to long-term progesterone effects.[120,130] No previous studies have investigated in detail the risk of cancer following VB-affected pregnancy.

Table 3. Summary of the literature: the association of *in utero* TAB exposure with long-term outcomes in children

Author, year	Setting, data sources, period	Study population, exposure	Outcome	Results and comments
Hua et al., 2014[47]	- Population-based cross-sectional study in China - The children of 160 classes from 15 randomly selected public nursery schools in five main districts in Suzhou City - Mothers of the children self-reported socio-demographic characteristics and pregnancy-related characteristics	- Population of children, n=4001 - Exposure: TAB at <20 weeks of gestation as reported by mothers	- DCD	- 18% of children with DCD were TAB-affected *in utero* vs 7% of children without DCD who were TAB-affected *in utero* - aOR* (95% CI): 2.72 (1.72-3.76) *adjusted for children's age, sex, and Kaup index computed as weight(kg)÷height(cm)^2×10^4
Wang et al., 2017[46]	- Systematic review and meta-analysis - Data on 37,634 autistic children and 12,081,416 non-autistic children from 17 studies - Countries: Tunisia, Sweden, China, Australia, USA, Canada, Turkey, Spain - Years of publication: 2002-2016	- Studies on TAB association with ASD included in the meta-analysis: Hadjkacem et al. 2016,[88] Zhang et al. 2010,[89] Glasson et al. 2004,[91] Say et al. 2016[90] - Total sample size in the meta-analysis was n=2249	- ASD	RR (95% CI): 2.28 (1.63-3.19)
Hadjkacem et al., 2016[88]	- Cross-sectional study in Tunisia - 2014	- Children diagnosed with ASD, n=50 and their unaffected siblings, n=51 - TAB-affected children with ASD, n=5 vs TAB-unaffected siblings without ASD, n=3 Exposure: TAB	- ASD	NA due to very small counts
Zhang et al., 2010[89]	- Case-control study of 190 Chinese children - 2007	- Cases with ASD, n=95 - Controls without ASD matched to cases on sex and the year of birth, n=95 - Exposure: TAB	- ASD	- 11.6% TAB-affected cases vs 2.1% TAB-affected controls - OR (95% CI): 6.09 (1.31-28.27)
Glasson et al., 2004[91]	- Case-control study in Australia - Children born in 1980-1995 and diagnosed with ASD by 1999	- Cases with ASD, n=501 - Controls matched for sex, n=1503 - Exposure: TAB at <20 weeks gestation	- ASD	OR (95% CI) for cases' vs controls' mothers to have had a TAB-affected pregnancy: 2.41 (1.56-3.73)
Say et al., 2016[90]	- Case-control study in Turkey - Children aged 3-18 years	- Cases with ASD, n=100 - Age- and sex-matched controls with ADHD, n=100 - Age- and sex-matched controls without ASD and ADHD - Exposure: TAB ("abortus threat")	- ASD - ADHD	- 20% of cases with ASD, 21% of controls with ADHD, and 11.2% of controls without ASD and ADHD were born from TAB-affected pregnancy

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; aOR, adjusted odds ratio; ASD, autism spectrum disorder; CI, confidence interval; DCD, developmental coordination disorder; NA, not available; OR, odds ratio; RR, risk ratio; TAB, threatened abortion

3. Objectives and hypotheses

The overall aim of this thesis was to investigate the potential long-term effects of *in utero* exposure to TAB on children's neurodevelopment and women's mortality and morbidity. We conducted four studies with the following objectives:

I. To investigate the association between being TAB-affected *in utero* and children's risks of epilepsy, cerebral palsy, and ADHD.
II. To examine the association of VB in pregnancy with all-cause and cause-specific mortality in women.
III. To examine the association between VB in pregnancy and cardiovascular morbidity in women.
IV. To examine the association between VB in pregnancy and subsequent risk of any and site-specific cancers in women.

In study I, we hypothesised that *in utero* TAB exposure, via pro-inflammatory cytokine-induced pathways, possibly impaired cytotrophoblast invasion, and progesterone insufficiency, may increase the risk of neurodevelopmental (ADHD) and neurological conditions (epilepsy, cerebral palsy) in children.

In studies II-IV, we hypothesised that VB in pregnancy may be associated with women's mortality, cardiovascular morbidity, and cancer due to the elevated levels of pro-inflammatory cytokines and decreased progesterone levels during the affected pregnancy.

4. Methods

4.1. Setting

Denmark provides universal tax-financed access to healthcare services to all its residents. Residents' interactions with healthcare providers are routinely registered through a network of health databases.[131] All Danish residents have a unique personal identifier (CPR number) assigned at birth or upon immigration. The CPR number is stored in the Danish civil registration system (CRS)[132] and allows unambiguous linkage of multiple administrative and health databases at individual level. We leveraged the unique healthcare and administrative data sources of Denmark[133] to conduct studies I-IV of this thesis.

4.2. Data sources

We used several administrative and healthcare registries, which prospectively and routinely capture individual-level data. The overview of all utilised data sources and other key characteristics of studies I-IV are presented in Table 4. In this section, we provide a brief description of the data sources used to conduct the studies.

4.2.1. The Danish Civil Registration System

Danish name: Det Centrale Personregister (CPR)

Studies: I-IV

Available period for the studies: 1971-2018

The Danish CRS[132] includes information on the unique personal identifier (CPR number) and vital, civil, and migration status. It permits data linkage at an individual level of all Danish administrative and healthcare databases, and it allows for virtually complete follow-up of Danish residents. Although the CRS was established in 1968, we had data on vital status starting from 1971,[134] on civil status from 1986,[135] and on migration status from 1971[136] according to availability on Statistics Denmark servers.[137]

4.2.2. The Danish Medical Birth Registry (MBR)

Danish name: Fødselsregisteret; from 1997 onward Det Medicinske Fødselsregister (MFR)

Available period for the studies: 1973-2017

The MBR[138,139] records all births in Denmark and comprises data on pregnancy, women's characteristics (age at delivery, parity, smoking during pregnancy starting in 1991, and body mass index (BMI) before conception starting in 2003; however, data on smoking and BMI during the first year of collection have considerable missingness), and neonatal characteristics (e.g. date of birth, gestational age, live vs stillbirth, sex, singleton vs multiple pregnancies, Apgar score, and birth weight). Each mother can be linked to her deliveries and each offspring. Before 2004, births starting from week 24 of gestation were recorded in the MBR. From 2004 onwards, births occurring between gestational weeks 22 and 24 have also been captured in the MBR.

4.2.3. The Danish National Patient Registry (DNPR)

Danish name: Landspatientregisteret (LPR)

Studies: I-IV

Available period for the studies: 1977-2018

The DNPR[140,141] collects data on inpatient contacts (since 1977), outpatient specialist clinic and emergency room encounters (since 1995), as well as surgical and other procedures and therapies (from 1977). The recorded information includes dates associated with the hospital encounter (admission and discharge dates for inpatient contacts and date of outpatient clinic or emergency room visit), primary and secondary diagnoses, and dates and codes of surgical procedures and therapies. From 1977 through 1993, the diagnoses were recorded using the International Classification of Diseases, Eighth Revision (ICD-8), and from 1994 forward using ICD-10. Surgical procedures are coded according to the Danish Classification of Surgical Procedures and Therapies (1977-1995) and the Danish version of the Nordic Medico-Statistical Committee Classification of Surgical Procedures (from 1996 onwards).

4.2.4. The Danish National Prescription Registry (NPR)

Danish name: Lægemiddeldatabasen (LMDB)

Studies: I-IV

Available period for the studies: 1995-2018

The NPR[142,143] stores data on all prescriptions redeemed at outpatient pharmacies and contains the medication user's unique personal identifier, data on the prescriber, and data on the dispensed drug. The latter comprises the date of dispensing, code of the product (Nordic article number), Anatomical Therapeutic Classification code, number of dispensed packages, the number of defined daily doses per package, strength per pill/unit, and number of pills/units per package dispensed. Information on drug indication, the duration of the prescription, and the dosing regimen is not recorded. Over-the-counter drugs, medications received during hospital admissions, and medications supplied by hospitals are also not captured by the NPR.

4.2.5. The Psychiatric Central Research Register (PCRR)

Danish name: Landspatientregisteret psykiatri (PSYK)

Studies: I-IV

Available period for the studies: 1995-2017

The PCRR[144] encompasses psychiatric inpatient, outpatient specialist clinic, and emergency room hospital encounters and includes data on the unique personal identifier, date of the encounter, discharge diagnoses, and the type of healthcare provider (hospital, department).

4.2.6. Danish education registers

Danish name: 'UDDA' - Uddannelser

Studies: I-IV

Available period for the studies: 1980-2018

Among other characteristics, the Danish education registers[145] at Statistics Denmark[137] capture data on the unique personal identifier and highest completed education from the Student Register.

4.2.7. Danish income registers

Danish name: 'IND' - Indkomst

Studies: I-IV

Available period for the studies: 1980-2018

The Danish income registries[146] capture data on personal A-taxable income (salaries, pensions, etc.), including the income from self-employment, before deductions from 1980 until 2013. After 2013, the data on total personal income have been recorded and include business and property income and other non-classifiable income that can be directly attributed to an individual, and before deduction of the labour market and pension contributions. Starting in 2016, income information is extracted from SKAT (Danish tax authority) registers.[147]

4.2.8. Danish registers on personal labour market affiliation (IDA)

Danish name: 'IDAP' - IDA persondata

Studies: I-IV

Available period for the studies: 1980-2018

IDA[148] contains data on individuals' employment and corresponding occupational code. The status of individuals outside of the labour market, e.g. retirees and people receiving state pension, is also recorded.

4.2.9. The Danish Register of Causes of Death (DRCD)

Danish names: Dødsårssagsregistret (DODSAARS); Dødsårsagsregister (DODSAASG)

Study: II

Available periods for the study: 1971-2001; 2002-2017

The DRCD[149] includes records of all deaths in Denmark since 1971; available data covers the date of death, cause of death (underlying cause and contributory causes I-III), and the manner of death (natural, accident, violence, suicide, or undetermined).

4.2.10. The Danish Cancer Registry (DCR)

Danish name: Cancerregisteret (CAR)

Study: IV

Available period for the study: 1979-2017

The DCR[150,151] captures records of all incident primary malignancies and several pre-cancerous conditions. The available data include the unique personal identifier, sex, age at diagnosis, date of diagnosis, and tumour characteristics (cancer site, ICD-10 diagnosis, cancer type, TNM stage at diagnosis, and histology).

4.3. Study designs

Studies I-IV were conducted using a cohort design. Study I additionally applied a sibling comparison design resulting in within-sibling relative risk estimates, additionally adjusted by design for confounders such as genetic make-up and environmental and other within-family time-invariant factors.[152]

4.4. Study populations

4.4.1. Study I

In study I, we included all singletons live-born between 1979 and 2010 to investigate the risk of epilepsy and cerebral palsy and those born between 1995 and 2010 to evaluate the risk of ADHD (Figure 5, Figure 6). We examined the risk of the neurodevelopmental outcomes in children TAB-affected vs TAB-unaffected *in utero* within 20 gestational weeks.

For the sibling comparison analyses, we ascertained three distinct populations of full siblings and maternal and paternal half-siblings. For each of these populations, we formed and kept the pairs of siblings discordantly exposed to TAB *in utero* within 20 gestational weeks. When several siblings had the same *in utero* TAB exposure status, we randomly chose one to complete a sibling or half-sibling pair. Paternal and full siblings could only be identified from 1986 onwards according to the availability of personal identifiers of children's fathers in the MBR and the CRS data on Statistics Denmark servers.

Figure 5. Population ascertainment for study I: children born between 1979 and 2010[153]

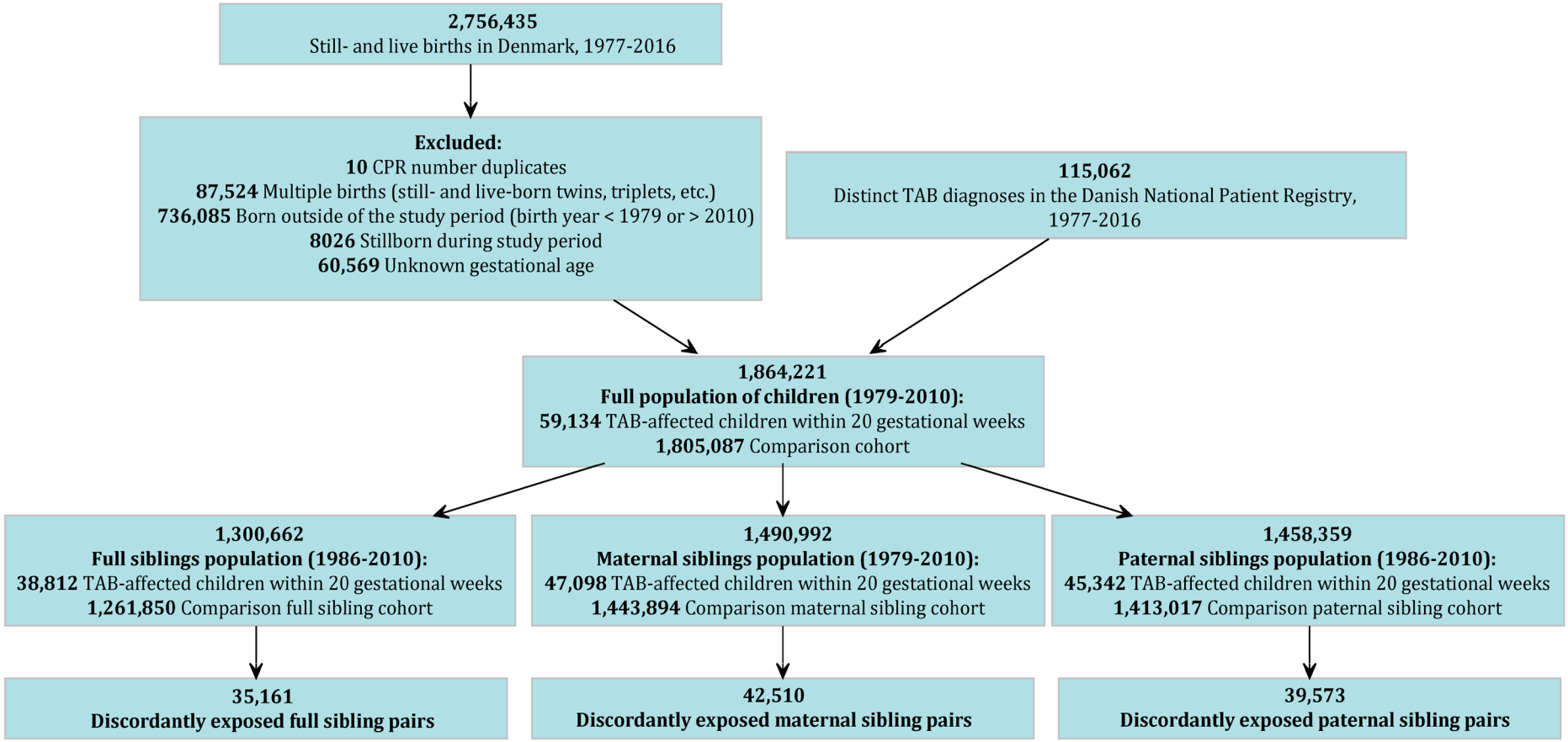
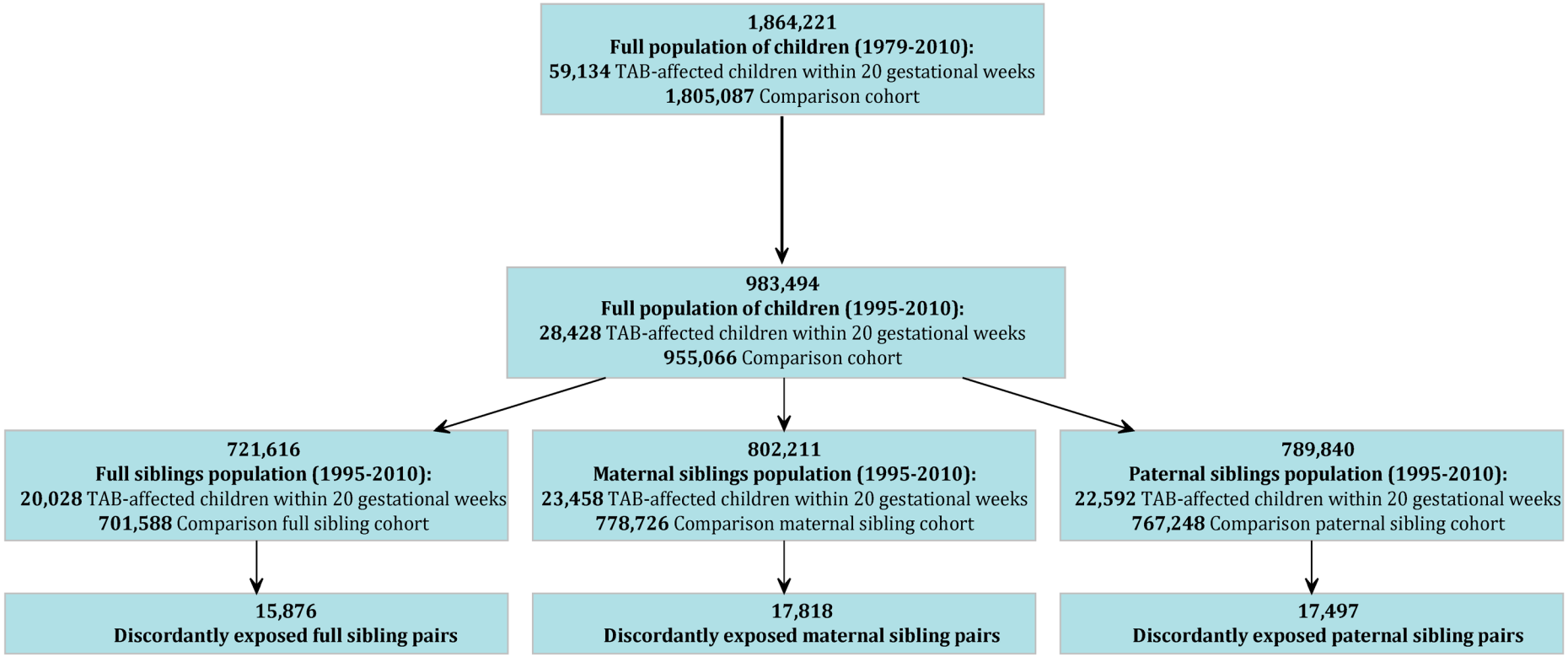


Figure 6. Population ascertainment for study I: children born between 1995 and 2010[a, 153]



[a]Data on ADHD medication prescriptions were available from 1995 onward

4.4.2. Studies II-IV

In study II, we included women with pregnancies between 1979 and 2017, whereas in studies III/IV, women with pregnancies between 1994/1995 and 2017, recorded in connection with childbirth or a hospital-based encounter due to pregnancy termination or miscarriage. We ascertained pregnancies ending in childbirth using delivery records from the MBR and pregnancies ending in a termination (medical and surgical abortions) or miscarriage using hospital diagnostic and procedure records from the DNPR. Pregnancies ending in a termination and pregnancies ending in a miscarriage formed two separate comparator cohorts, in addition to the comparator cohort of VB-unaffected pregnancies ending in a delivery. To identify pregnancies affected by VB and ending in childbirth, we used primary and secondary hospital-based diagnoses of haemorrhage specified as due to TAB from the DNPR recorded within the first 20 weeks of gestation (Figure 7, Figure 8, Figure 9).

We investigated mortality, diabetes and cardiovascular morbidity, and cancer risk following VB-affected vs VB-unaffected pregnancy ending in childbirth. In two separate sets of analyses, we compared the risk of the outcomes following pregnancy affected by VB and ending in childbirth with that of pregnancy ending in a termination or miscarriage. We repeated the analyses in a data subset of the first identifiable pregnancy of a woman.

Figure 7. Population ascertainment for study II, 1979-2017

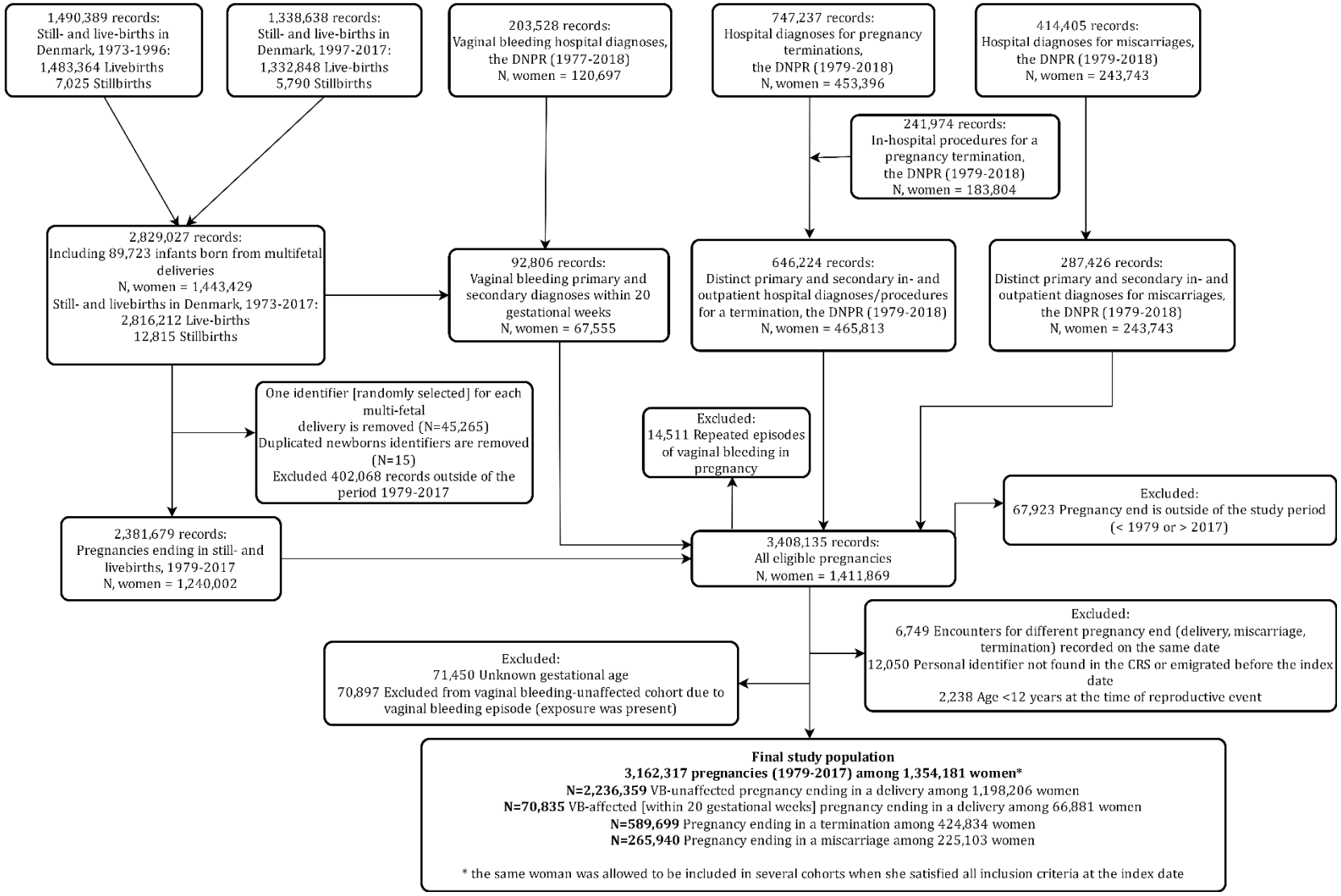


Figure 8. Population ascertainment for study III, 1994-2017[154]

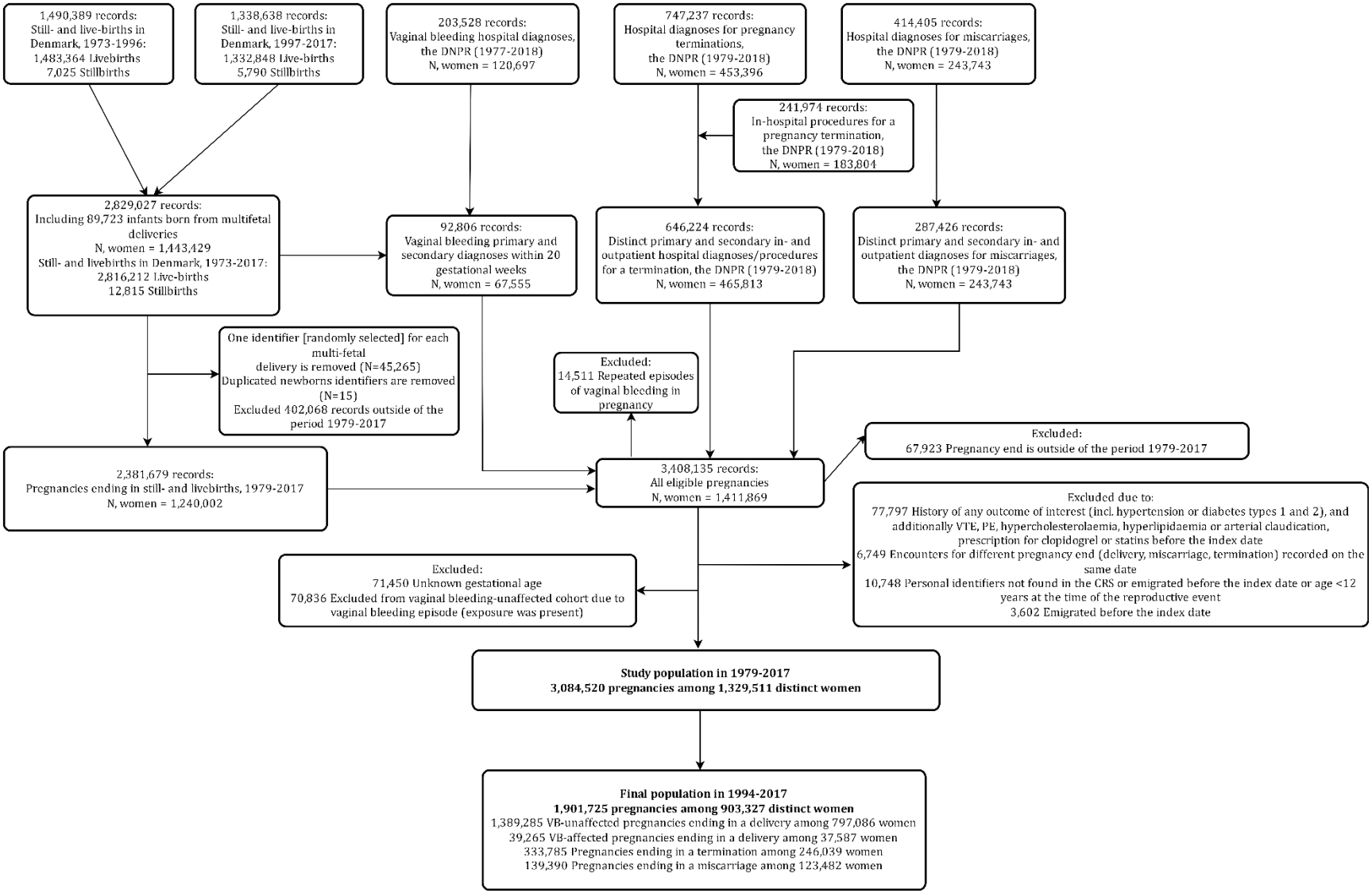
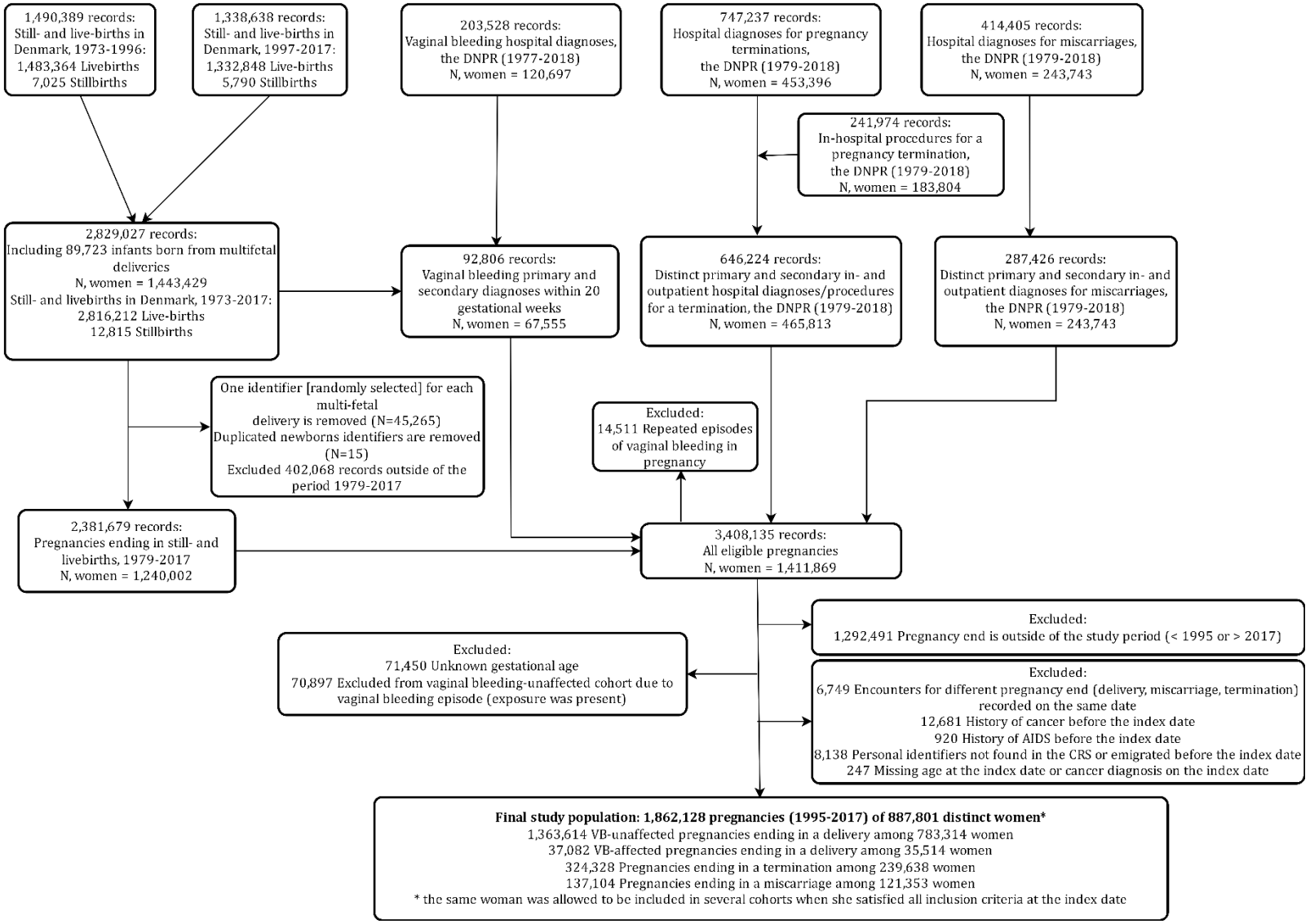


Figure 9. Population ascertainment for study IV, 1995-2017[155]



4.5. Exposure: threatened abortion (vaginal bleeding in pregnancy)

In main analyses of studies I-IV, the exposure was VB within the first 20 weeks of pregnancy. It was identified using primary and secondary diagnoses of haemorrhage specified as due to TAB recorded at any hospital-based visit in the DNPR and occurring within the first 20 gestational weeks of pregnancy ending in childbirth as recorded in the MBR.

4.5.1. Child's *in utero* exposure to threatened abortion: study I

Using the MBR, we identified pregnancies ending in a birth of a live singleton in Denmark in 1979-2010. To classify children as TAB-affected vs TAB-unaffected *in utero*, we ascertained diagnoses of TAB occurring within 20 gestational weeks via ICD-8 and ICD-10 codes in the DNPR.

4.5.2. Vaginal bleeding in pregnancy: studies II-IV

Using the MBR, we ascertained all pregnancies ending in delivery in Denmark in 1979-2017 (study II) or in 1994/1995-2017 (studies III/IV). To identify pregnancies with VB within the first 20 weeks of gestation, we ascertained ICD-8 and ICD-10 (according to the study period) codes from the DNPR for hospital-based diagnoses of haemorrhage specified as due to TAB in this calendar period.

4.6. Outcomes

In study I, the outcomes were cerebral palsy, epilepsy, and ADHD. From the DNPR, we ascertained primary and secondary diagnoses at discharge from hospital encounters for cerebral palsy or epilepsy between 1979 and 2016. For ADHD risk assessment, we used the PCRR and DNPR to identify records of primary and secondary ADHD diagnoses at discharge; from the NPR, we ascertained dispensings of ADHD medication. The date of the ADHD outcome was the earliest of the discharge diagnosis date or medication dispensing date. Due to data availability for ADHD outcomes starting in 1995, we narrowed the study population to children born in 1995-2010 (Appendix I, Supplementary Table S1 and Table S2).

In study II, the outcomes were all-cause and cause-specific mortality. For the all-cause mortality investigation, we ascertained the women's vital status from the CRS between 1979 and 2018. To examine the cause-specific mortality, we ascertained the women's causes of death listed as the underlying cause of death in the DRCD between 1979 and 2017. We investigated the women's risk of death due to medically-related (natural) and non-natural causes. We also examined the women's risk of death due to specific natural causes: cancer, cardiovascular diseases including myocardial infarction and stroke, respiratory, endocrine, and metabolic conditions, and nervous system conditions. In a separate analysis, we divided the non-natural causes of death into motor vehicle accidents, non-motor vehicle accidents or violence, and suicides (Appendix II).

In study III, the outcomes were incident diabetes type 1 and type 2, hypertension, ischaemic heart disease including myocardial infarction, heart failure, ischaemic stroke, and haemorrhagic stroke. From the DNPR between 1994 and 2018, we ascertained the following incident outcomes using primary and secondary diagnoses recorded due to inpatient, outpatient specialist clinic, or emergency room hospital visits: myocardial infarction, ischaemic heart disease, ischaemic stroke, heart failure, and haemorrhagic stroke. Hypertension outcome was defined as the earliest of either the diagnosis record in the DNPR or the first of 2+ redeemed prescriptions for drugs from different antihypertensives classes (alpha-blockers, non-loop diuretics, vasodilators, beta-blockers, calcium channel blockers, or renin-angiotensin system (RAS)-acting agents) within 180 days of each other in the NPR. Diabetes mellitus type 1 and type 2 outcomes were defined as the incident primary or secondary diagnosis in the DNPR or prescription of the drugs used in diabetes from the NPR, whichever was recorded first. Coronary artery bypass grafting and percutaneous coronary intervention were additional outcomes investigated in a sensitivity analysis among women with a record of pregnancy ending in a delivery, termination or miscarriage between 1977 and 1993 and were identified via the procedure codes (since 1999) from the DNPR. Various outcomes did not censor one another (Appendix III).[156–158]

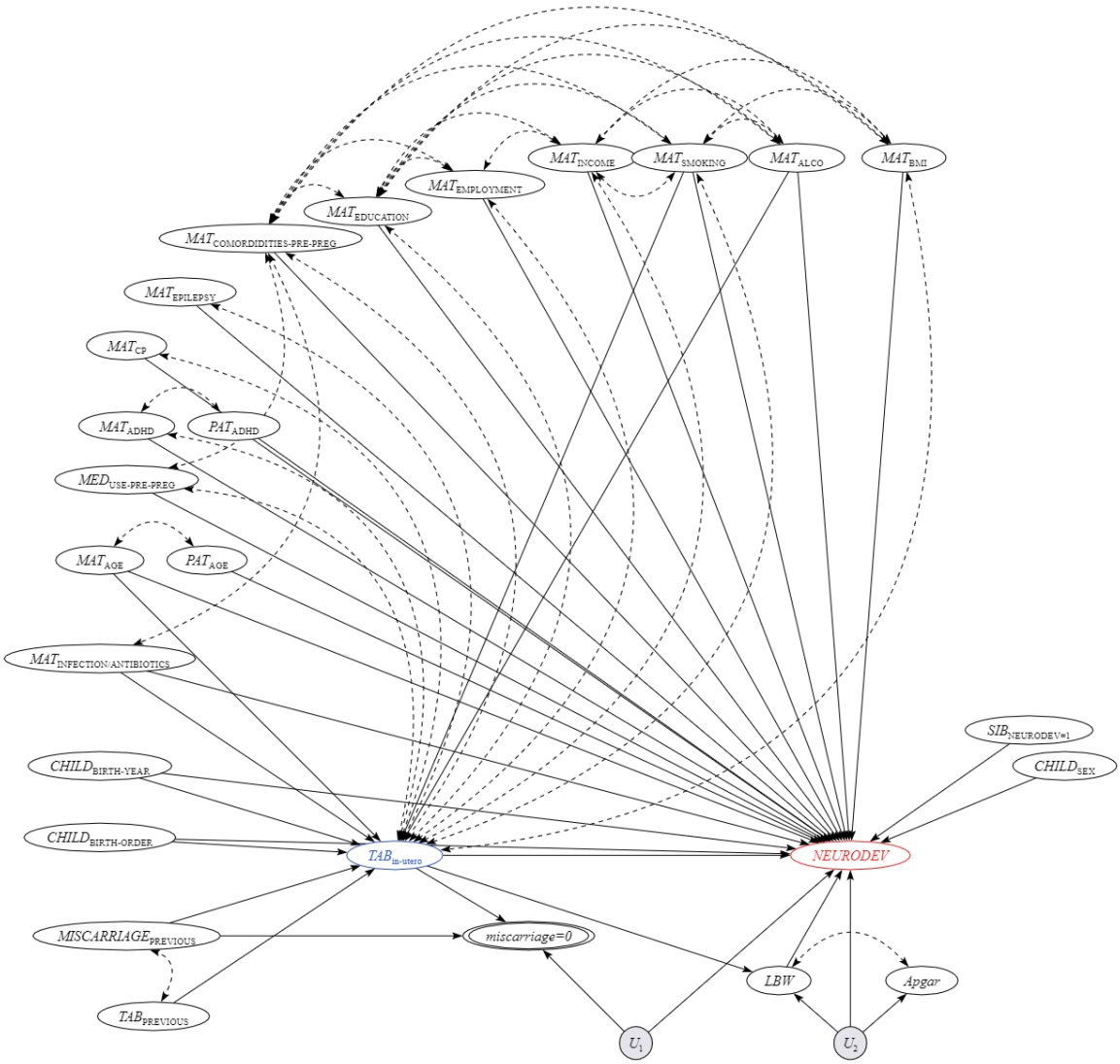
In study IV, the outcomes were incident primary cancer at any location and site-specific cancers ascertained from the DCR between 1995 and 2018 (Appendix IV).

4.7. Directed acyclic graphs (DAGs)

In all studies, we used DAGs, or causal diagrams, to depict the measured and unmeasured factors and their relations with the exposure, outcomes, and among themselves (Figure 10, Figure 11).[159–163] We selected the set of covariables sufficient to remove measured confounding between the exposure and the outcomes based on the assumed structure on the causal diagram following the rule: "control for each covariate that is a cause of the exposure, or of the outcome, or both; excluding from this set any variable known to be an instrumental variable".[159,163,164]

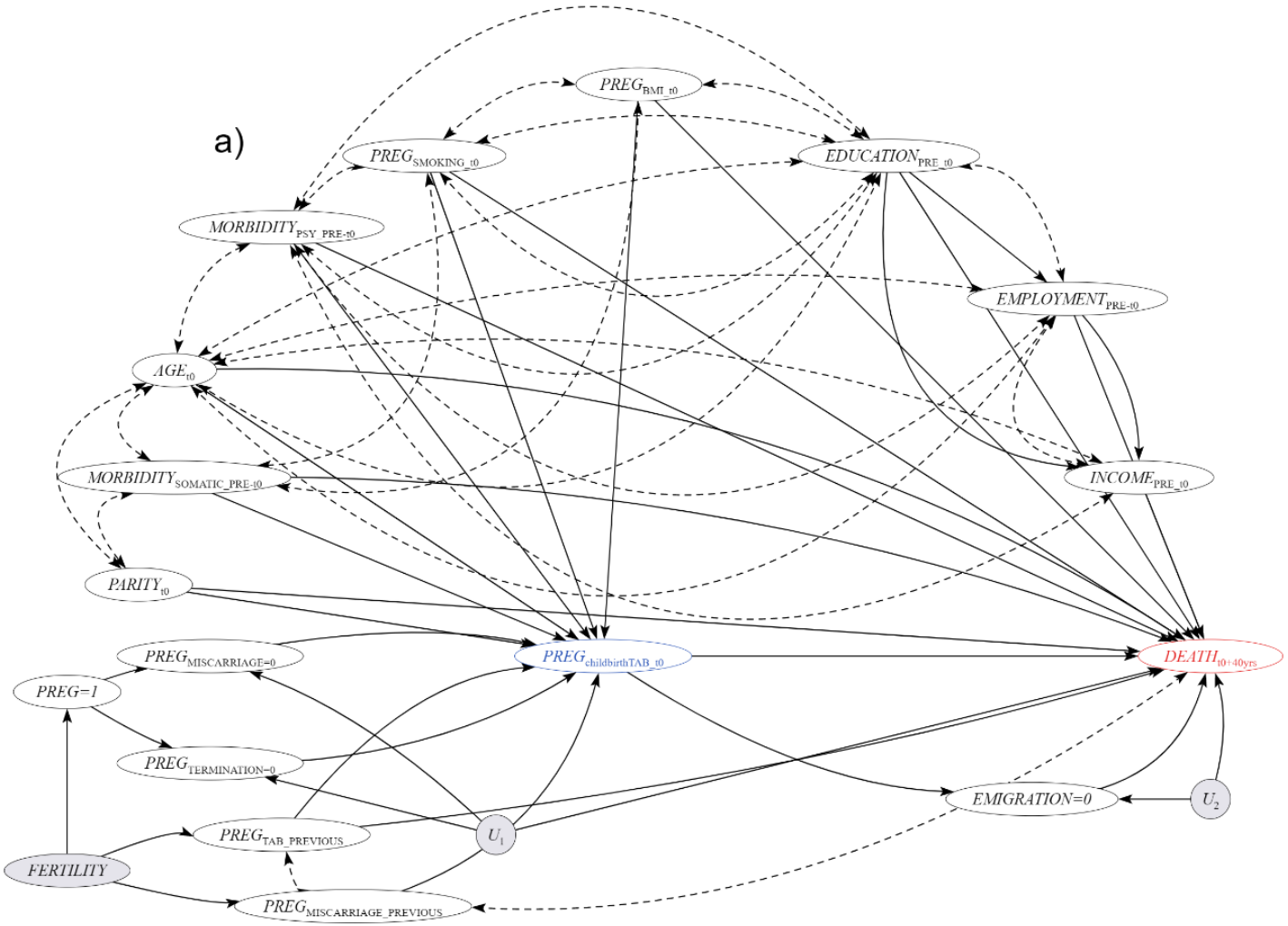
In study I, we strived against conditioning on potential mediators of the effects of TAB *in utero* on the child's neurodevelopment and, therefore, did not adjust for factors following the last menstrual period date, including those coinciding with gestation period (infections, gestational diabetes, preeclampsia-eclampsia, and neonatal characteristics such as Apgar score, birth weight, and gestational age at birth). In studies II-IV, we did not investigate the mediation pathways of observed total associations and, similarly to study I, did not condition on potential mediators of VB effect on the outcomes while controlling for pre-pregnancy factors. However, in studies II, III and IV, we adjusted for hypertensive pregnancy conditions, placenta praevia, and abruption placentae at the on-study pregnancy for the contrast of women having VB-affected vs VB-unaffected pregnancy ending in a delivery. We assumed that hypertensive pregnancy conditions, placenta praevia, and abruption placentae were not mediators but proxies of the underlying condition potentially also leading to VB, which manifested post-exposure.

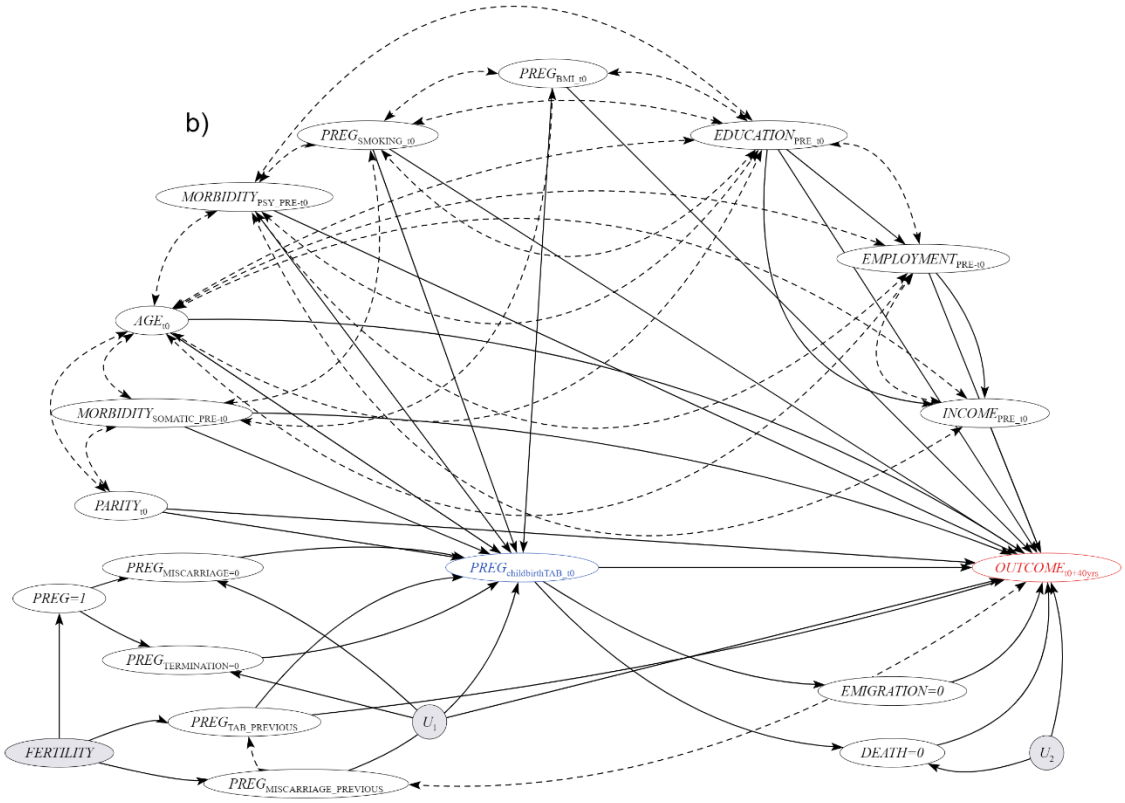
Figure 10. Directed acyclic graph for study I: the effect of exposure to TAB *in utero* on the neurological and neurodevelopmental outcomes (cerebral palsy, epilepsy, attention-deficit/hyperactivity disorder) in children



Abbreviations: ALCO, alcohol abuse-related conditions; BMI, body mass index; LBW, low birth weight; MAT, marks maternal covariables; NEURODEV, neurodevelopmental conditions; PAT, marks paternal covariables; PREG, pregnancy; TAB, threatened abortion; U_1, an unmeasured shared cause of the collider node "miscarriage" and NEURODEV; U_2, an unmeasured shared cause of LBW (a mediator of the TAB exposure *in utero*) and NEURODEV
Exposure: *in utero* TAB exposure ($TAB_{in\text{-}utero}$)
Outcome: neurodevelopmental conditions (NEURODEV)
Directed solid line arrows: causal association (including temporality, e.g. A → B means that A causes and precedes B)
Bi-directed dashed line arrows: associations confounded by the unmeasured common shared causes (A ⟷ B means A ←U→ B, where U is unmeasured)
DAG tool: https://causalfusion.net/app[161]

Figure 11. Directed acyclic graph for studies II-IV: the effect of vaginal bleeding-affected pregnancy on women's (a) mortality, (b) cerebrovascular outcomes, or cancer





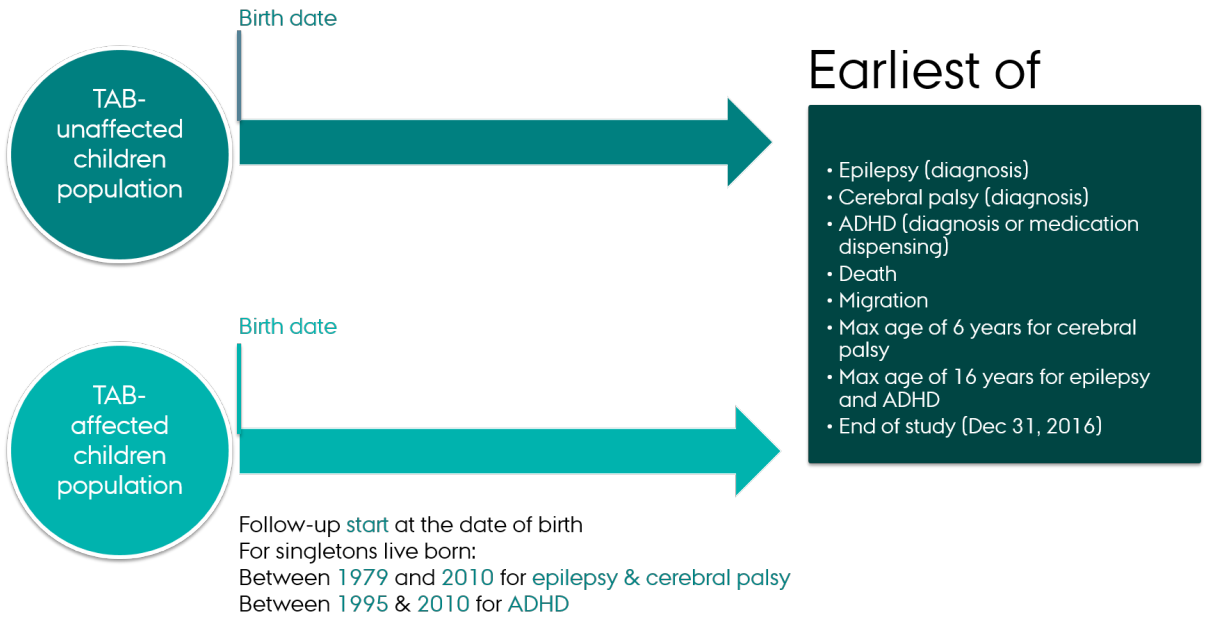
Abbreviations: PREG, pregnancy; $PREG_{BMI\_t0}$, body mass index at the index pregnancy (at time zero); PRE-t0, marks covariables measured before the index pregnancy (before time zero); TAB, threatened abortion (vaginal bleeding); U_1, an unmeasured shared cause for both miscarriage and pregnancy termination and the $OUTCOME_{t0+40yrs}$; U_2, an unmeasured factor increasing the risk of death and of the $OUTCOME_{t0+40yrs}$ (death is a competing event and the outcomes are only observed among those living "DEATH=0"); FERTILITY is unmeasured (latent node)
Exposure: TAB-affected pregnancy within the first 20 weeks of gestation ($PREG_{childbirthTAB\_t0}$)
Outcome: a) mortality (study II); b) CVD events (study III) or cancer (study IV)[155]
Directed solid line arrows: causal association (including temporality, e.g. A → B means that A causes and precedes B)
Bi-directed dashed line arrows: associations confounded by the unmeasured common shared causes (A ↔ B is the same as A ←U→ B, where U is unobserved)
DAG tool: https://causalfusion.net/app[161]

4.8. Statistical analyses

In study I, we followed TAB-affected and TAB-unaffected *in utero* children from the date of birth until the earliest of each of the outcomes, the pre-determined maximum age (16th birthday for epilepsy and ADHD, 6th birthday for cerebral palsy), emigration, death, or end of the study on 31 December 2016. We constructed cumulative incidence curves and computed the incidence rates per 1000 person-years (PYs) of each outcome of interest at the end of follow-up (Figure 12) separately for the full and each of the sibling populations. For each outcome, we calculated the HR with 95% CI using Cox proportional hazards regression to adjust for potential confounders. These included maternal factors (age at childbirth in categories, pre-pregnancy conditions

[hypertension, diabetes mellitus types 1 and 2, obesity, thyroid disorders, rheumatic diseases, epilepsy, cerebral palsy, and any psychiatric disorder]; the number of hospital and outpatient contacts and number of distinct diagnoses received within 12 months before pregnancy as an indicator of healthcare utilisation; medication use within 12 months before pregnancy; pre-pregnancy highest attained education, employment status, and income level in yearly quartiles of the full population), paternal factors (age at childbirth in categories, neurological and psychiatric disorders before the woman's last menstrual period date), and characteristics related to the child (year of birth, birth order, and also sex at birth for the ADHD investigation). To adjust for time-invariant family-shared confounding, we computed adjusted HRs within sibling pairs and separately within maternal and paternal half-sibling pairs using stratified Cox proportional hazards regression with one stratum per family cluster. We computed the 95% CIs using a robust "jackknife" standard error estimator with *survival* R package.[165–167] Proportionality of hazards assumption was visually assessed using log[–log(survival probability)] over time; no deviations from proportionality were observed.

Figure 12. Design features and follow-up for study I: risk of the neurological and neurodevelopmental outcomes in children TAB-affected vs TAB-unaffected *in utero*



In studies II-IV, we constructed the cumulative incidence curves using cumulative incidence function treating death (except for study II, where all-cause mortality was one of the outcome of interest) and emigration as competing risks and computed the incidence rates per 10,000 PYs of each outcome of interest at the end of follow-up. The confounders and outcome predictors in these studies were women's age, calendar year of pregnancy, socioeconomic factors (civil status, employment, highest attained education, year-specific income in quartiles computed based on the full population), reproductive factors (parity, number of previous identifiable pregnancies ending in delivery, termination or miscarriage, history of 1+ pregnancy termination, history of 1+ miscarriage, and history of VB in previous pregnancies, history of preeclampsia-eclampsia and other hypertensive disorders of pregnancy and placental complications), chronic conditions and comorbidities (obesity, chronic kidney and liver disease, chronic obstructive pulmonary disease, cancer, and psychiatric conditions according to data availability). Analyses were also adjusted for the history of cardiovascular conditions and diabetes mellitus in studies II and IV, but not in study III, since the population was restricted to pregnancies of women without such comorbidities as recorded in the DNPR before the index date. We also adjusted the analyses for medication use history to capture potential subtle differences in the underlying pre-pregnancy health of the women (mood disorders medication, antipsychotics, antiepileptics, non-steroid anti-inflammatory drugs, steroids, anti-infectives, and antihypertensives). The observations with missing data on women's age at pregnancy end (~0.3% of all eligible records) were excluded. Other variables with missing data entered the analyses categorised as "missing". In studies I and IV, we used conventional multivariable Cox proportional hazards regression, while in studies II and III we also used stabilised inverse probability of treatment (IPT) weights[159,168,169] truncated at 99th percentile when the IPT weights were above 50.[170,171]

Formulas for IPT weights computation:

- stabilised IPT weights: $\frac{\Pr[VB=1]}{\Pr[VB=1|L]}$ for VB-affected observations and $\frac{1-\Pr[VB=1]}{1-\Pr[VB=1|L]}$ for VB-unaffected observations[159,168–170]

where VB denotes exposure to VB within 20 gestational weeks and L denotes measured confounding factors and outcome predictors. The computations of IPT weights were performed via R package Weighting for Covariate Balance in Observational Studies.[171] The covariables balance between exposed and unexposed for each contrast was evaluated using standardised mean difference.[172]

In studies II-IV, in the analyses of all ascertained pregnancies, each identified pregnancy of each woman was treated as an observation. Each identified pregnancy of each woman had an index date and was followed until the earliest of the outcomes of interest, death, emigration, or end of study. Each next pregnancy of the same woman, while being treated as a separate observation, accounted for the changes in the woman's parity, comorbidities, medication use, socioeconomic status, and other covariables. To account for multiple pregnancies of the same woman, we computed robust standard errors and associated 95% CIs using *survival and WeightIt* R packages.[165–167,171] In these studies, we also repeated the main analyses following a woman's first identified pregnancy, which was classified as either VB-affected or VB-unaffected pregnancy ending in a delivery, pregnancy ending in a termination or pregnancy ending in a miscarriage. In the latter analyses, each pregnancy observation corresponded to one unique woman.

4.9. Sensitivity analyses

In study I, we performed six sensitivity analyses. First, we constructed the cumulative incidence curves for every outcome of interest treating death and emigration of children as competing events. Second, we added maternal smoking only and both maternal smoking and BMI to the adjustment set in the analyses of children born starting in 1995 and 2004, respectively. Third,

we used several different definitions of VB in pregnancy as exposure irrespective of the current clinical definition of TAB (VB any time during pregnancy, during the first 12 weeks of gestation, between gestational weeks 13 and 20, and starting from gestational week 13 and any time later until childbirth). Fourth, we narrowed the ADHD outcome to diagnoses following hospital encounters regardless of the ADHD medication dispensing. Fifth, we employed a wider definition for epilepsy outcome using the earliest of hospital-based epilepsy diagnosis or prescription of antiepileptic medication. Sixth, we allowed a follow-up of up to 16 years from the date of birth for the cerebral palsy outcome.

In study II, we utilised the data available starting in 1994 with the most complete information on somatic and psychiatric conditions and medication use for all pregnancies; and information on smoking for pregnancies ending in childbirth. In study III, we additionally conducted analyses of pregnancies identified between 1979 and 1993, which allowed up to 40 years of follow-up, and computed the HRs for two additional long-term cardiovascular outcomes, i.e. coronary artery bypass grafting and percutaneous coronary intervention. In studies II-IV, in analyses of pregnancies ending in childbirth, we investigated how additional adjustment for lifestyle proxy such as BMI (using data from 2004 onward) in pregnancy would change adjusted HRs. In studies II and IV, we investigated the risk of the outcomes in a subset of women whose last identifiable pregnancy was recorded at the age of 35+ years and 40+ years, respectively.

For studies II-IV, we conducted landmark analyses[173–175] to answer a research question "*Is vaginal bleeding in pregnancy associated with a risk of <death, CVD, cancer> after landmark age in women with one child?*". This set of analyses included women having had one delivery as recorded in the Danish Medical Birth Registry before the landmark age of 35 years in Study II and 40 years in Studies III and IV and simultaneously remaining alive, not emigrated, and outcome-free by the landmark age. In these landmark analyses, we followed women from the date of turning 35/40 years until the earliest of the outcome of interest, emigration or death (excluding study II, where the risk of death was the outcome of interest), and history of chronic conditions was re-ascertained taking into account new index date.

In all studies, we masked small counts to comply with the personal data protection regulations of Statistics Denmark.[137] The summary of the methodological aspects of all studies is available in Table 4.

4.10. Statistical software

All analyses were performed on the servers of Statistics Denmark via RStudio[176] and R[177] versions 3.6.0-4.1.0 using a collection of packages *tidyverse*,[178] *ggplot2*,[179(p2)] *survival*,[165] *flextable*,[180] *gtsummary*,[181] *patchwork*,[182] *purrr*,[183] *broom*,[184] *cmprsk*,[185] *prodlim*,[186] *epiR*,[187] *WeightIt*[171] and their dependencies.

4.11. Ethics

No patient consent or ethical approval is required for registry-based research according to Danish legislation. The studies included in this thesis were reported to the Danish Data Protection Agency[188] through registration at Aarhus University (record number: AU-2016-051-000001, sequential number 605).

Table 4. Summary of applied methods in studies I-IV (design, data sources, and statistical analyses)

	Study I	Study II	Study III	Study IV
Objectives	To investigate the association of TAB exposure *in utero* with children's long-term neurodevelopment measured by risk of cerebral palsy, epilepsy, and ADHD	To investigate the association of vaginal bleeding due to threatened abortion with all-cause and cause-specific mortality in women	To investigate the association between vaginal bleeding due to threatened abortion and cardiovascular morbidity in women	To investigate the association between vaginal bleeding due to threatened abortion and subsequent risk of cancer in women
Design	Cohort study	Cohort study	Cohort study	Cohort study
Data sources	CRS, MBR, DNPR, NPR, PCRR, Danish education registries, Danish income registries	CRS, MBR, DNPR, NPR, Danish education registries, Danish income registries, DRCD	CRS, MBR, DNPR, NPR, Danish education registries, Danish income registries	CRS, MBR, DNPR, NPR, DCR, Danish education registries, Danish income registries
Study period	1979-2016	• All-cause mortality: 1979-2018 • Cause-specific mortality: 1979-2017	1994-2018	1995-2017
Study population	Live-born singletons (1979-2010)	1. Pregnancies ending in a miscarriage, termination, still- or live birth (1979-2017) (multiple observations of the same woman were allowed) 2. Women with a first identifiable pregnancy ending in a miscarriage, termination, still- or live birth (1979-2017) (one observation per woman was allowed)	3. Pregnancies ending in a miscarriage, termination, still- or live birth (1994-2017) (multiple observations of the same woman were allowed) 1. Women with a first identifiable pregnancy ending in a miscarriage, termination, still- or live birth (1994-2017) (one observation per woman was allowed)	Women with a pregnancy ending in a miscarriage, termination, still- or live birth (1995-2017) (multiple observations of the same woman were allowed)
Exposure definition	*In utero* exposure to TAB within the first 20 gestational weeks (computed as the period between the last menstrual period date and [last menstrual period date + 140 days])	Pregnancy affected by vaginal bleeding within the first 20 gestational weeks (last menstrual period date + 140 days) and ending in delivery	Pregnancy affected by vaginal bleeding within the first 20 gestational weeks (last menstrual period date + 140 days) and ending in delivery	Pregnancy affected by vaginal bleeding within the first 20 gestational weeks (last menstrual period date + 140 days) and ending in delivery
Outcomes	Cerebral palsy, epilepsy, ADHD	• All-cause mortality • Cause-specific mortality from natural and non-natural causes	• Main outcomes: diabetes mellitus type 1 and type 2 and cardiovascular outcomes (hypertension, atrial fibrillation or flutter, heart failure, ischaemic stroke, haemorrhagic stroke,	• Any cancer • Cancers classified as hormone-related, haematological, immune-related, smoling or alcohol-related, obesity-

	Study I	Study II	Study III	Study IV
		• Cause-specific mortality due to: any CVD condition; CVD conditions other than myocardial infarction and stroke, myocardial infarction, stroke, respiratory disease, endocrine, nutritional, and metabolic conditions, motor vehicle accidents, non-motor vehicle accidents and violence, suicides, and all other causes	ischaemic heart disease including myocardial infarction) • Additional outcomes in the sensitivity analysis (includes pregnancies with index dates in 1979-1993 and follows them through 2018): coronary artery bypass graft, and percutaneous coronary intervention	related, cancers of neurological origin, and other sites • Site-specific cancers (focusing on premenopausal breast cancer, cervical cancer, ovary and fallopian tube cancer, and uterine cancer)
Covariables	Maternal factors: age at childbirth, pre-pregnancy comorbidities (hypertension, diabetes mellitus, obesity, thyroid disorders, rheumatic diseases; history of epilepsy, cerebral palsy, behavioural disorders incl. ADHD, schizophrenia, mood disorders, autism spectrum disorder, neurotic disorders, eating disorders, personality disorders, intellectual disorders, alcohol abuse); the number of hospital admissions, outpatient contacts, and distinct diagnoses within 12 months before the last menstrual period date; highest attained education, employment, year-specific income in quartiles; pre-pregnancy medication use. Paternal factors: age at childbirth; history of neurological and psychiatric morbidity (epilepsy, cerebral palsy, behavioural disorders incl. ADHD, any psychiatric disorder; history of use of epilepsy or ADHD medication). Child's factors: birth year, birth order, and sex for ADHD outcome.	Reproductive history (parity, number of previous pregnancies, history of at least one pregnancy termination or miscarriage, hypertensive disorders of pregnancy, placenta praevia, abruptio placentae), a woman's age, and calendar year of recorded pregnancy, socioeconomic factors (civil status, highest completed education, employment, and personal year-specific income), history of chronic conditions (obesity, polycystic ovary syndrome, thyroid disorders, rheumatic disorders with heart involvement, chronic obstructive pulmonary disease, chronic liver and kidney disease, hyperlipidaemia, hypercholesterolemia, any cardiovascular or metabolic conditions [diabetes mellitus type 1 and 2, hypertension, deep vein thrombosis, pulmonary embolism, stroke, ischaemic heart disease, atrial fibrillation or flutter, heart failure], any cancer), and history of psychiatric conditions.	Women's age, calendar year of pregnancy end, parity (nulli- or primiparous vs multiparous) as the number of delivered live and stillborn infants, pre-index date number of identifiable pregnancies ending in delivery, termination or miscarriage, civil status, employment status, highest attained education, annual women's income in quartiles; reproductive history (VB diagnosis irrespective of a later delivery, history of VB before 20th gestational week of pregnancy ending in a childbirth, histories of at least one termination or miscarriage, history of preeclampsia-eclampsia, placenta praevia, abruptio placentae, gestational diabetes), obesity, COPD, chronic kidney and liver diseases, cancer, rheumatic conditions with heart involvement, and psychiatric conditions before the index date. As a proxy for the underlying long-term health, we also ascertained the medication use (antipsychotics, mood disorders medication, antiepileptics, non-steroid anti-inflammatory drugs [NSAIDs], steroids for systemic use, anti-infectives) history before the end of 12th gestational week for pregnancies ending in a childbirth and before the index date for pregnancies ending in termination or miscarriage.	Women's age, calendar year of pregnancy end, women's parity, number of previous identifiable pregnancies, socioeconomic factors (education, employment, and year-specific income level divided into quartiles), reproductive history [pre-index date vaginal bleeding, termination, or miscarriages, placental complication in previous and current deliveries], chronic somatic conditions [obesity, polycystic ovary syndrome, thyroid disorders, chronic kidney disease, chronic liver disease, hyperlipidemia, diabetes, hypertension, any connective tissue disorder] and any psychiatric condition using the entire available lookback period; use of NSAIDs and steroids for systemic use before the 12 weeks of gestation for VB-affected and VB-unaffected pregnancies and before the index date for terminations and miscarriages. In the comparisons of pregnancies ending in childbirth, we additionally controlled for placental complications not only at the previous deliveries but also at the index pregnancy as a late manifesting proxy of potential placental dysfunction during pregnancy.

	Study I	Study II	Study III	Study IV
Follow-up start (index date, or time zero)	Child's date of birth	Date of delivery for pregnancies ending in a delivery (both VB-affected and VB-unaffected); date of diagnostic record or procedure date, whichever was the earliest, for pregnancies ending in termination or miscarriage		
Maximal pre-determined follow-up	16 years for epilepsy and ADHD risk investigation; 6 years for cerebral palsy risk investigation.	No restrictions		
Follow-up end	Child's 6th (cerebral palsy) or 16th (epilepsy, ADHD) birthday, the outcome of interest, death, emigration, or study end, whichever occurred first	The earliest of death, emigration, or study end	The earliest of the outcome of interest, death, emigration, or study end	The earliest of cancer, death, emigration, or study end
Time scale	Years since the birth date	Years since the index date	Years since the index date	Years since the index date
Outcome frequency/risk measures	IR per 1000 PYs, cumulative incidence at the end of the follow-up	Mortality rate per 10,000 PYs	IR per 10,000 PYs and cumulative incidence at the end of up to 25 years of follow-up	IR per 10,000 PYs and cumulative incidence at the end of up to 24 years of follow-up
Measure of association	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
Statistical methods	Cox proportional hazards regression	Cox proportional hazards regression	Cox proportional hazards regression	Cox proportional hazards regression
Confounding control	Multivariable adjustment; sibling pairs analysis	IPT-weighting with large (> 50) weights truncated at 99th percentile; conventional multivariable adjustment	IPT-weighting with large (> 50) weights truncated at 99th percentile; conventional multivariable adjustment	Multivariable adjustment
Sensitivity analyses	1. Treating death and emigration among children as competing events for cumulative incidence computation 2. Additional adjustment	<u>All-cause mortality:</u> 1. Analyses of women with pregnancies occurring in the ICD-10 era (from 1994 onwards) with more complete data while additionally adjusted for	1. Analyses of pregnancies occurring in the ICD-8 era (from 1979 through 1993) 2. Analyses additionally adjusted for pre-pregnancy BMI for pregnancies	1. Additional adjustment for maternal smoking and pre-pregnancy BMI for pregnancies ending in childbirth starting in 2004 2. Analyses of additional cancer sites

Study I	Study II	Study III	Study IV
a. for maternal smoking among children born starting in 1995 b. for maternal smoking and BMI among children born starting in 2004 3. Varying exposure definition (vaginal bleeding at any time during the gestational period, within the first 12 gestational weeks, between the 13th and 20th gestational weeks, and in the 13th gestational week or any time later until delivery) 4. ADHD defined as the hospital-based diagnosis only 5. Epilepsy defined as the earliest of diagnosis record or antiepileptic treatment record 6. Extended follow-up for CP outcome until 16th birthday	smoking for pregnancies ending in childbirth 2. Analyses of the last identifiable pregnancy recorded at the age of 35+ years 3. Landmark analyses of women with one lifetime childbirth	ending in childbirth (starting in 2004) 3. Landmark analyses of women with one lifetime childbirth	3. Analyses of last recorded pregnancies at the age of 40+ years 4. Landmark analyses of women with one lifetime childbirth

Abbreviations: ADHD, Attention-deficit/hyperactivity disorder; BMI, Body mass index, kg/m2; CI, Confidence interval; COPD, Chronic obstructive pulmonary disease; CRS, The Danish Civil Registration System; CVD, Cardiovascular diseases; DCR, The Danish Cancer Registry; DRCD, Danish Register of Causes of Death; DNPR, The Danish National Patient Registry; HR, Hazard ratio; IPT, Inverse probability of treatment; IR, Incidence rate; MBR, Medical Birth Registry; NPR, National Prescription Registry; NSAIDs, non-steroid anti-inflammatory drugs; PYs, Person-years; PCRR, The Psychiatric Central Research Registry; RAS, renin-angiotensin system TAB, threatened abortion (vaginal bleeding in pregnancy within the first 20 gestational weeks); VB, vaginal bleeding

5. Results

5.1. Study I

Between 1979 and 2010, 1,864,221 singletons were live-born in Denmark. Of these, 3.2% (N=59,134) were TAB-affected *in utero*. Of 983,494 singletons live-born between 1995 and 2010, 2.9% (N=28,428) were TAB-affected *in utero*. Mothers of TAB-affected *in utero* children were more likely than mothers of TAB-unaffected children to smoke in pregnancy, to be diagnosed with any psychiatric disorder before pregnancy, and to utilise the healthcare system within the 12 months before the last menstrual period date. TAB-affected and TAB-unaffected *in utero* children did not differ in terms of distribution of maternal and paternal age, birth order, most of the maternal comorbidities, and pre-pregnancy medication use (Appendix I, Table 1).[153] At the end of follow-up, the cumulative incidence for TAB-affected vs TAB-unaffected *in utero* children was 2.2% (95% CI: 2.1-2.3) vs 1.6% (95% CI: 1.6-1.6) for epilepsy, 5.5% (95% CI: 5.2-5.8) vs 4.2% (95% CI: 4.2-4.2) for ADHD, and 0.4% (95% CI: 0.3-0.4) vs 0.2% (95% CI: 0.2-0.3) for cerebral palsy (Appendix I, Figure 1). TAB-affected and unaffected *in utero* children had similar incidence rates of neurological and neurodevelopmental outcomes with incidence rate differences not higher than 1 per 1000 PYs for all outcomes (Table 5). Compared with TAB-unaffected children, TAB-affected *in utero* children were at a 25% increased hazard of epilepsy (HR: 1.25, 95% CI: 1.16-1.34), 42% increased hazard of cerebral palsy (HR: 1.42, 95% CI: 1.20-1.68), and 21% increased hazard of ADHD (HR: 1.21, 95% CI: 1.14-1.29). When we adjusted for family-shared confounding in sibling-comparison analyses, associations with epilepsy and ADHD attenuated towards the null value, while the associations with cerebral palsy shifted away from the null value; however, the estimates were imprecise (Table 5).[153]

Table 5. Incidence rates and hazard ratios for epilepsy, cerebral palsy, and ADHD among TAB-affected and TAB-unaffected *in utero* live-born singletons in the full population of children and among siblings and half-siblings[153]

Population	Cohorts, N[a]		Events, N[a]		Incidence rate per 1000 Person-Years			HR (95% CI)	
	TAB-affected	TAB-unaffected	TAB-affected	TAB-unaffected	TAB-affected	TAB-unaffected	Difference	Crude	Adjusted[b]
Epilepsy[c]									
Full population	59,135	1,805,085	1225	26,105	1.41	1.02	0.39	1.40	1.25 (1.16-1.34)
Maternal siblings	42,510	42,510	965	920	1.54	1.48	0.05	1.05	1.04 (0.90-1.20)
Paternal siblings[d]	39,575	39,575	880	790	1.51	1.37	0.13	1.11	1.08 (0.93-1.25)
Full siblings[d]	35,160	35,160	725	705	1.40	1.38	0.02	1.03	0.96 (0.82-1.12)
Cerebral palsy[c]									
Full population	59,135	1,805,085	225	4395	0.64	0.41	0.23	1.57	1.42 (1.20-1.68)
Maternal siblings	42,510	42,510	155	110	0.61	0.44	0.17	1.38	2.03 (1.15-3.57)
Paternal siblings[d]	39,575	39,575	140	95	0.59	0.41	0.18	1.45	3.28 (1.36-7.91)
Full siblings[d]	35,160	35,160	120	90	0.58	0.42	0.16	1.38	2.92 (1.33-6.39)
ADHD[e]									
Full population	28,430	955,065	1225	28,680	3.19	2.35	0.84	1.31	1.21 (1.14-1.29)
Maternal siblings	17,820	17,820	735	660	3.06	2.83	0.23	1.03	1.04 (0.90-1.20)
Paternal siblings	17,495	17,495	720	610	3.05	2.68	0.37	1.09	1.08 (0.93-1.25)
Full siblings	15,875	15,875	585	520	2.72	2.49	0.23	1.05	1.08 (0.92-1.27)

[a] Counts are clouded to nearest 5

[b] In the analyses investigating epilepsy and cerebral palsy outcomes, adjusted for characteristics of the mother (age at childbirth, pre-pregnancy comorbidities [somatic, neurologic, psychiatric], healthcare utilisation, medication use, income, education, and employment); the father (age on the date the child's birth, psychiatric comorbidities, and the history of epilepsy, cerebral palsy, and ADHD); and the child (birth year, birth order). Additionally adjusted for maternal cigarette smoking in the analyses investigating ADHD outcome.

[c] Among children born between 1979 and 2010

[d] Paternal personal identifier was available from 1986 onwards

[e] Among children born between 1995 and 2010 due to unavailability of data on ADHD outpatient diagnoses and medication dispensings before 1995

Abbreviations: ADHD, Attention-deficit/hyperactivity disorder; CI, Confidence interval; HR, Hazard ratio; TAB, Threatened abortion

When accounting for children's death and emigration in sensitivity analysis 1, we observed results virtually the same as in the main analyses (Appendix I, Supplementary Figure S18). In sensitivity analysis 2, the additional adjustment for maternal smoking and BMI in pregnancy for the full population of children slightly attenuated the HRs towards the null value for epilepsy and ADHD, but not for cerebral palsy (Table 6; Appendix I, Supplementary Figure S19). In sensitivity analysis 3, examining different definitions of VB in pregnancy did not meaningfully change HR estimates compared to the main results (Table 7; Appendix I, Supplementary Figure S20). In sensitivity analysis 4, a more specific definition of ADHD outcome resulted in HRs shifting towards unity, especially when additionally adjusting for maternal smoking and BMI in analyses of children born in 2004-2010 (HR: 1.06, 95% CI: 0.90-1.25); among siblings, the results were virtually the same as in the main analyses and showed no association with ADHD (Appendix I, Supplementary Figure S21). In sensitivity analysis 5, widening the definition of epilepsy outcome did not result in meaningful changes in HR estimates (HR: 1.23, 95% CI: 1.15-1.32 for the full population and HR: 1.02, 95% CI: 0.87-1.20 for the full siblings; the associations for half-sibling pairs were also unity) (Appendix I, Supplementary Figure S22). In sensitivity analysis 6, extending the follow-up for cerebral palsy to the maximum of 16 years resulted in narrower 95% CIs for within-sibling comparisons and HRs shifted towards the null value in comparison with the estimates from the main analyses (HR: 1.16, 95% CI: 0.89-1.52 for maternal half-siblings, HR: 1.30, 95% CI: 0.96-1.77 for paternal half-siblings, and HR: 1.18, 95% CI: 0.84-1.64 for full siblings) (Appendix I, Supplementary Figure S22).

Table 6. Sensitivity analysis 2: incidence rates and hazard ratios for epilepsy, cerebral palsy, and ADHD among TAB-affected and TAB-unaffected *in utero* live-born singletons in the full population of children[153]

	Cohorts, N[a]		Events, N[a]		Incidence rate per 1000 Person-Years					
Calendar period	**TAB-affected**	**TAB-unaffected**	**TAB-affected**	**TAB-unaffected**	**TAB-affected**	**TAB-unaffected**	**Difference**	**Model**	**Adjusted for**	**HR (95% CI)**
Epilepsy										
1979-2010	59,135	1,805,080	1225	26,105	1.41 (1.33-1.49)	1.02 (1.01-1.03)	0.39	Adjusted	Set 1[b]	1.25 (1.16-1.34)
								Crude		1.40 (1.32-1.48)
1995-2010	28,430	955,065	445	11,065	1.16 (1.06-1.27)	0.91 (0.89-0.92)	0.26	Adjusted	Set 1[b] + Maternal smoking	1.22 (1.10-1.36)
								Crude		1.30 (1.18-1.42)
2004-2010	9,085	414,345	110	3680	1.26 (1.04-1.52)	0.94 (0.91-0.97)	0.32	Adjusted	Set 1[b] + Maternal smoking + BMI	1.21 (0.98-1.49)
								Crude		1.35 (1.12-1.63)
Cerebral palsy										
1979-2010	59,135	1,805,085	225	4395	0.64 (0.56-0.73)	0.41 (0.39-0.42)	0.23	Adjusted	Set 1[b]	1.42 (1.20-1.68)
								Crude		1.57 (1.37-1.80)
1995-2010	28,430	955,065	95	2210	0.57 (0.47-0.69)	0.39 (0.37-0.40)	0.18	Adjusted	Set 1[b] + Maternal smoking	1.43 (1.14-1.78)
								Crude		1.48 (1.21-1.81)
2004-2010	9,085	414,345	35	900	0.66 (0.47-0.91)	0.36 (0.34-0.39)	0.30	Adjusted	Set 1[b] + Maternal smoking + BMI	1.69 (1.18-2.42)
								Crude		1.83 (1.31-2.56)
ADHD										
1995-2010	28,430	955,065	1225	28,680	3.19 (3.02-3.38)	2.35 (2.33-2.38)	0.84	Adjusted	Set 1[b] + Maternal smoking	1.21 (1.14-1.29)
								Crude		1.31 (1.24-1.39)
2004-2010	9,085	414,345	240	8395	2.75 (2.42-3.11)	2.14 (2.10-2.19)	0.60	Adjusted	Set 1[b] + Maternal smoking + BMI	1.10 (0.96-1.27)
								Crude		1.26 (1.11-1.43)

[a] Counts are clouded to nearest 5

[b] Set 1: characteristics of the mother (age at childbirth, pre-pregnancy comorbidities [somatic, neurologic, psychiatric], healthcare utilisation, medication use, income, education, and employment); the father (age on the date the child's birth, psychiatric comorbidities, and the history of epilepsy, cerebral palsy, and ADHD); and the child (birth year, birth order, and sex for ADHD outcome)

Abbreviations: ADHD, Attention-deficit/hyperactivity disorder; CI, Confidence interval; HR, Hazard ratio; TAB, Threatened abortion

Table 7. Sensitivity analysis 3: risk of epilepsy, cerebral palsy, and ADHD among TAB-affected and TAB-unaffected *in utero* live-born singletons in the full population of children and among siblings and half-siblings[153]

Exposure	Year[a]	Population	HR (95% CI)[b]
Epilepsy			
Vaginal bleeding in 0-20 gestational weeks[c]	1979-2010	Full population	1.25 (1.16-1.34)
		Maternal siblings	1.04 (0.90-1.20)
	1986-2010	Paternal siblings	1.08 (0.93-1.25)
		Full siblings	0.96 (0.82-1.12)
Vaginal bleeding any time during the gestational period	1979-2010	Full population	1.24 (1.16-1.33)
		Maternal siblings	0.97 (0.84-1.11)
	1986-2010	Paternal siblings	1.05 (0.92-1.21)
		Full siblings	0.96 (0.83-1.12)
Vaginal bleeding in 0-12 gestational weeks	1979-2010	Full population	1.22 (1.12-1.32)
		Maternal siblings	1.06 (0.94-1.19)
	1986-2010	Paternal siblings	1.05 (0.93-1.19)
		Full siblings	0.99 (0.87-1.13)
Vaginal bleeding in 13-20 gestational weeks	1979-2010	Full population	1.32 (1.14-1.51)
		Maternal siblings	0.99 (0.84-1.18)
	1986-2010	Paternal siblings	1.12 (0.93-1.36)
		Full siblings	0.97 (0.80-1.18)
Vaginal bleeding within 13 gestational weeks-birth	1979-2010	Full population	1.27 (1.13-1.44)
		Maternal siblings	1.05 (0.91-1.22)
	1986-2010	Paternal siblings	1.14 (0.97-1.33)
		Full siblings	1.05 (0.89-1.24)
Cerebral palsy			
Vaginal bleeding in 0-20 gestational weeks[c]	1979-2010	Full population	1.42 (1.20-1.68)
		Maternal siblings	2.03 (1.15-3.57)
	1986-2010	Paternal siblings	3.28 (1.36-7.91)
		Full siblings	2.92 (1.33-6.39)
Vaginal bleeding any time during the gestational period	1979-2010	Full population	1.47 (1.25-1.72)
		Maternal siblings	2.31 (1.30-4.11)
	1986-2010	Paternal siblings	1.88 (1.14-3.11)
		Full siblings	2.36 (1.35-4.15)
Vaginal bleeding in 0-12 gestational weeks	1979-2010	Full population	1.43 (1.18-1.72)
		Maternal siblings	1.71 (1.21-2.40)
	1986-2010	Paternal siblings	1.66 (1.16-2.38)
		Full siblings	2.03 (1.35-3.03)
Vaginal bleeding in 13-20 gestational weeks	1979-2010	Full population	1.37 (0.98-1.91)
		Maternal siblings	1.27 (0.74-2.18)
	1986-2010	Paternal siblings	1.08 (0.61-1.91)
		Full siblings	0.88 (0.49-1.60)
Vaginal bleeding within 13 gestational weeks-birth	1979-2010	Full population	1.52 (1.16-1.99)
		Maternal siblings	1.51 (0.98-2.34)
	1986-2010	Paternal siblings	0.92 (0.60-1.41)
		Full siblings	1.27 (0.75-2.15)
ADHD			
Vaginal bleeding in 0-20 gestational weeks[c]	1995-2010	Full population	1.21 (1.14-1.29)
		Maternal siblings	1.04 (0.90-1.20)
		Paternal siblings	1.08 (0.93-1.25)
		Full siblings	1.08 (0.92-1.27)
Vaginal bleeding any time during the gestational period	1995-2010	Full population	1.21 (1.14-1.28)
		Maternal siblings	1.06 (0.93-1.22)
		Paternal siblings	1.09 (0.95-1.25)
		Full siblings	1.07 (0.92-1.25)
Vaginal bleeding in 0-12 gestational weeks	1995-2010	Full population	1.21 (1.13-1.30)
		Maternal siblings	1.04 (0.89-1.21)
		Paternal siblings	1.08 (0.92-1.26)
		Full siblings	1.04 (0.87-1.24)
Vaginal bleeding in 13-20 gestational weeks	1995-2010	Full population	1.19 (1.05-1.35)
		Maternal siblings	1.20 (0.92-1.56)
		Paternal siblings	1.09 (0.83-1.44)

Exposure	Year[a]	Population	HR (95% CI)[b]
		Full siblings	1.07 (0.79-1.46)
Vaginal bleeding within 13 gestational weeks-birth	1995-2010	Full population	1.18 (1.06-1.32)
		Maternal siblings	1.16 (0.92-1.46)
		Paternal siblings	1.07 (0.84-1.36)
		Full siblings	1.04 (0.80-1.35)

[a] For children born in 1979-2010, the paternal identifier was available starting in 1986; analyses for ADHD outcome were carried out for children born in 1995-2010

[b] Due to the small number of outcomes among TAB-affected *in utero* children, adjusted models for vaginal bleeding in 0-12 gestational weeks, vaginal bleeding within 13 gestational weeks to birth, and vaginal bleeding in 13-20 gestational weeks among sibling populations accounted for a reduced set of covariables (maternal age at birth, year of child's birth, number of maternal pre-pregnancy hospital contacts, income, education, employment, maternal history of epilepsy, cerebral palsy, and any psychiatric disorder).

[c] Same as the main analysis

Abbreviations: ADHD, Attention-deficit/hyperactivity disorder; CI, Confidence interval; HR, Hazard ratio; TAB, Threatened abortion

5.2. Study II

Of 3,162,317 included pregnancies (among 1,354,181 unique women), 2.2% (N=70,835) were pregnancies affected by VB within the first 20 weeks of gestation, 70.7% (N=2,236,359) were pregnancies unaffected by VB, 18.7% (N=589,969) were terminations, and 8.4% (N=265,940) were miscarriages. Overall, chronic somatic conditions and drug utilisation among women before the index date were rare. Compared with VB-unaffected pregnancy, VB-affected pregnancy, termination, and miscarriage were more prevalent in women with a history of the psychiatric condition (6.8%, 8.5%, 11.0%, and 7.6%, respectively). Termination was more likely to occur in younger women with basic and high school or equivalent education, and first income quartile. A history of at least one miscarriage was more prevalent among women with VB-affected pregnancy (26.0%) and among women with pregnancy ending in a miscarriage (16.0%) than among women with VB-unaffected pregnancy (12.0%) or pregnancy ending in a termination (9.7%) (Appendix II, Table 1).

The median follow-up for all pregnancies was 20 years (25th-75th percentile, 10.8-28.9 years). By the end of data in 2018, there were 2,320 deaths from any cause following VB-affected pregnancy cohort; 55,030 deaths following VB-unaffected pregnancy cohort; 27,500 deaths following termination cohort, and 10,865 deaths following a miscarriage cohort. The corresponding mortality rates per 10,000 PY were 15.2 (95% CI: 14.6-15.9) for the VB-affected pregnancy cohort, 12.7 (95% CI: 12.6-12.8) VB-unaffected pregnancy cohort, 21.9 (95% CI: 21.6-22.1) for the termination cohort, and 19.2 (95% CI: 18.8-19.6) for the miscarriage cohort. We observed a slightly higher women's mortality rate per 10,000 PY from natural causes (10.6, 95% CI: 10.1-11.1) following VB-affected pregnancy vs VB-unaffected pregnancy (9.0, 95% CI: 8.9-9.1). Mortality rate from natural causes was 14.8 (95% CI: 14.6-15.0) per 10,000 PY following a termination and 13.2 (95% CI: 12.9-13.5) per 10,000 PY following a miscarriage (Appendix II, Table 2).

The results of crude analyses are presented in Table 8. In adjusted analyses, contrasting VB-affected vs VB-unaffected pregnancy the HR for all-cause mortality was 1.14 (95% CI: 1.09-1.19). Of note, the HR for all-cause mortality following VB-affected vs VB-unaffected pregnancy indicated no association (HR: 1.00, 95% CI: 0.90-1.12) when we adjusted for co-medication use and smoking in pregnancy in the sensitivity analysis using the ICD-10 era data subset. Similarly, associations with mortality from natural causes (HR: 1.15, 95% CI: 1.09-1.22), including overall cardiovascular diseases (HR: 1.15, 95% CI: 0.99-1.34), stroke (HR: 1.29, 95% CI: 0.93-1.80), myocardial infarction (HR: 1.15, 95% CI: 0.80-1.65), and nervous system conditions (1.19, 0.89-1.61) disappeared in the sensitivity analysis, where HRs became 0.98 (0.85-1.14) for natural causes mortality, 1.08 (0.73-1.60) for cardiovascular diseases, 0.98 (0.33-2.87) for stroke, 0.34 (0.08-1.37) for myocardial infarction, and 0.85 (0.34-2.12) nervous system conditions (Table 9). Associations with mortality from respiratory disease and endocrine, nutritional, and metabolic conditions did not shift to the null value. However, due to the small number of events in the analyses of the ICD-10 era data subset, the precision of several estimates was low.

There was no evidence for an association between VB-affected pregnancy and mortality from cancer (HR: 1.07, 95% CI: 1.00-1.15). An association between VB-affected pregnancy and mortality from non-natural causes (HR: 1.27, 95% CI: 1.08-1.48) also disappeared in the sensitivity analysis (HR: 1.04, 95% CI: 0.72-1.51), however, was imprecise. There was no meaningful association with motor vehicle accidents (HR: 1.00, 0.71-1.41); associations with mortality from non-motor vehicle accidents or violence (HR: 1.43, 95% CI: 1.13-1.81) and with suicide (HR: 1.36, 95% CI: 1.05-1.76) became null in the sensitivity analysis (Table 9).

Comparisons of VB-affected pregnancy with a termination or miscarriage showed slightly reduced risks of all-cause and natural causes mortality, including cancer and cardiovascular mortality. The HRs for non-natural mortality were compatible with no association for comparisons of VB-affected pregnancy with pregnancy ending in termination or miscarriage (Table 9). In general, the associations in the analyses of the first identifiable pregnancy of a

woman were consistent with analyses of all identifiable pregnancies of a woman (Table 9) and the results of a sensitivity analysis following the last pregnancy of a woman at the age of 35+ years supported no association with increased mortality risk for the comparison of VB-affected pregnancy vs VB-unaffected pregnancy (Appendix II, Table 5 in Supplementary data).

Results of landmark analyses of women with one childbirth before the age of 35 years were in line with the results of the main analyses (Figure 13).

Table 8. Crude associations between vaginal bleeding-affected pregnancy and all-cause and cause-specific mortality in women

Outcome	Population	Calendar period: 1979-2017/2018 (main analysis)			Calendar period: 1994-2017/2018 (sensitivity analysis)		
		HRs (95% CI)			HRs (95% CI)		
		VB-affected vs VB-unaffected pregnancy	VB-affected pregnancy vs termination	VB-affected pregnancy vs miscarriage	VB-affected vs VB-unaffected pregnancy	VB-affected pregnancy vs termination	VB-affected pregnancy vs miscarriage
All-cause mortality[a]	All pregnancies	1.21 (1.16-1.26)	0.76 (0.73-0.79)	0.83 (0.79-0.87)	1.09 (0.98-1.21)	0.66 (0.60-0.74)	0.74 (0.66-0.83)
	First pregnancy	1.25 (1.15-1.36)	0.73 (0.67-0.80)	0.77 (0.70-0.84)	1.15 (0.93-1.43)	0.88 (0.71-1.10)	0.82 (0.65-1.04)
Mortality from natural causes[b, c]	All pregnancies	1.19 (1.13-1.26)	0.79 (0.75-0.84)	0.85 (0.80-0.89)	1.03 (0.90-1.18)	0.74 (0.64-0.85)	0.78 (0.67-0.90)
	First pregnancy	1.23 (1.10-1.37)	0.73 (0.66-0.82)	0.76 (0.68-0.85)	-	-	-
Cancer	All pregnancies	1.08 (1.01-1.16)	0.80 (0.74-0.86)	0.85 (0.79-0.91)	0.90 (0.76-1.08)	0.74 (0.61-0.89)	0.74 (0.61-0.90)
	First pregnancy	1.12 (0.98-1.29)	0.73 (0.63-0.84)	0.76 (0.66-0.88)	-	-	-
Overall CVD	All pregnancies	1.24 (1.07-1.44)	0.79 (0.68-0.91)	0.87 (0.75-1.01)	1.17 (0.81-1.69)	0.83 (0.57-1.22)	0.93 (0.63-1.37)
	First pregnancy	1.13 (0.83-1.54)	0.63 (0.46-0.86)	0.73 (0.53-1.00)	-	-	-
CVD other than myocardial infarction and stroke	All pregnancies	1.20 (1.00-1.44)	0.79 (0.66-0.95)	0.86 (0.71-1.04)	1.33 (0.88-2.01)	0.96 (0.62-1.48)	1.07 (0.69-1.65)
	First pregnancy	1.17 (0.81-1.69)	0.69 (0.48-1.00)	0.83 (0.56-1.23)	-	-	-
Myocardial infarction	All pregnancies	1.30 (0.89-1.89)	0.78 (0.53-1.14)	0.99 (0.68-1.45)	0.55 (0.14-2.17)	0.32 (0.08-1.29)	0.64 (0.14-2.86)
	First pregnancy	0.89 (0.37-2.15)	0.42 (0.17-1.02)	0.52 (0.21-1.30)	-	-	-
Stroke	All pregnancies	1.33 (0.96-1.86)	0.78 (0.56-1.10)	0.80 (0.57-1.12)	0.98 (0.36-2.65)	0.77 (0.27-2.18)	0.57 (0.20-1.61)
	First pregnancy	1.22 (0.60-2.47)	0.64 (0.32-1.30)	0.59 (0.29-1.23)	-	-	-
Respiratory diseases	All pregnancies	1.65 (1.33-2.04)	0.84 (0.67-1.04)	0.90 (0.72-1.13)	1.70 (0.87-3.35)	0.85 (0.43-1.68)	0.96 (0.45-2.02)
	First pregnancy	1.78 (1.13-2.82)	0.70 (0.45-1.11)	0.69 (0.43-1.12)	-	-	-
Endocrine, nutritional, metabolic conditions	All pregnancies	1.39 (0.98-1.97)	0.79 (0.56-1.12)	0.70 (0.49-1.00)	2.23 (1.09-4.59)	1.34 (0.62-2.87)	0.99 (0.45-2.19)
	First pregnancy	2.27 (1.30-3.97)	1.15 (0.66-2.02)	1.10 (0.61-1.99)	-	-	-
Nervous system conditions	All pregnancies	1.27 (0.95-1.70)	0.73 (0.55-0.98)	0.92 (0.68-1.26)	0.85 (0.35-2.05)	0.47 (0.19-1.16)	0.83 (0.31-2.24)
	First pregnancy	1.21 (0.67-2.21)	0.68 (0.37-1.23)	0.67 (0.36-1.23)	-	-	-
Mortality from non-natural causes[b, c]	All pregnancies	1.45 (1.25-1.68)	0.67 (0.57-0.78)	0.89 (0.76-1.04)	1.38 (0.98-1.93)	0.54 (0.38-0.75)	0.78 (0.55-1.12)
	First pregnancy	1.45 (1.08-1.94)	0.64 (0.47-0.85)	0.80 (0.58-1.08)	-	-	-
Motor vehicle accidents	All pregnancies	1.18 (0.84-1.65)	0.83 (0.59-1.19)	1.01 (0.72-1.43)	1.33 (0.75-2.37)	0.69 (0.38-1.25)	1.10 (0.58-2.07)
	First pregnancy	1.10 (0.57-2.14)	0.71 (0.36-1.39)	0.91 (0.45-1.85)	-	-	-
	All pregnancies	1.63 (1.30-2.05)	0.61 (0.49-0.77)	0.82 (0.65-1.04)	1.49 (0.82-2.69)	0.44 (0.25-0.78)	0.65 (0.36-1.18)

Outcome	Population	Calendar period: 1979-2017/2018 (main analysis)			Calendar period: 1994-2017/2018 (sensitivity analysis)		
		HRs (95% CI)			HRs (95% CI)		
		VB-affected vs VB-unaffected pregnancy	VB-affected pregnancy vs termination	VB-affected pregnancy vs miscarriage	VB-affected vs VB-unaffected pregnancy	VB-affected pregnancy vs termination	VB-affected pregnancy vs miscarriage
Non-motor vehicle accidents or violence	First pregnancy	1.69 (1.08-2.65)	0.66 (0.42-1.03)	0.84 (0.52-1.35)	-	-	-
Suicides	All pregnancies	1.54 (1.21-1.97)	0.69 (0.54-0.89)	0.98 (0.76-1.26)	1.31 (0.72-2.36)	0.55 (0.30-1.01)	0.72 (0.38-1.39)
	First pregnancy	1.56 (0.97-2.49)	0.61 (0.38-0.97)	0.75 (0.46-1.23)	-	-	-
Other	All pregnancies	1.40 (1.24-1.59)	0.77 (0.68-0.87)	0.81 (0.71-0.92)	1.30 (0.92-1.84)	0.63 (0.44-0.89)	0.71 (0.49-1.03)
	First pregnancy	1.43 (1.12-1.83)	0.78 (0.61-1.00)	0.74 (0.57-0.97)	-	-	-

[a] All-cause mortality data were available through 2018

[b] Cause-specific mortality data were available through 2017

[c] The list of ICD-8 and ICD-10 codes for the cause-specific mortality outcomes is available in Appendix II, Table 1 in Supplementary data

Abbreviations: CI, Confidence interval; CVD, Cardiovascular diseases; HR, Hazard ratio; VB, Vaginal bleeding

Table 9. Adjusted associations between vaginal bleeding-affected pregnancy and all-cause and cause-specific mortality in women

Outcome	Population	Calendar period: 1979-2017/2018 (main analysis)			Calendar period: 1994-2017/2018 (sensitivity analysis)		
		HRs (95% CI)[a]			HRs (95% CI)[b]		
		VB-affected vs VB-unaffected pregnancy	VB-affected pregnancy vs termination	VB-affected pregnancy vs miscarriage	VB-affected vs VB-unaffected pregnancy	VB-affected pregnancy vs termination	VB-affected pregnancy vs miscarriage
All-cause mortality[c]	All pregnancies	1.14 (1.09-1.19)	0.85 (0.81-0.90)	0.85 (0.80-0.90)	1.00 (0.90-1.12)	0.74 (0.64-0.86)	0.72 (0.62-0.85)
	First pregnancy	1.19 (1.09-1.30)	0.83 (0.75-0.92)	0.88 (0.80-0.97)	1.12 (0.89-1.40)	0.83 (0.61-1.13)	0.83 (0.65-1.08)
Mortality from natural causes[d, e]	All pregnancies	1.15 (1.09-1.22)	0.86 (0.80-0.92)	0.88 (0.82-0.95)	0.78 (0.66-0.94)	0.78 (0.64-0.96)	0.78 (0.66-0.94)
	First pregnancy	1.17 (1.05-1.31)	0.80 (0.71-0.91)	0.86 (0.77-0.97)	-	-	-
Cancer	All pregnancies	1.07 (1.00-1.15)	0.82 (0.75-0.89)	0.90 (0.81-0.99)	0.90 (0.75-1.09)	0.68 (0.54-0.86)	0.69 (0.52-0.90)
	First pregnancy	1.09 (0.95-1.26)	0.73 (0.61-0.87)	0.85 (0.72-0.99)	-	-	-
Overall CVD	All pregnancies	1.15 (0.99-1.34)	0.84 (0.69-1.01)	0.82 (0.67-1.01)	1.08 (0.73-1.60)	0.95 (0.61-1.48)	0.85 (0.51-1.41)
	First pregnancy	1.04 (0.76-1.43)	0.70 (0.48-1.00)	0.80 (0.56-1.13)	-	-	-
CVD other than myocardial infarction and stroke	All pregnancies	1.11 (0.93-1.33)	0.83 (0.67-1.04)	0.83 (0.64-1.07)	1.24 (0.82-1.90)	1.16 (0.72-1.87)	1.10 (0.62-1.97)
	First pregnancy	1.06 (0.72-1.55)	0.77 (0.50-1.19)	0.90 (0.59-1.37)	-	-	-
Myocardial infarction	All pregnancies	1.15 (0.80-1.65)	0.97 (0.63-1.49)	0.98 (0.59-1.65)	0.34 (0.08-1.37)	0.46 (0.11-1.90)	0.28 (0.06-1.32)
	First pregnancy	0.85 (0.35-2.11)	0.74 (0.28-1.92)	0.79 (0.31-2.04)	-	-	-
Stroke	All pregnancies	1.29 (0.93-1.80)	0.73 (0.49-1.08)	0.70 (0.43-1.13)	0.98 (0.33-2.87)	0.50 (0.15-1.62)	0.33 (0.08-1.32)
	First pregnancy	1.15 (0.56-2.36)	0.42 (0.17-1.04)	0.54 (0.25-1.16)	-	-	-
Respiratory diseases	All pregnancies	1.53 (1.22-1.92)	1.02 (0.78-1.34)	0.99 (0.72-1.36)	1.42 (0.67-3.00)	1.42 (0.64-3.17)	1.27 (0.45-3.58)
	First pregnancy	1.68 (1.03-2.72)	0.97 (0.59-1.60)	0.90 (0.54-1.49)	-	-	-
Endocrine, nutritional, metabolic conditions	All pregnancies	1.29 (0.89-1.86)	1.16 (0.77-1.77)	0.96 (0.61-1.52)	2.04 (0.89-4.67)	2.01 (0.78-5.18)	1.89 (0.69-5.20)
	First pregnancy	1.95 (1.11-3.44)	1.45 (0.75-2.79)	1.12 (0.60-2.12)	-	-	-

Outcome	Population	Calendar period: 1979-2017/2018 (main analysis)			Calendar period: 1994-2017/2018 (sensitivity analysis)		
		HRs (95% CI)[a]			HRs (95% CI)[b]		
		VB-affected vs VB-unaffected pregnancy	VB-affected pregnancy vs termination	VB-affected pregnancy vs miscarriage	VB-affected vs VB-unaffected pregnancy	VB-affected pregnancy vs termination	VB-affected pregnancy vs miscarriage
Nervous system conditions	All pregnancies	1.19 (0.89-1.61)	0.75 (0.52-1.08)	1.06 (0.71-1.60)	0.85 (0.34-2.12)	0.77 (0.29-2.06)	1.90 (0.63-5.77)
	First pregnancy	0.97 (0.52-1.79)	0.66 (0.33-1.29)	0.66 (0.35-1.24)	-	-	-
Mortality from non-natural causes[d, e]	All pregnancies	1.27 (1.08-1.48)	0.86 (0.71-1.03)	0.90 (0.73-1.11)	1.04 (0.72-1.51)	0.77 (0.51-1.17)	0.90 (0.54-1.50)
	First pregnancy	1.37 (1.02-1.85)	1.09 (0.79-1.50)	1.10 (0.79-1.52)	-	-	-
Motor vehicle accidents	All pregnancies	1.00 (0.71-1.41)	1.04 (0.70-1.56)	0.93 (0.57-1.50)	1.11 (0.59-2.11)	1.01 (0.49-2.07)	1.08 (0.43-2.74)
	First pregnancy	1.02 (0.51-2.03)	1.32 (0.65-2.70)	1.21 (0.58-2.53)	-	-	-
Non-motor vehicle accidents or violence	All pregnancies	1.43 (1.13-1.81)	0.83 (0.63-1.09)	0.81 (0.58-1.12)	1.00 (0.54-1.84)	0.78 (0.42-1.45)	1.11 (0.50-2.49)
	First pregnancy	1.52 (0.96-2.43)	1.11 (0.67-1.84)	1.14 (0.69-1.87)	-	-	-
Suicides	All pregnancies	1.36 (1.05-1.76)	0.84 (0.62-1.14)	1.10 (0.78-1.56)	1.01 (0.54-1.90)	0.57 (0.26-1.21)	0.47 (0.21-1.01)
	First pregnancy	1.58 (0.98-2.54)	1.00 (0.59-1.69)	1.08 (0.64-1.82)	-	-	-
Other	All pregnancies	1.35 (1.18-1.53)	0.91 (0.78-1.06)	0.78 (0.65-0.94)	1.11 (0.78-1.58)	0.76 (0.50-1.16)	0.66 (0.41-1.05)
	First pregnancy	1.39 (1.08-1.80)	1.00 (0.75-1.33)	0.94 (0.71-1.25)	-	-	-

[a] Analyses were adjusted via IPTW for age at the pregnancy end, calendar year of pregnancy, reproductive history and pre-existing comorbidities (obesity, thyroid disorders, chronic obstructive pulmonary disease, chronic liver and kidney disease, hyperlipidaemia, hypercholesterolemia, cardiovascular, and metabolic diseases), and psychiatric conditions. Analyses of pregnancies ending in childbirth were additionally adjusted for placenta-related conditions at the index pregnancy.

[b] Analyses were additionally adjusted for medication use any time before 12 gestational weeks for pregnancies ending in childbirth or index date for pregnancies ending in termination or miscarriage. Medication dispensing data were available from the Danish National Prescription Registry starting from 1995. Analyses including the first identifiable pregnancy of a woman were not performed due to the small number of accrued events

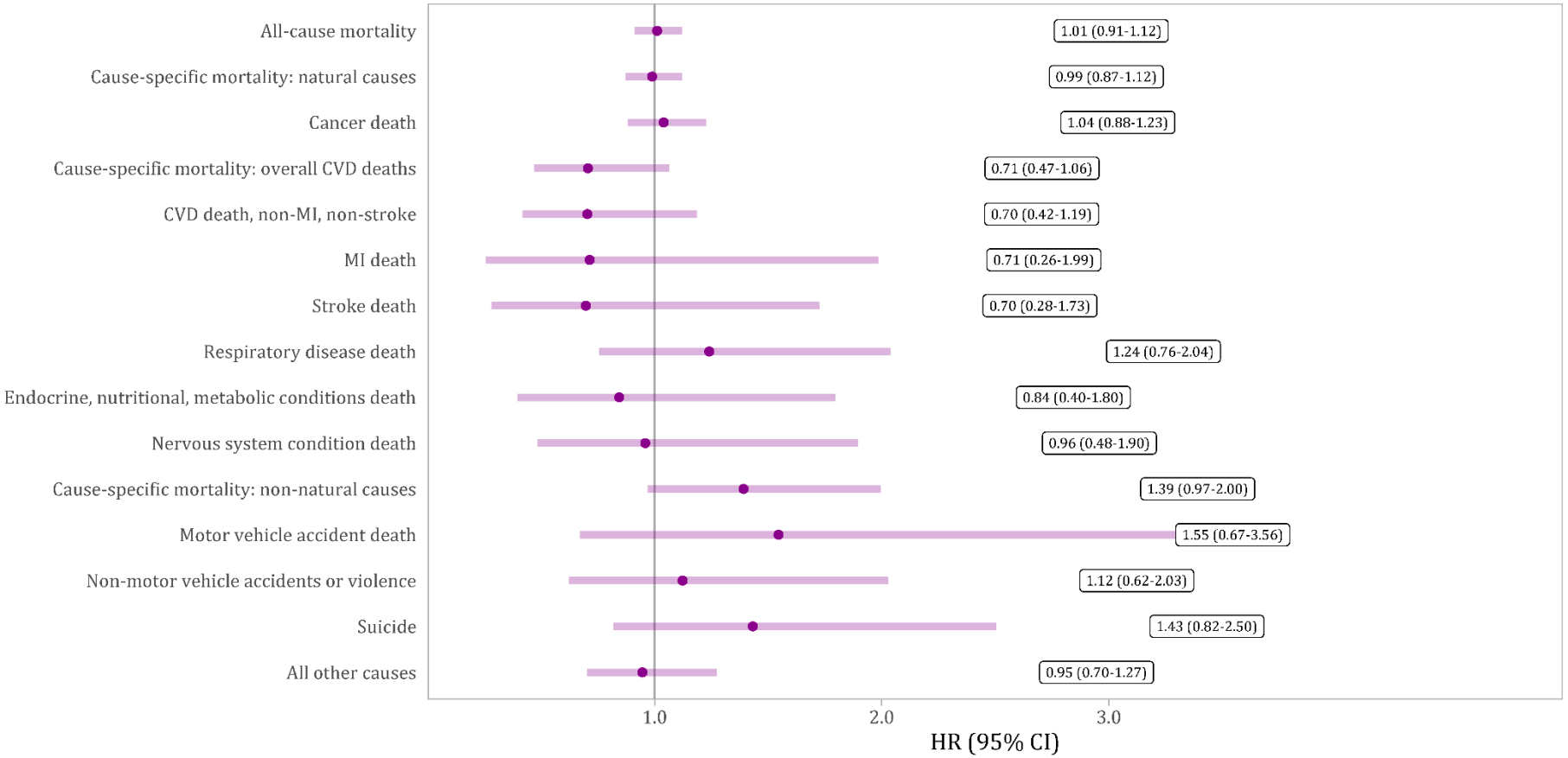
[c] All-cause mortality data were available through 2018

[d] Cause-specific mortality data were available through 2017

[e] The list of ICD-8 and ICD-10 codes for the cause-specific mortality outcomes is available in Appendix II, Table 1 in Supplementary data

Abbreviations: CI, Confidence interval; CVD, Cardiovascular diseases; HR, Hazard ratio; VB, Vaginal bleeding

Figure 13. Association between vaginal bleeding-affected pregnancy and mortality in women with one childbirth before 35 years



CVD, cardiovascular diseases; MI, myocardial infarction; VB, vaginal bleeding
By design, women having VB-exposed and VB-unexposed pregnancy and not surviving or emigrating before 35 years of age are excluded
Follow-up starts at 35 years of age in VB-exposed and VB-unexposed cohorts and stops at the date of emigration or death
Study period: 1979-2017/2018

5.3. Study III

We identified 1,901,725 pregnancies (among 903,327 women) recorded in 1994-2017. Of all pregnancies, 2.1% (N=39,265) were VB-affected and ended in a delivery, 73.1% (N=1,389,285) were VB-unaffected and ended in a delivery, 17.6% (N=333,785) ended in a termination, and 7.3% (N=139,390) ended in a miscarriage. Similarly to study II, women's pre-pregnancy history of chronic conditions was rare. The pre-pregnancy psychiatric conditions were more prevalent among women with VB-affected pregnancy (13.0%), pregnancy ending in a termination (18.0%), or miscarriage (12.0%) than among women with VB-unaffected pregnancy (9.8%). Women with VB-affected pregnancy (28.0%) and pregnancy ending in a termination (15.0%) or miscarriage (30.0%) were also less likely to have achieved higher education than women with VB-affected pregnancy (34.0%). All cohorts but women with pregnancy ending in a termination had a nearly equal distribution of year-specific income in quartiles (Appendix III, Table 1).[154]

The median follow-up (25-75 percentile) in this study was 13.3 (7.2-19.2) years and varied slightly by the on-study outcome. At the end of follow-up, among women with VB-affected pregnancy there were 365 events of diabetes type 1 (incidence rate: 6.1, 95% CI: 5.5-6.8 per 10,000 PY); 1,425 events of diabetes type 2 (24.4, 95% CI: 23.1-25.6); 2,120 events of hypertension (36.5, 95% CI: 34.9-38.0); 720 events of ischaemic heart disease (12.2, 95% CI: 11.4-13.1), including 180 events of myocardial infarction (3.0, 95% CI: 2.6-3.5); 235 events of atrial fibrillation or flutter (4.0, 95% CI: 3.5-4.5); 100 events of heart failure (1.7, 95% CI: 1.4-2.0); 390 ischaemic stroke events (6.6, 95% CI: 5.9-7.2) and 165 haemorrhagic stroke events (2.8, 95% CI: 2.4-3.3). Incidence rates were similar although numerically lower for women with VB-unaffected pregnancy (Appendix III, Table 2).

Crude associations between VB in pregnancy and CVD outcomes across comparators are presented in Figure 14. In analyses allowing for multiple pregnancies of the same woman, VB-affected pregnancy was associated with a 15% increased hazard of diabetes mellitus type 1 (IPTW adjusted HR [aHR]: 1.15, 95% CI: 1.03-1.28), 19% increased hazard of diabetes type 2

(IPTW aHR: 1.19, 1.13-1.26), hypertension (IPTW aHR: 1.19, 1.14-1.25), 26% increased hazard of ischaemic heart disease (IPTW aHR: 1.26, 1.16-1.37) and 21% increased hazard of specifically myocardial infarction (IPTW aHR: 1.21, 1.03-1.42), 32% increased hazard of atrial fibrillation or flutter (IPTW aHR: 1.32, 1.14-1.51), 23% increased hazard of heart failure (IPTW aHR: 1.23, 0.99-1.52), 41% increased hazard of ischaemic (IPTW aHR: 1.41, 1.26-1.57) and 46% increased hazard of haemorrhagic stroke (IPTW aHR: 1.46, 1.23-1.72) when compared with women having VB-unaffected pregnancy (Figure 15a). The elevated hazards for the outcomes were observed across analyses restricted to the first identifiable pregnancy per woman and analyses using conventional multivariable Cox proportional hazards regression to adjust for measured confounding.

Comparisons with women having pregnancy ending in a termination showed increased hazards of 25-29% for diabetes mellitus type 1; 33-37% for diabetes mellitus type 2, 16-35% for hypertension, 19-20% for ischaemic heart disease, and 7-18% for ischaemic or haemorrhagic stroke (Figure 15b).

When we contrasted the long-term risk of cardiovascular outcomes in women following VB-affected pregnancy with that following pregnancy ending in a miscarriage, the HRs were below unity or unity for all outcomes of interest across analyses of all and first identifiable pregnancy of a woman (Figure 15c). Results were largely consistent across a set of sensitivity analyses (Appendix III, Table 4 and Table 5 in the Supplementary material). Furthermore, the investigation of additional cardiovascular outcomes among women having a pregnancy recorded between 1979 through 1993 and maximum follow-up of 40 years showed 28% and 20% increased risk of coronary artery bypass grafting and percutaneous coronary intervention, respectively, for women with VB-affected vs VB-unaffected pregnancy; the associations remained following a first identifiable pregnancy of a woman (Appendix III, Table 4 in the Supplementary material).

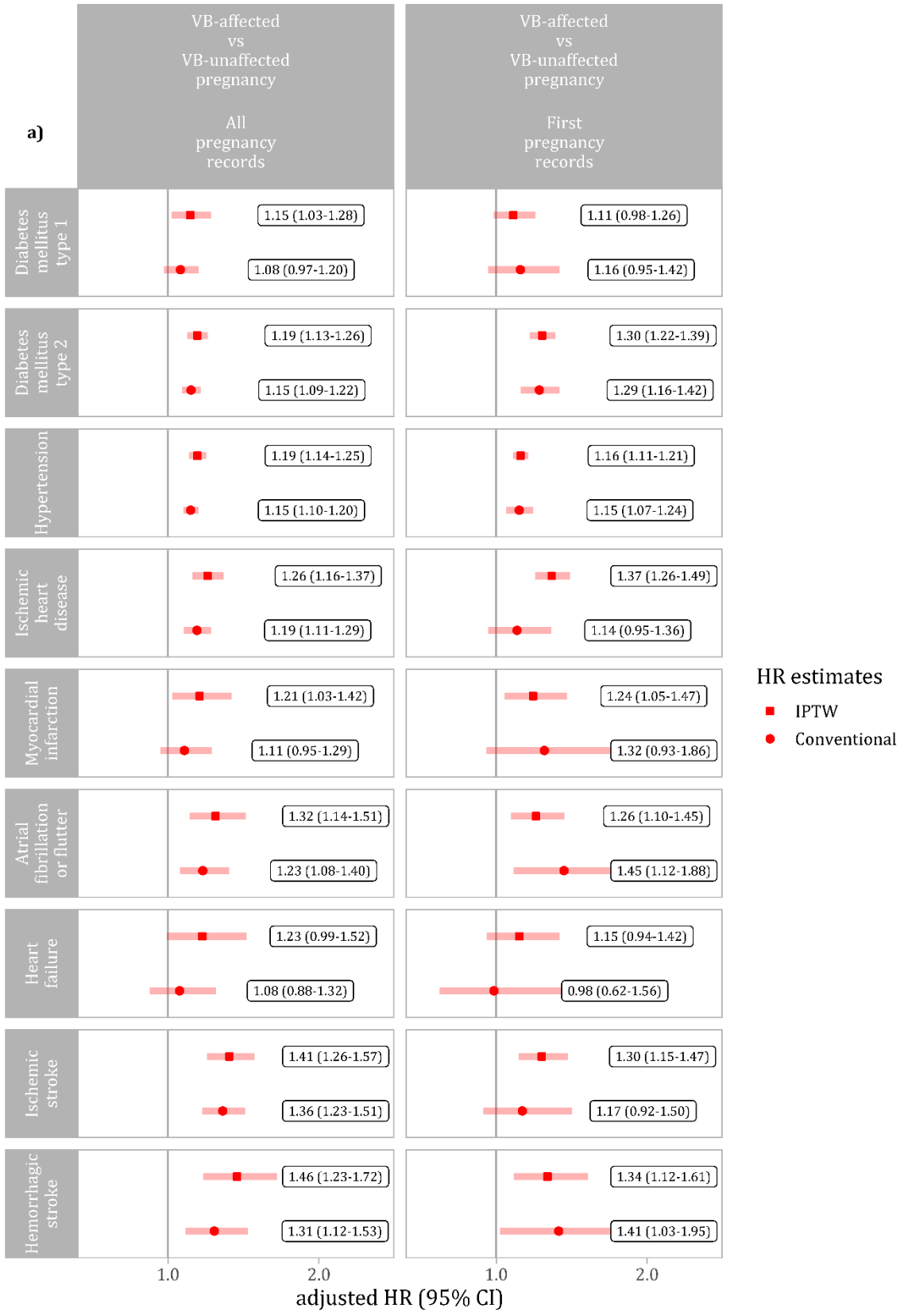
In landmark analyses, women with one VB-affected vs VB-unaffected pregnancy ending in childbirth before the age of 40 were at a 1.2 to 1.5-fold increased risk of diabetes type 2 and multiple CVD outcomes in line with the results of the main analyses (Figure 16).

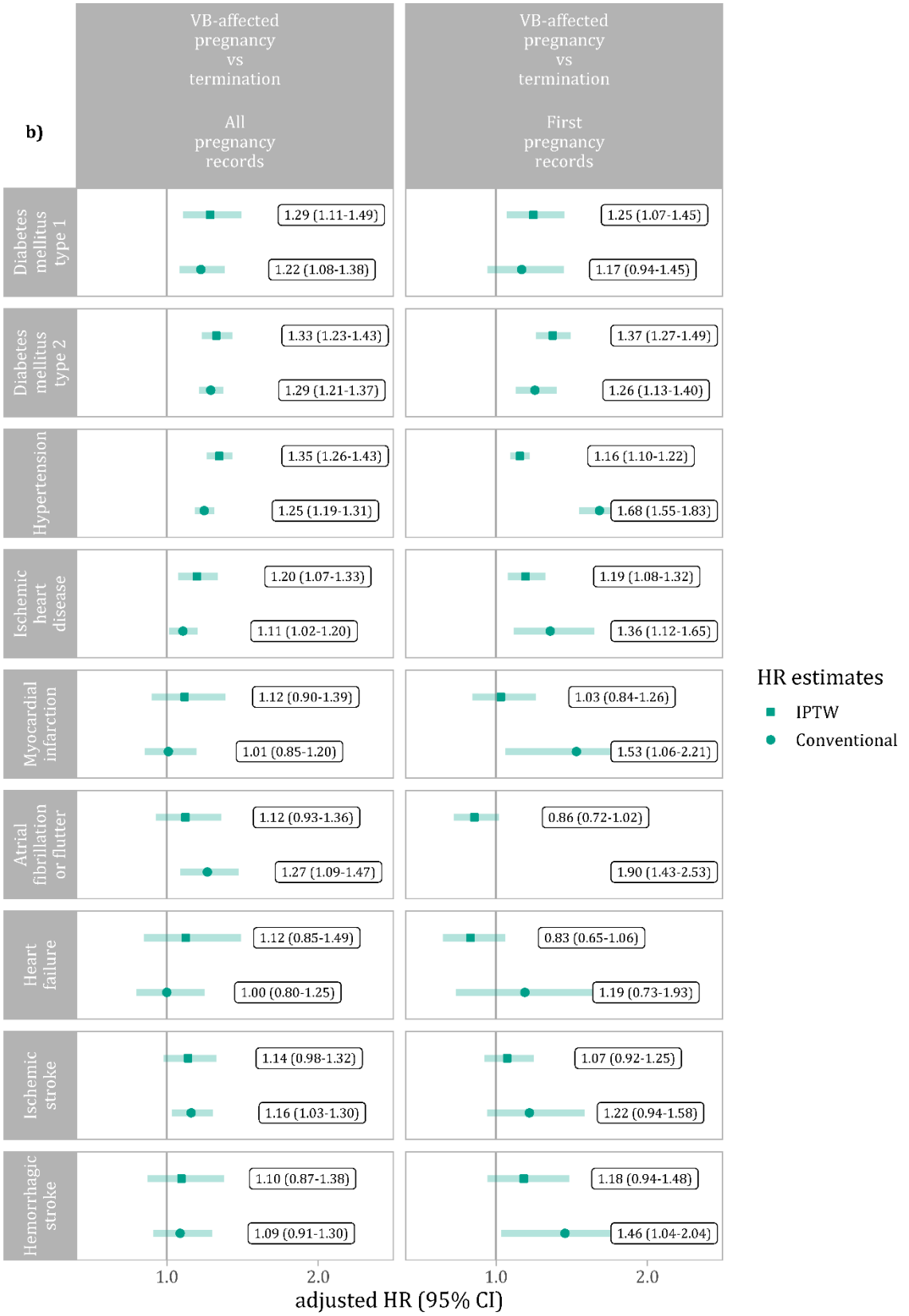
Figure 14. Crude associations between vaginal bleeding-affected pregnancy and cardiovascular outcomes in women: vaginal bleeding-affected vs vaginal bleeding-unaffected pregnancy (a), vaginal bleeding-affected pregnancy vs termination (b), vaginal bleeding-affected pregnancy vs miscarriage (c)

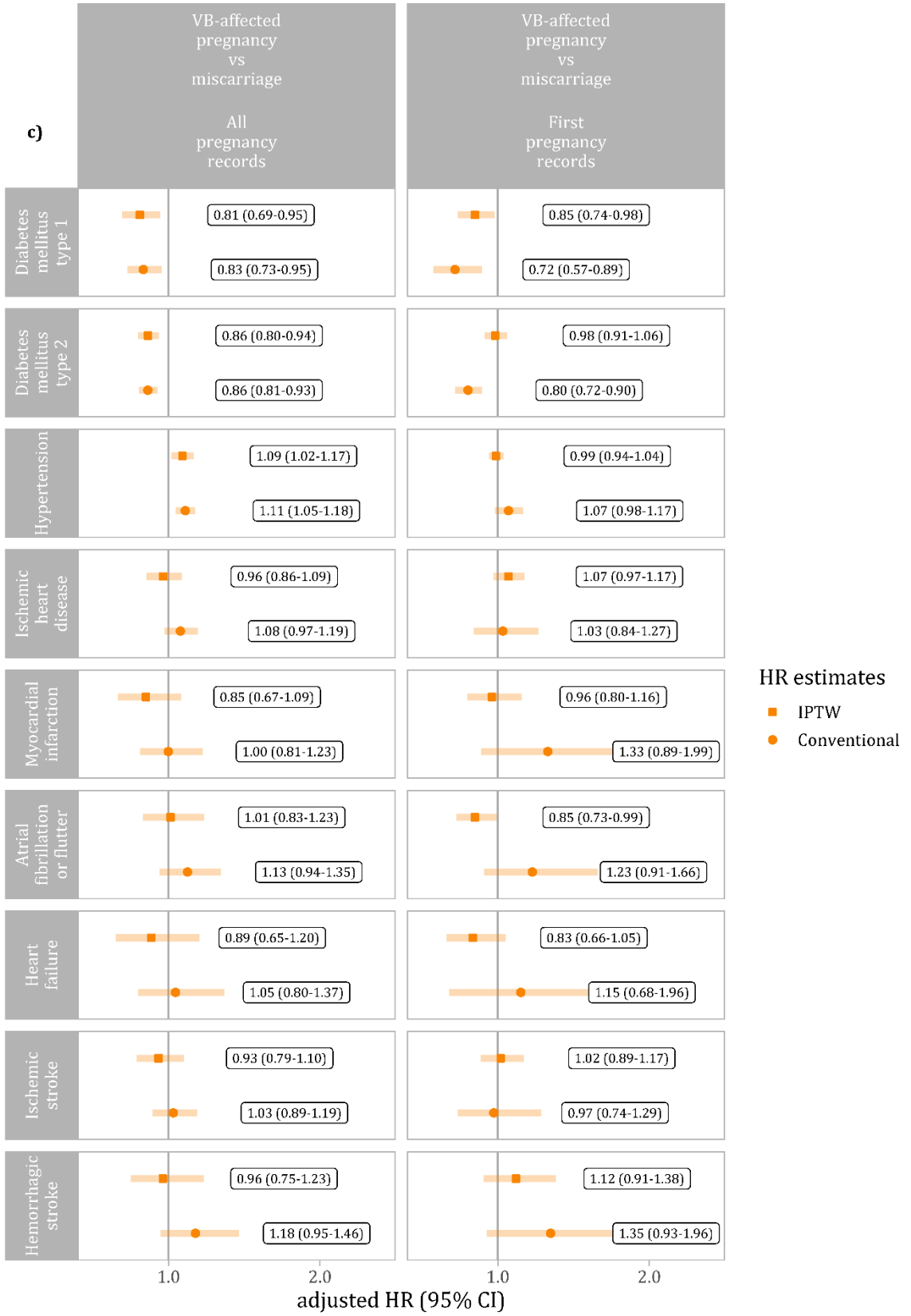

b)
VB-affected pregnancy vs termination
All pregnancy records
VB-affected pregnancy vs termination
First pregnancy records
Diabetes mellitus type 1
1.08 (0.97-1.20)
1.24 (1.01-1.53)
Diabetes mellitus type 2
1.19 (1.12-1.25)
1.40 (1.26-1.56)
Hypertension
1.29 (1.23-1.35)
1.83 (1.69-1.99)
Ischemic heart disease
1.07 (0.99-1.15)
1.41 (1.17-1.71)
Myocardial infarction
0.99 (0.84-1.15)
1.56 (1.09-2.25)
Atrial fibrillation or flutter
1.22 (1.06-1.40)
2.02 (1.52-2.67)
Heart failure
0.92 (0.75-1.14)
1.18 (0.73-1.90)
Ischemic stroke
1.13 (1.02-1.26)
1.30 (1.00-1.68)
Hemorrhagic stroke
1.05 (0.89-1.23)
1.43 (1.02-2.01)
1.0
2.0
1.0
2.0
crude HR (95% CI)


c)
VB-affected pregnancy vs miscarriage
All pregnancy records
VB-affected pregnancy vs miscarriage
First pregnancy records
Diabetes mellitus type 1
0.79 (0.70-0.88)
0.75 (0.60-0.93)
Diabetes mellitus type 2
0.88 (0.83-0.93)
0.85 (0.76-0.95)
Hypertension
1.03 (0.98-1.08)
1.10 (1.01-1.20)
Ischemic heart disease
1.01 (0.93-1.10)
1.04 (0.85-1.27)
Myocardial infarction
0.97 (0.82-1.15)
1.30 (0.87-1.94)
Atrial fibrillation or flutter
1.08 (0.93-1.25)
1.23 (0.91-1.66)
Heart failure
0.93 (0.74-1.17)
1.16 (0.69-1.96)
Ischemic stroke
1.15 (1.02-1.29)
1.00 (0.76-1.32)
Hemorrhagic stroke
1.18 (0.99-1.41)
1.33 (0.92-1.93)
1.0
2.0
1.0
2.0
crude HR (95% CI)

Figure 15. Adjusted associations between vaginal bleeding-affected pregnancy and cardiovascular outcomes in women: vaginal bleeding-affected vs vaginal bleeding-unaffected pregnancy (a), vaginal bleeding-affected pregnancy vs termination (b), vaginal bleeding-affected pregnancy vs miscarriage (c)

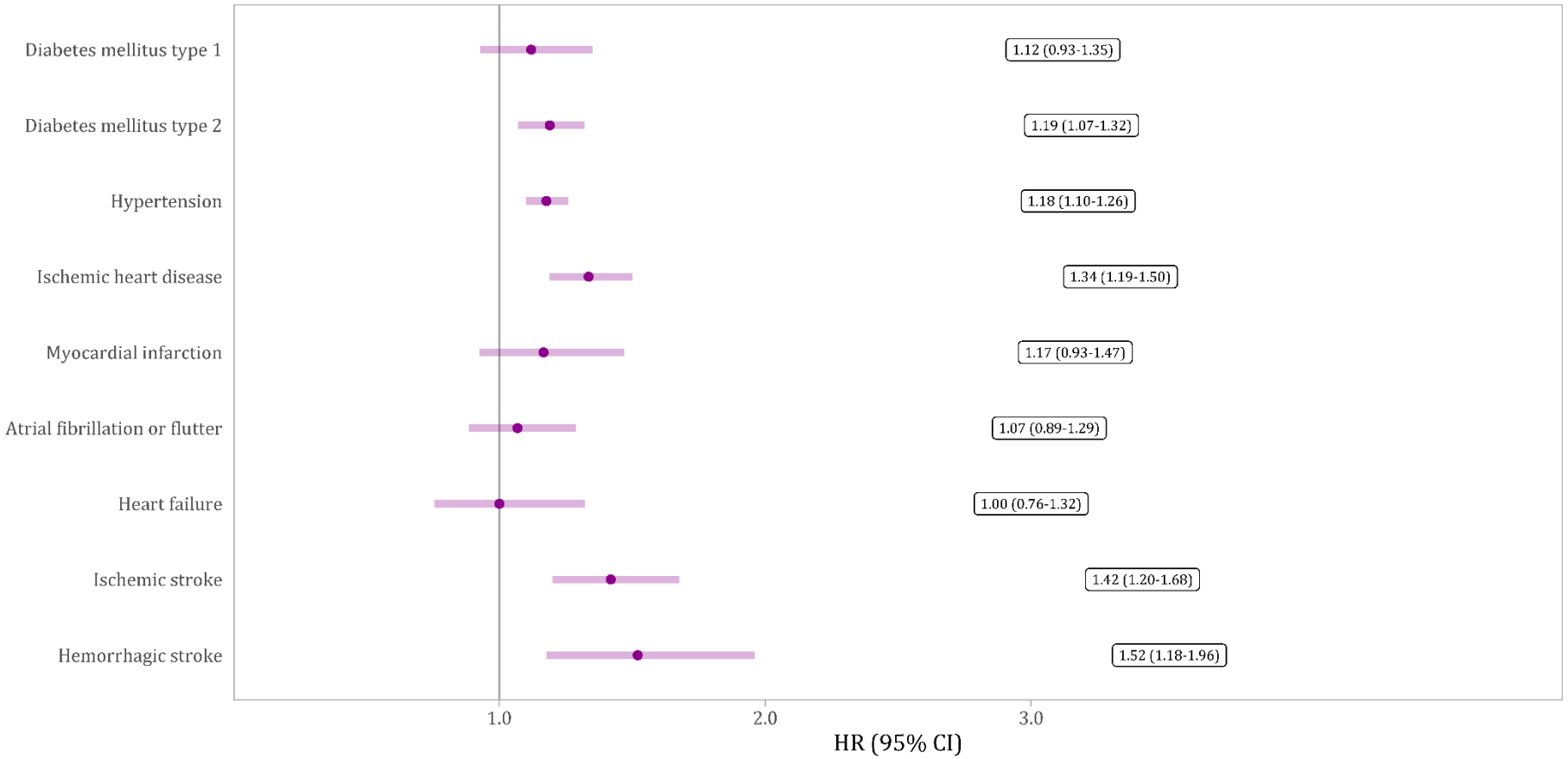



b)
VB-affected pregnancy vs termination
All pregnancy records
VB-affected pregnancy vs termination
First pregnancy records
Diabetes mellitus type 1
1.29 (1.11-1.49)
1.22 (1.08-1.38)
1.25 (1.07-1.45)
1.17 (0.94-1.45)
Diabetes mellitus type 2
1.33 (1.23-1.43)
1.29 (1.21-1.37)
1.37 (1.27-1.49)
1.26 (1.13-1.40)
Hypertension
1.35 (1.26-1.43)
1.25 (1.19-1.31)
1.16 (1.10-1.22)
1.68 (1.55-1.83)
Ischemic heart disease
1.20 (1.07-1.33)
1.11 (1.02-1.20)
1.19 (1.08-1.32)
1.36 (1.12-1.65)
Myocardial infarction
1.12 (0.90-1.39)
1.01 (0.85-1.20)
1.03 (0.84-1.26)
1.53 (1.06-2.21)
Atrial fibrillation or flutter
1.12 (0.93-1.36)
1.27 (1.09-1.47)
0.86 (0.72-1.02)
1.90 (1.43-2.53)
Heart failure
1.12 (0.85-1.49)
1.00 (0.80-1.25)
0.83 (0.65-1.06)
1.19 (0.73-1.93)
Ischemic stroke
1.14 (0.98-1.32)
1.16 (1.03-1.30)
1.07 (0.92-1.25)
1.22 (0.94-1.58)
Hemorrhagic stroke
1.10 (0.87-1.38)
1.09 (0.91-1.30)
1.18 (0.94-1.48)
1.46 (1.04-2.04)
1.0
2.0
1.0
2.0
adjusted HR (95% CI)
HR estimates
IPTW
Conventional



Abbreviations: CI, Confidence interval; HR, Hazard ratio; IPTW, Inverse probability of treatment weighting

Figure 16. Adjusted association between vaginal bleeding-affected pregnancy and cardiovascular outcomes in women with one childbirth before the age of 40 years



CVD, cardiovascular diseases; MI, myocardial infarction; VB, vaginal bleeding
By design, women with VB-exposed and VB-unexposed pregnancy not surviving, emigrating or experiencing CVD outcome before 40 years of age are excluded
Follow-up starts at 40 years of age in VB-exposed and VB-unexposed cohorts and stops at the earliest of CVD outcome, emigration, or death
Study period: 1979-2018

5.4. Study IV

We identified 1,862,128 recorded pregnancies among 887,801 women in 1995-2017. Of all identified pregnancies, 37,082 were VB-affected childbirths, 1,363,614 were VB-unaffected pregnancies, 324,328 were terminations, and 137,104 were miscarriages. At baselibe, women with VB-affected pregnancy were less likely than women with VB-unaffected pregnancy to have a higher level of attained education (29% and 35%), were more likely to be smokers (19% vs 15%), to have had at least one VB episode (13% vs 5%), and miscarriage (22% vs 12%). Compared with all other pregnancy cohorts, women with pregnancy ending in a termination were more likely to be younger, unmarried, and unemployed with a lower level of the highest attained education and in the first income quartile (Appendix IV, Table 1).[155]

At the end of the 24-year follow-up period, we observed 1,320 events of any cancer among women with VB-affected pregnancy; 40,420 events among women with VB-unaffected pregnancy; 10,300 events among women with termination, and 4,790 events among women with a miscarriage (Appendix IV, Table 2). The cumulative incidence of any cancer at end of the follow-up period following VB-affected and VB-unaffected pregnancy was 7.4% and 7.5%, respectively, while the cumulative incidence for women with pregnancy ending in a termination or miscarriage was 7.3% and 7.9%, respectively (Appendix IV). The cumulative incidence of hormone-related cancers was 3.2% (60 events), 3.0% (1,585 events), 2.6% (1,340 events), and 3.0% (535 events) following VB-affected, VB-unaffected, termination, or miscarriage, respectively; of haematological cancers was 0.4% for all pregnancy cohorts with 65 events following VB-affected pregnancy, 2,025 following VB-unaffected pregnancy, 580 events following a miscarriage and 260 events following a termination. The cumulative incidence of immune-related cancers was 1.7% (360 events) following VB-affected pregnancy, 1.9% (11,660 events) following VB-unaffected pregnancy, 1.8% (2,915 events) following a termination, and 1.7% (1,175 events) following a miscarriage. Smoking or alcohol-related cancers were rare with 0.5% cumulative incidence at the end of the follow-up among both women with VB-affected (65 events) and VB-unaffected (1,885 events) pregnancy, 0.8% (770 events) among women with a

termination, and 0.6% (300 events) among women with a miscarriage. The cumulative incidence of obesity-related cancers was 0.9% (160 events) at the end of follow-up among women with VB-affected pregnancy, 1.1% (4,855 events) among women with a VB-unaffected pregnancy, 1.1% (1,315 events) among women with a termination, and 1.3% (615 events) among women with a miscarriage. The cumulative incidence of cancers of neurological origin and other cancers was <1.0% among all pregnancy cohorts.

The incidence rate per 10,000 PYs of premenopausal breast cancer was 9.2 (95% CI: 8.4-10.1) with 480 events following VB-affected pregnancy, 8.7 (95% CI: 8.6-8.9) with 14,655 events following VB-unaffected pregnancy, 7.7 (95% CI: 7.4-8.0) with 3,315 events following a termination, and 9.9 (95% CI: 9.5-10.4) with 1,770 events following a miscarriage. For cervical cancer, the incidence rate per 10,000 PYs was 2.0 (95% CI: 1.6-2.4) with 100 events following VB-affected pregnancy, 2.1 (95% CI: 2.0-2.1) with 3,455 events following VB-unaffected pregnancy, 2.2 (95% CI: 2.1-2.4) with 955 events following a termination, and 1.8 (95% CI: 1.6-2.0) with 320 events following a miscarriage. For ovary and fallopian tube cancer, the incidence rate per 10,000 PYs was 0.6 (95% CI: 0.4-0.9) with 30 events following VB-affected childbirth, 0.5 (95% CI: 0.4-0.5) with 770 events following VB-unaffected pregnancy, 0.5 (95% CI: 0.4-0.6) with 220 events following a termination, and 0.6 (95% CI: 0.5-0.8) with 110 events following a miscarriage. Uterine cancer was rare with an incidence rate of 0.3 (95% CI: 0.2-0.4) per 10,000 PYs (15 events) following VB-affected childbirth, 0.3 (95% CI: 0.3-0.3) per 10,000 PYs (500 events) following VB-unaffected pregnancy, 0.3 (95% CI: 0.2-0.3) per 10,000 PYs (110 events) following a terminations, and 0.4 (95% CI: 0.3-0.5) per 10,000 PYs (65 events) following a miscarriage (Appendix IV, Table 2).

Table 10 shows the crude HRs for associations between VB in pregnancy and cancer. Adjusted HRs for any cancer in the VB-affected pregnancy cohort indicated no association in analyses with all comparators and were 1.03 (95% CI: 0.97-1.08) vs VB-unaffected pregnancy cohort, 1.03 (95% CI: 0.97-1.09) vs termination cohort, and 0.90 (95% CI: 0.84-0.95) vs miscarriage

cohort (Table 11). The results suggested no association between having VB-affected pregnancy and subsequent risk of hormone-related, haematological, immune-related, obesity-related, cancers of neurological origin, or other cancers when contrasted with having a VB-unaffected pregnancy as well as a termination or miscarriage in analyses of all identifiable pregnancies of a woman (Table 11). While there was no association between VB and smoking- or alcohol-related cancers in the analyses of all identifiable pregnancies of a woman (HR: 0.98, 95% CI: 0.76-1.25), there was an association in the analyses of a first VB-affected vs VB-unaffected pregnancy of a woman (HR: 1.29, 95% CI: 0.78-2.13; Table 11).

Table 10. Crude associations between vaginal bleeding-affected pregnancy and groups of site-specific cancers in women, 1995-2018

		VB-affected vs VB-unaffected pregnancy	VB-affected pregnancy vs termination	VB-affected pregnancy vs miscarriage
Outcome	**Observations**	**HR (95% CI)**		
Any cancer	All identifiable pregnancies	1.02 (0.96-1.08)	1.05 (0.99-1.11)	0.91 (0.86-0.97)
	First pregnancy	1.09 (0.98-1.21)	1.51 (1.35-1.69)	1.08 (0.96-1.22)
Hormone-related cancers	All identifiable pregnancies	1.02 (0.93-1.11)	1.18 (1.07-1.29)	0.90 (0.81-0.99)
	First pregnancy	1.11 (0.93-1.32)	1.89 (1.57-2.28)	1.06 (0.87-1.29)
Haematological cancers	All identifiable pregnancies	0.98 (0.76-1.26)	0.89 (0.69-1.16)	0.80 (0.61-1.05)
	First pregnancy	0.99 (0.60-1.63)	1.23 (0.73-2.08)	1.02 (0.58-1.79)
Immune-related cancers	All identifiable pregnancies	0.98 (0.88-1.09)	1.02 (0.91-1.13)	1.03 (0.92-1.16)
	First identifiable	1.06 (0.88-1.29)	1.26 (1.03-1.54)	1.23 (0.99-1.54)
Smoking or alcohol-related cancers	All identifiable pregnancies	1.09 (0.86-1.39)	0.71 (0.56-0.92)	0.72 (0.55-0.94)
	First identifiable	1.45 (0.88-2.39)	1.63 (0.96-2.78)	1.07 (0.61-1.89)
Obesity-related cancers	All identifiable pregnancies	1.01 (0.86-1.19)	0.99 (0.84-1.16)	0.85 (0.71-1.01)
	First identifiable	1.15 (0.84-1.57)	1.80 (1.29-2.51)	1.04 (0.73-1.47)
Cancers of neurological origin	All identifiable pregnancies	1.11 (0.93-1.33)	1.12 (0.93-1.35)	0.98 (0.81-1.20)
	First identifiable	0.98 (0.68-1.42)	1.18 (0.80-1.73)	0.87 (0.58-1.31)
Other cancers	All identifiable pregnancies	1.03 (0.71-1.48)	0.99 (0.68-1.45)	0.89 (0.59-1.33)
	First identifiable	0.90 (0.43-1.91)	1.23 (0.56-2.70)	0.97 (0.42-2.23)

Table 11. Adjusted associations between vaginal bleeding-affected pregnancy and groups of site-specific cancers in women, 1995-2018[155]

		VB-affected vs VB-unaffected pregnancy[a]	VB-affected pregnancy vs termination[b]	VB-affected pregnancy vs miscarriage[b]
Outcome	**Observations**	**HR (95% CI)**		
Any cancer	All identifiable pregnancies	1.03 (0.97-1.08)	1.03 (0.97-1.09)	0.90 (0.84-0.95)
	First pregnancy	1.06 (0.95-1.18)	1.03 (0.91-1.17)	1.11 (0.98-1.25)
Hormone-related cancers	All identifiable pregnancies	1.02 (0.94-1.12)	1.01 (0.91-1.12)	0.98 (0.87-1.10)
	First pregnancy	1.06 (0.89-1.26)	1.08 (0.88-1.33)	1.08 (0.88-1.33)
Haematological cancers	All identifiable pregnancies	0.95 (0.74-1.23)	0.91 (0.69-1.20)	0.85 (0.61-1.17)
	First pregnancy	0.96 (0.58-1.57)	0.83 (0.47-1.47)	1.08 (0.60-1.91)
Immune-related cancers	All identifiable pregnancies	1.02 (0.92-1.14)	0.90 (0.80-1.02)	1.06 (0.92-1.22)
	First identifiable	1.06 (0.87-1.28)	0.97 (0.78-1.21)	1.22 (0.97-1.54)
Smoking or alcohol-related cancers	All identifiable pregnancies	0.98 (0.76-1.25)	0.84 (0.65-1.09)	0.77 (0.57-1.04)
	First identifiable	1.29 (0.78-2.13)	1.19 (0.67-2.10)	1.25 (0.70-2.24)
Obesity-related cancers	All identifiable pregnancies	1.01 (0.86-1.18)	0.99 (0.83-1.18)	0.88 (0.72-1.08)
	First identifiable	1.11 (0.81-1.51)	1.10 (0.76-1.60)	1.07 (0.74-1.54)
Cancers of neurological origin	All identifiable pregnancies	1.14 (0.96-1.36)	1.21 (0.99-1.48)	1.00 (0.79-1.27)
	First identifiable	1.00 (0.69-1.45)	1.04 (0.68-1.59)	0.89 (0.58-1.35)
Other cancers	All identifiable pregnancies	1.00 (0.70-1.44)	0.97 (0.64-1.47)	0.99 (0.61-1.61)
	First identifiable	0.90 (0.42-1.90)	1.62 (0.68-3.85)	1.15 (0.48-2.74)

[a] Analyses were adjusted for age, calendar year of pregnancy end, women's parity and number of previous identifiable pregnancies, socioeconomic factors (education, employment, and year-specific income level divided into quartiles based on all included pregnancies), smoking in pregnancy, reproductive history (pre-index date vaginal bleeding, termination, or miscarriages, placental complication in previous and current deliveries), history of chronic somatic conditions, any psychiatric condition, and use of NSAIDs [lookback of 365 days] and steroids for systemic use [all available lookback] before the 12 weeks of gestation for VB-affected and VB-unaffected pregnancies and before the index date for terminations and miscarriages

[b] Analyses could not be adjusted for smoking in pregnancy since the data were unavailable for pregnancies ending in a termination or miscarriage

For women having VB-affected vs VB-unaffected pregnancy, we found no increased hazard of premenopausal breast cancer (HR: 1.01, 95% CI: 0.92-1.11), cervical cancer (HR: 0.95, 0.78-1.16), ovarian and fallopian tube cancer (HR: 1.20, 0.84-1.71), and uterine cancer (HR: 0.83, 0.49-1.42) (Table 12).

Table 12. Crude and adjusted associations between vaginal bleeding-affected pregnancy and selected site-specific cancers in women, 1995-2018[155]

		VB-affected vs VB-unaffected pregnancy[a,b]	VB-affected pregnancy vs termination[a,c]	VB-affected pregnancy vs miscarriage[a,c]
Outcome	**Model**	**HR (95% CI)**		
Breast cancer, premenopausal	Crude	1.00 (0.92-1.10)	1.17 (1.06-1.29)	0.89 (0.81-0.99)
	Adjusted	1.01 (0.92-1.11)	1.07 (0.97-1.18)	1.04 (0.93-1.17)
Cervical cancer	Crude	0.95 (0.78-1.16)	0.88 (0.72-1.08)	1.09 (0.87-1.37)
	Adjusted	0.95 (0.78-1.16)	0.82 (0.66-1.02)	1.01 (0.77-1.32)
Ovary and fallopian tube cancer	Crude	1.28 (0.90-1.83)	1.21 (0.84-1.76)	0.96 (0.64-1.42)
	Adjusted	1.20 (0.84-1.71)	0.96 (0.65-1.42)	0.88 (0.56-1.38)
Uterine cancer	Crude	0.85 (0.50-1.45)	1.01 (0.58-1.76)	0.70 (0.40-1.26)
	Adjusted	0.83 (0.49-1.42)	0.96 (0.54-1.72)	0.77 (0.40-1.48)

[a] Computed using all identifiable pregnancies of a woman
[b] Adjusted for age, calendar year of pregnancy end, women's parity and number of previous identifiable pregnancies, socioeconomic factors (education, employment, and year-specific income level divided into quartiles based on all included pregnancies), smoking in pregnancy, reproductive history (pre-index date vaginal bleeding, termination, or miscarriages, placental complication in previous and current deliveries), history of chronic somatic conditions, any psychiatric condition, and use of NSAIDs and steroids for systemic use before the 12 weeks of gestation for VB-affected and VB-unaffected pregnancies and before the index date for terminations and miscarriages
[c] Analyses could not be adjusted for smoking in pregnancy since the data were unavailable for pregnancies ending in termination or miscarriage
Abbreviations: CI, Confidence interval; HR, Hazard ratio; VB, vaginal bleeding

The results of a sensitivity analysis of a subset of women with pregnancies in 2004-2017 while additionally controlling for women's BMI was in line with the main analyses and suggested no association between VB-affected pregnancy and any cancer. HRs for groups of site-specific cancers as well as individual cancers at specific sites were close to the null value of 1 with wide 95% CIs in this analysis due to the small number of accrued events (Table 13 and Appendix IV, Table 4 and Table 5 in the Electronic Supplementary material).

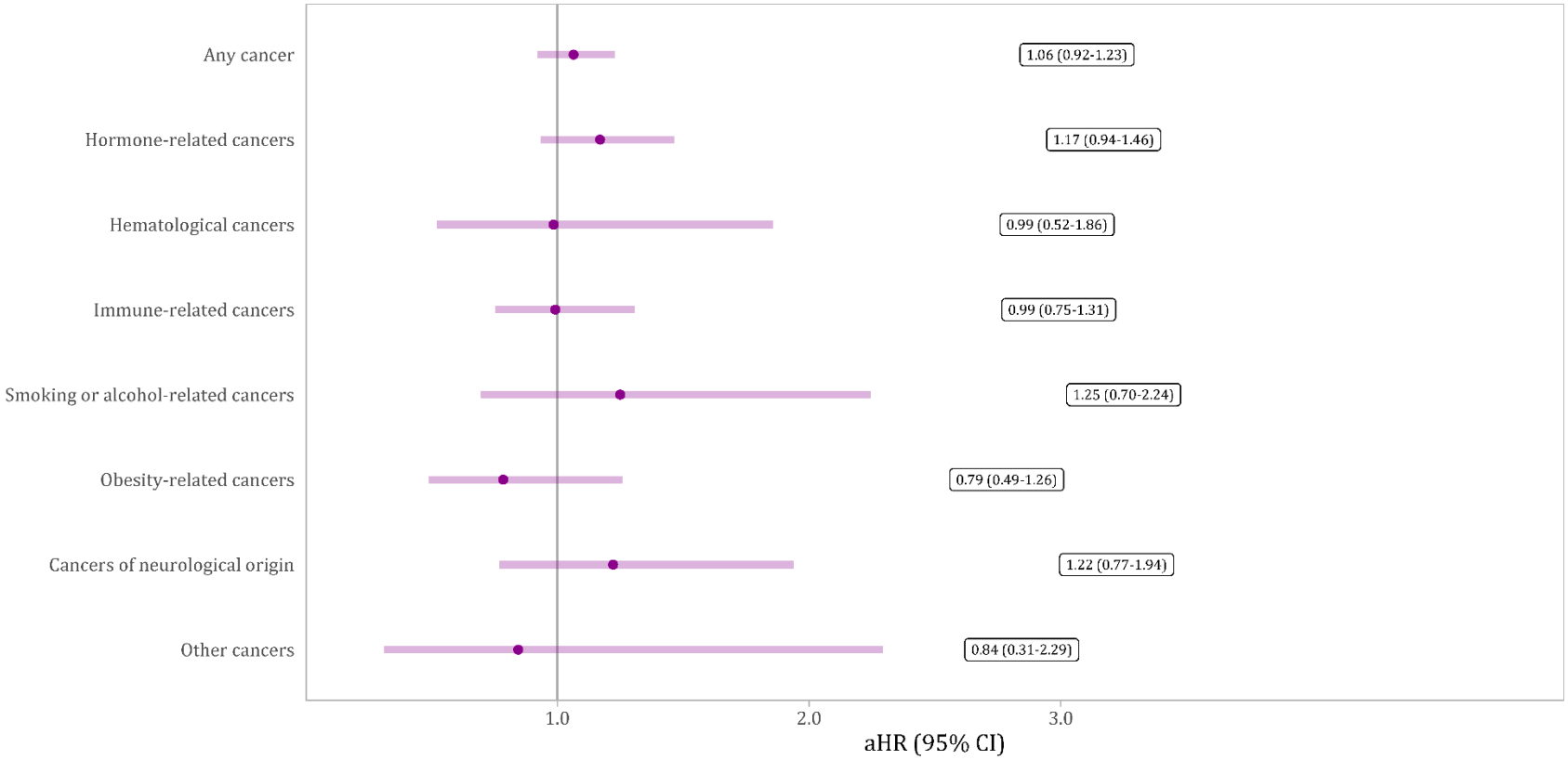
In landmark analyses, women with one VB-affected vs VB-unaffected pregnancy ending in childbirth before the age of 40 were not at increased risk of any cancer and groups of site-specific cancers, except for smoking or alcohol-related cancers (Figure 17).

Table 13. Adjusted associations between vaginal bleeding-affected pregnancy and cancer in women when additionally adjusted for BMI, 2004-2018 [155]

		VB-affected vs VB-unaffected pregnancy[a]
Outcome	**Observations**	**HR (95% CI)[a]**
Any cancer	All identifiable pregnancies	0.96 (0.78-1.18)
	First pregnancy	1.23 (0.85-1.79)
Hormone-related cancers	All identifiable pregnancies	0.62 (0.32-1.20)
	First pregnancy	0.94 (0.35-2.52)
Hematological cancers	All identifiable pregnancies	1.13 (0.92-1.38)
	First pregnancy	0.84 (0.55-1.28)
Immune-related cancers	All identifiable pregnancies	0.94 (0.47-1.90)
	First pregnancy	0.48 (0.07-3.46)
Smoking or alcohol-related cancers	All identifiable pregnancies	0.77 (0.51-1.15)
	First pregnancy	0.50 (0.19-1.33)
Obesity-related cancers	All identifiable pregnancies	1.07 (0.75-1.53)
	First pregnancy	1.19 (0.62-2.31)
Cancers of neurological origin	All identifiable pregnancies	1.59 (0.85-2.99)
	First pregnancy	2.61 (1.06-6.41)
Other cancers	All identifiable pregnancies	0.96 (0.78-1.18)
	First pregnancy	1.23 (0.85-1.79)

[a] Adjusted for age, calendar year of pregnancy end, women's parity and number of previous identifiable pregnancies, socioeconomic factors (education, employment, and year-specific income level divided into quartiles based on all included pregnancies), smoking in pregnancy, BMI, reproductive history (pre-index date vaginal bleeding, termination, or miscarriages, placental complication in previous deliveries), history of chronic somatic conditions, any psychiatric condition, and use of NSAIDs and steroids for systemic use before the 12 weeks of gestatioin for VB-affected and VB-unaffected pregnancies and before the index date for terminations and miscarriages.
BMI, body mass index; CI, Confidence interval; HR, Hazard ratio; VB, vaginal bleeding

Figure 17. Adjusted associations between vaginal bleeding-affected pregnancy and groups of site-specific cancers in women with one childbirth before the age of 40 years



VB, vaginal bleeding
By design, women with VB-exposed and VB-unexposed pregnancy not surviving, emigrating or experiencing cancer outcome before 40 years of age are excl
Follow-up starts at 40 years of age in VB-exposed and VB-unexposed cohorts and stops at the earliest of cancer outcome, emigration, or death
Study period: 1995-2018

6. Discussion

6.1. Main findings

1. Exposure to TAB *in utero* was not associated with an increased hazard of ADHD and epilepsy in children; however, it was associated with a 1.4-fold increased hazard of cerebral palsy in conventional cohort analyses and with a 2 to 3-fold increased hazard in the analyses of full and half-sibling pairs. The absolute risk of cerebral palsy was low for both TAB-affected and TAB-unaffected *in utero* children. The association of TAB exposure *in utero* with a 1.2 to 1.3-fold increased hazard of epilepsy and ADHD in children observed in full cohort analyses disappeared after removing time-invariant family-shared confounding in sibling comparisons.
2. In comparison of women with VB-affected vs VB-unaffected pregnancy, the association with all-cause (HR: 1.14, 95% CI: 1.09-1.19), natural-causes (HR: 1.15, 95% CI: 1.09-1.22) and non-natural causes (HR: 1.27, 95% CI: 1.08-1.48) of mortality disappeared after additional adjustment for medication use and lifestyle proxies in a sensitivity analysis of a data subset ascertained in 1994-2017 with HRs becoming 1.00 (95% CI: 0.90-1.12), 0.98 (95% CI: 0.85-1.14), and 1.04 (95% CI: 0.71-1.51), respectively. Comparisons with pregnancies not ending in childbirth showed slightly decreased risks of all-cause and cause-specific mortality; while associations with non-natural causes mortality were close to the null value of 1.
3. VB-affected pregnancy was associated with a 1.2 to 1.3-fold increased risk of diabetes types 1 and 2, hypertension, ischaemic heart disease, including myocardial infarction, atrial fibrillation or flutter, and heart failure; the risk was 1.4 and 1.5-fold increased for ischaemic and haemorrhagic stroke, respectively, when compared with having VB-unaffected pregnancy. Following a woman's first identifiable pregnancy, the HRs remained similarly increased for women with VB-affected pregnancy vs VB-unaffected pregnancy. When we compared having VB-affected pregnancy with a termination, the hazards were up to 1.3-fold increased for diabetes and hypertension. When we compared having VB-affected pregnancy with a miscarriage, no increased hazards for the outcomes were observed.

4. We found no association between having a VB-affected pregnancy and any cancer as compared with having a VB-unaffected pregnancy, termination or miscarriage. We observed no association of VB-affected pregnancy with later hazards of haematological, immune-related, obesity-related and other cancers across all comparisons. An association between VB and smoking- or alcohol-related cancers found in the analysis including a first pregnancy of a woman disappeared in a sensitivity analysis with additional adjustment for BMI. There was no increased hazard of premenopausal breast cancer, cervical cancer, ovary and fallopian tube cancer, or uterine cancer for women with VB-affected vs VB-unaffected pregnancy, termination or miscarriage. Furthermore, we found no association with other cancers at specific sites across all comparisons and in sensitivity analyses.

6.2. Comparison with existing literature

The cumulative incidences of neurological conditions computed in study I were in line with previous Danish studies. We reported a cumulative incidence of epilepsy at age 16 of 1.6% for the population of TAB-unaffected *in utero* and live-born singletons in Denmark. This estimate is close to 1.3-1.4% cumulative incidence of epilepsy at age 15 among all children in Denmark born in 1977-2002.[189] In line with our expectation, the incidence rate of epilepsy in study I (1.41 per 1000 PYs) was lower than the incidence rate of 1.56 per 1000 PYs previously reported for preterm-born children,[190] who are at increased risk of epilepsy. Another Danish study showed that the incidence rate of epilepsy for term-born children was 1.05 per 1000 PYs and 1.74 per 1000 PYs for preterm-born children,[191] positioning the cumulative incidence of epilepsy from study I between these two estimates.

The cumulative incidence of cerebral palsy among all unexposed singletons at 6 years of age in study I was 0.2% (95% CI: 0.2-0.3) and was close to previously reported estimates of 0.3% (95% CI: 0.2-0.4) for moderately and late-preterm infants (born at 32-36 gestational weeks) computed at 8 years of age.[192] The difference in cumulative incidence of cerebral palsy in these studies may stem from the differences in the study populations, e.g. children born preterm are

at a higher risk of cerebral palsy compared with the general population of live-born singletons in study I, most of whom were born at term. When we carried out the follow-up for cerebral palsy until 16 years of age, the cumulative incidence of cerebral palsy was 0.4%. This estimate was close but as expected lower than the cumulative incidence at the same age among moderately and late preterm infants (0.5%).[192]

The cumulative incidence of ADHD at age 16 was 5.5% among TAB-affected *in utero* children and 4.2% among TAB-unaffected children. The latter estimate is in line with ADHD cumulative incidence in the general population of children in the Nordics. A Swedish study reported a cumulative incidence of ADHD at age 13 of 1.3% among girls (N=23,656) and 5.2% among boys (N=5440).[193] The cumulative incidence of ADHD at age 13 for the population of both sexes was 3.5%, which was similar to the cumulative incidence of ADHD at the same age of 3.4% in study I. One Danish study showed that the cumulative incidence of ADHD at age 18 was 3.0% among young women and 6.0% among young men, who constituted 71% of the population with ADHD events.[194] Given these estimates, the average cumulative incidence of ADHD at age 18 for both sexes was 5.1%. Assuming the same sex distribution among ADHD cases at 16 years of age as at 18 years of age and taking sex-specific cumulative incidences of 2.3% among female adolescents and 5.0% among male adolescents from the plotted curves,[194] overall cumulative incidence of ADHD at age 16 was 4.2% (the same as in study I for TAB-unaffected children).

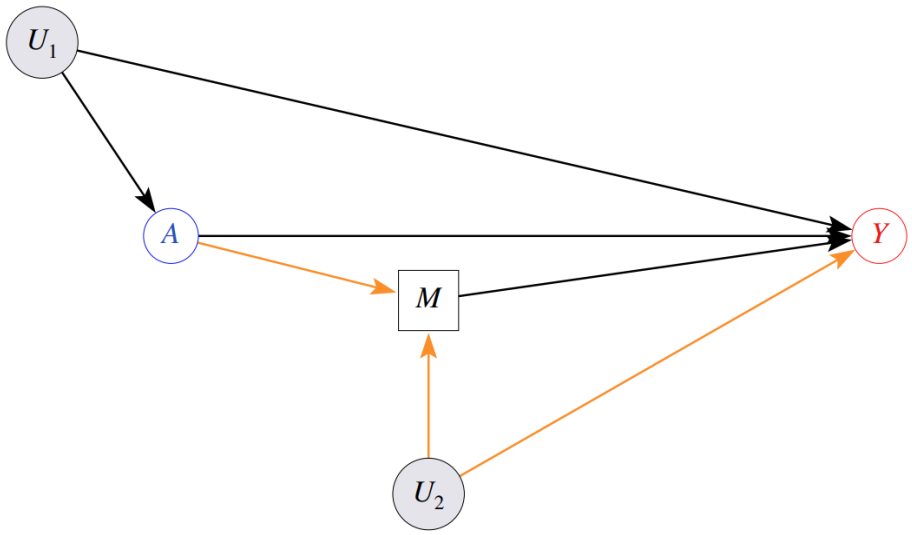
Previous literature only reported associations of TAB with neurological and neurodevelopmental conditions for full populations of children[46,47] and is, therefore, subject to family-shared confounding, which we addressed with the sibling design in study I.

One previous Danish study reported mortality among the general population of women giving birth in 1979-2010 of 1.27 (95% CI: 1.24-1.29) per 1000 PYs,[195] which was comparable to the mortality rate in our study among women without VB in pregnancy in 1979-2018 (1.27; 95% CI: 1.26-1.28 per 1000 PYs) and to women's mortality rate in The Nurses' Health Study in 1993-2017 (1.24 per 1000 PYs).[109] The mortality rate among women with VB (1.52 per 1000 PYs)

was similar to that reported in women with other undesired pregnancy outcomes, e.g. among women who gave birth to an infant with a major congenital anomaly (1.60 per 1000 PYs)[195] or among women with a history of multiple miscarriages (1.47 per 1000 PYs).[46]

A previous Danish study[48] reported a 25-60% higher relative risk of ischaemic heart disease, hypertension, stroke, thrombotic events, and diabetes in women having a pregnancy with VB before 12 weeks of gestation; however, the associations were adjusted for post-exposure factors (preterm delivery, prelabour rupture of membranes, foetal growth restriction, placental abruption, and stillbirth) and were not controlled for pre-exposure confounders (Figure 18).

Figure 18. Simplified directed acyclic graph depicting how an inflated association between vaginal bleeding and cardiovascular outcomes may arise when conditioning on a post-exposure variable in the presence of shared causes of post-exposure variable and the outcome



A: Pregnancy with vaginal bleeding before 12 weeks of gestation;
Y: outcomes (ischaemic heart disease, hypertension, stroke, thrombotic events, diabetes);
M: preterm delivery, prelabour rupture of membranes, foetal growth restriction, placental abruption and stillbirth;
U_1: pre-pregnancy women's morbidity;
U_2: a common cause of placental complications in pregnancy, preterm delivery, prelabour rupture of membranes, and cardiovascular outcomes (unadjusted[48]).
The paths 1) A → [M] ← U_2 → Y (in orange: collider stratification bias leading to inflation of the association between A and Y due to unmeasured U_2) and 2) A ←U_1→ Y (open backdoor path) are biasing the association of A on Y.
DAG tool: https://causalfusion.net/app[161]

6.3. Methodological considerations

The results of this thesis must be interpreted in the context of the biases (selection bias, information bias, confounding), constraints of the applied analytical strategies, and assumptions.

6.3.1. Selection bias

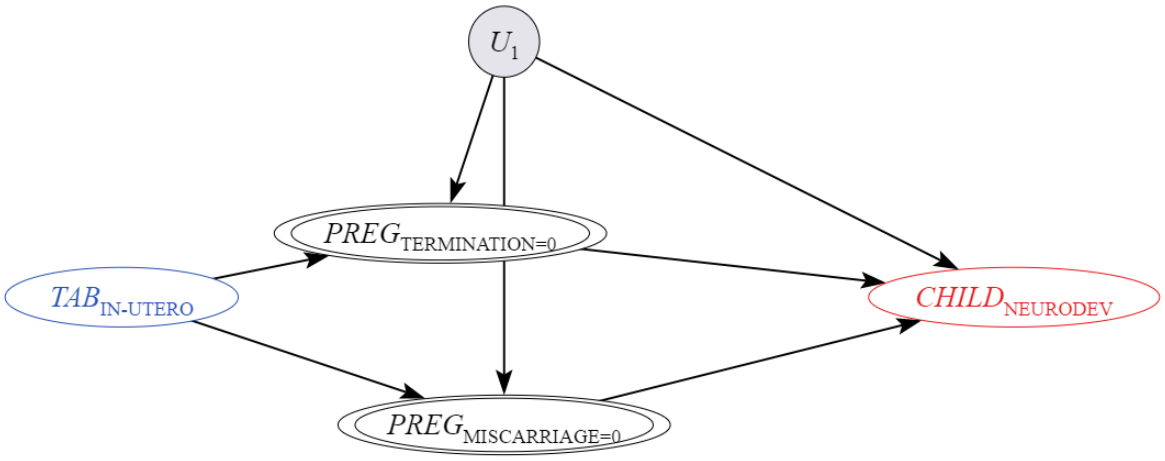
The tax-funded and universal healthcare system of Denmark allows for every resident free and virtually fully equal access to healthcare reducing the risk of selection bias due to differential healthcare access. The registry-based studies conducted in Denmark also allow for nearly complete follow-up from birth until death or emigration, minimising the risk of informative loss-to-follow-up and associated with it selection bias.[196]

6.3.1.1. Live birth bias

In study I, the selection (collider stratification) bias due to live births could have occurred if there had been unmeasured factors (e.g. U_1, Figure 19), which simultaneously would increase the risk of a pregnancy loss or termination before giving birth and of the children's neurodevelopmental and neurological outcomes. Conditioning on live births (not observing potential neurodevelopmental outcomes in pregnancies ending in a termination or miscarriage) in Figure 19 opens biasing paths 1) $TAB_{IN\text{-}UTERO} \rightarrow [PREG_{TERMINATION=0}] \leftarrow U_1 \rightarrow CHILD_{NEURODEV}$ and 2) $TAB_{IN\text{-}UTERO} \rightarrow [PREG_{MISCARRIAGE=0}] \leftarrow U_1 \rightarrow CHILD_{NEURODEV}$. Previous studies suggest that 15-31% of pregnancies with VB end in pregnancy loss.[10,14,30,31] Although VB increases the risk of pregnancy loss,[17] the relationship is not deterministic, and most VB-affected pregnancies continue gestation.

Although we cannot eliminate live birth bias in study I, the results can be interpreted as the association partly mediated by the competing events (early and late pregnancy losses and terminations). The presented estimates may be of the most interest to clinicians and parents, who would be concerned about the children's long-term risk of neurodevelopmental outcomes given a VB-affected pregnancy ended in a live birth.

Figure 19. Directed acyclic graph illustrating selection bias arising from conditioning on foetal survival until birth ("live-birth bias")



Abbreviations: NEURODEV, neurodevelopmental conditions; PREG, pregnancy; TAB, threatened abortion; U1, an unmeasured confounding factor (common cause) for the relationship between pregnancies ending in miscarriage or termination and neurodevelopmental conditions in children. $PREG_{TERMINATION}$ and $PREG_{MISCARRIAGE}$ are colliders on the pathway from $TAB_{IN\text{-}UTERO}$ to $CHILD_{NEURODEV}$.
In text $[PREG_{TERMINATION}=0]$ and $[PREG_{MISCARRIAGE}=0]$: square brackets [ ] around variables mean that these variables have been conditioned on (we restrict on observing the outcomes only among pregnancies not ending in miscarriages or terminations);
Conditioning on the colliders opens previously closed biasing paths:
1) $TAB_{IN\text{-}UTERO} \rightarrow [PREG_{TERMINATION=0}] \leftarrow U_1 \rightarrow CHILD_{NEURODEV}$
2) $TAB_{IN\text{-}UTERO} \rightarrow [PREG_{MISCARRIAGE=0}] \leftarrow U_1 \rightarrow CHILD_{NEURODEV}$
DAG tool: https://causalfusion.net/app[161]

6.3.1.2. Loss-to-follow-up as the source of selection bias

Due to virtually complete administrative follow-up from birth until death or emigration, the selection bias is not expected starting from the delivery date for children and during the entire follow-up for women.

HRs are inherently problematic to be endowed with causal meaning. The hazard function is the conditional probability of experiencing the endpoint within the next unit of time given that the event has not yet occurred.[197] Even when all causal assumptions such as exchangeability (absence of confounding), consistency, positivity,[159] no selection and information bias, and no interference hold (an unlikely scenario), HRs are subject to "built-in selection bias".[198] Each new subset of the study population at risk of the outcome at the current time is the subset of "survivors" during the previous period.

6.3.2. Data quality and information bias

The Danish registries are a unique source[133] of routinely and prospectively collected healthcare and administrative data, reducing the risk of differential misclassification of exposure in regards to the outcome and vice versa. The differential misclassification of the exposure in regards to the outcome (recall bias) is impossible; the differential misclassification of the outcome in regards to exposure (surveillance bias) is possible, although, in this study, we believe, is minimal. The expected influence of the non-differential (and non-dependent) misclassification of the binary exposure and/or outcome on the investigated associations of interest is their dilution, or shift towards or beyond the null value.[197] Information bias on confounding variables would lead to residual confounding.

6.3.2.1. Exposure: Threatened abortion (vaginal bleeding in pregnancy)

In the analyses of all pregnancies (studies II-IV), VB-affected pregnancies were overascertained among women with a higher baseline risk of previous miscarriage and, therefore, also possibly of cardiovascular outcomes (study III) and mortality from non-natural causes (study II).

The recording of VB diagnosis in pregnancy was not validated in the DNPR at the time of writing this thesis. Hospital records of pregnancy terminations are expected to have high validity. In general, procedures recorded in the DNPR have high validity; additionally, the induced abortion diagnoses had a PPV of 94% in a different registry.[141] Miscarriage (spontaneous abortion) diagnoses in the DNPR were previously validated and had a PPV of 97.4% (95% CI: 92.7-99.5%) in the period 1980-2008.[199] The PPV was high for both ICD-8 and ICD-10 revisions (94.4% and 98.8%, respectively).[199] We see no reasons to believe that TAB diagnoses would have a strikingly lower PPV than spontaneous abortion diagnoses.

In Denmark, 98% of women with self-reported VB in pregnancy had an ultrasound screening according to a study conducted in 1989-1991.[8] It is not unreasonable to assume that the proportion of ultrasounds performed at VB presentation has increased over time and that most of the TAB diagnoses recorded in the DNPR are following clinical criteria resulting in few

exposure false-positives. Only data on diagnoses at hospital encounters were available. We had no information on VB episodes leading to general practitioner visits and VB episodes not leading to any healthcare contact. Based on the literature review (Table 1), the prevalence of VB varied between <5%[6] and 30%[17] with recent cohort studies reporting a prevalence of 23%.[14–16] According to an earlier Danish study of women who reported a VB in pregnancy, 14% had a hospital admission. The admission was related to heavy bleeding, longer episode duration, and pain.[8] Assuming that the true prevalence of VB in the Danish population of pregnancies is closer to 20%, we missed a major proportion of VB-affected pregnancies by using a registry-based approach. Since we assume few false-positives among VB-affected pregnancies, the expected direction of bias due to non-differential misclassification of exposure is a dilution of an association (studies II and III). Of note, we do not believe that the null association with cancer in the comparison with VB-unaffected pregnancys in study IV can be explained by non-differential misclassification of VB status in pregnancy. Although we cannot rule out that the registered VB diagnoses are systematically more severe than unregistered ones, we have no evidence that the potential systematic capture of more severe VB can fully explain away the observed associations, especially, in study III.

6.3.2.1.1. Threatened abortion (vaginal bleeding in pregnancy) in multiple successive pregnancies

This thesis did not investigate the longitudinal (cumulative) effect of VB in multiple successive pregnancies on the long-term outcomes in women. The use of advanced methods is necessary to address research questions investigating the longitudinal effects of time-varying exposures and time-varying confounders affected by past exposure.[159,200,201] As discussed by Wilcox and Skjaerven,[202] previous pregnancy complications are strong risk factors for the complications in subsequent pregnancies[203] and, simultaneously, are often risk factors for long-term outcomes in women such as CVD. Adverse pregnancy outcomes and complications may also be associated with fewer successive births.[202]

To further reason about the susceptibility of conducted analyses to potential methodological challenges due to repeated complications in subsequent pregnancies in the same woman, we conducted several descriptive post-hoc analyses. First, we investigated the risks of VB recurrence in successive pregnancies. Second, we tabulated the maximum number of observed childbirths in women following the first VB-affected vs VB-unaffected pregnancy. Third, we illustrated how the prevalence of covariables and reproductive factors changed by VB status from a woman's 1st to 2nd to 3rd successive childbirth (Table 16).

The risk of VB occurrence in second childbirth following the first VB-affected pregnancy was increased 3-fold when compared to women with no VB in the first pregnancy ending in childbirth (Table 14). However, the first childbirth from a VB-affected pregnancy did not reduce the number of subsequent births. When classifying women according to the VB status at the end of their first delivery, the distribution of the maximum number of births per woman was similar with 36%/35% having had 1 childbirth among VB-affected/VB-unaffected women, 45%/44% having had 2 childbirths and 16%/16% having had 3 childbirths (Table 15). These results do not suggest an association between VB in the first pregnancy with fewer subsequent childbirths (as a measure of fertility) in the data used for this thesis. Although the "accumulation" of the prevalence of placental complications was slightly more prominent for VB-affected pregnancies, absolute risk differences remained small (<1%) in each successive pregnancy for women with VB-affected vs VB-unaffected pregnancies (Table 16).

Table 14. Vaginal bleeding recurrence in successive pregnancies, 1979-2017

Pregnancy status at the time of the 1st childbirth			**n[a]**	**%**
VB-unaffected			1,151,145	96.91
VB-affected			**36,715**	**3.09**
Pregnancy status at the time of the 2nd childbirth, conditional on the status of 1st childbirth				
1st childbirth	**2nd childbirth**		**n**	**%**
VB-unaffected	VB-unaffected		723,420	62.84
VB-unaffected	**VB-affected**		**21,165**	**1.84**
VB-unaffected	censored/no 2nd delivery in the study period		406,565	35.32
VB-affected	VB-unaffected		21,185	57.70
VB-affected	**VB-affected**		**2,245**	**6.11**
VB-affected	censored/no 2nd delivery in the study period		13,290	36.20
Pregnancy status at the time of the 3rd childbirth, conditional on the status of 1st and 2nd childbirths				
1st childbirth	**2nd childbirth**	**3rd childbirth**	**n**	**%**
VB-unaffected	VB-unaffected	VB-unaffected	211,480	29.23
VB-unaffected	**VB-unaffected**	**VB-affected**	**5,950**	**0.82**
VB-unaffected	VB-unaffected	censored/no 3rd delivery in the study period	505,990	69.94
VB-unaffected	VB-affected	VB-unaffected	6,290	29.73
VB-unaffected	**VB-affected**	**VB-affected**	**600**	**2.84**
VB-unaffected	VB-affected	censored/no 3rd delivery in the study period	14,270	67.43
VB-unaffected	censored/no 2nd delivery in the study period	censored/no 3rd delivery in the study period	406,565	100.00
VB-affected	VB-unaffected	VB-unaffected	6,175	29.15
VB-affected	**VB-unaffected**	**VB-affected**	**430**	**2.02**
VB-affected	VB-unaffected	censored/no 3rd delivery in the study period	14,580	68.83
VB-affected	VB-affected	VB-unaffected	655	29.29
VB-affected	**VB-affected**	**VB-affected**	**110**	**4.90**
VB-affected	VB-affected	censored/no 3rd delivery in the study period	1,475	65.80
VB-affected	censored/no 2nd delivery in the study period	censored/no 3rd delivery in the study period	13,290	100.00

[a]All counts are rounded to the nearest 5 to adhere to Statistics Denmark data confidentiality requirements.
Notes: The population includes women with at least 1 childbirth record in the Danish Medical Birth Registry. The data are cut at the time of a woman's 3rd childbirth irrespective of later childbirths (4th, etc.)

Table 15. Maximun number of childbirths in women according to vaginal bleeding exposure status at the end of the first childbirth, 1979-2017

Pregnancy status at the end of 1st childbirth (irrespective of later childbirths)	Maximum observed number of childbirths per woman	Number of women[a] (%)
N=1,187,860[a]		
VB-unaffected, N=1,115,145[a]	1	406,565 (35.3)
	2	520,260 (45.2)
	3	178,905 (15.5)
	>3	45,420 (4.0)
VB-affected, N=36,715[a]	1	13,290 (36.2)
	2	16,055 (43.7)
	3	5,790 (15.8)
	>3	1,580 (4.3)

[a] All counts are clouded to the nearest 5 to adhere to Statistics Denmark data confidentiality requirements and prevent the identification of individuals.
Notes: Each row in a dataset was a unique woman.

Table 16. Descriptive characteristics of VB-affected pregnancies within 20 gestational weeks vs VB-unaffected pregnancies at the time of 1st, 2nd, and 3rd childbirths, 1979-2017

	Pregnancy status at the time of the 1st childbirth of a woman		Pregnancy status at the time of the 2nd childbirth of a woman		Pregnancy status at the time of the 3rd childbirth of a woman	
Characteristic	**VB-affected, N=36,715**	**VB-unaffected, N=1,151,145**	**VB-affected, N=23,405**	**VB-unaffected, N=744,600**	**VB-affected, N=7,090**	**VB-unaffected, N=224,600**
Vaginal bleeding prevalence, %	**3.09**		**3.05**		**3.06**	
	n[a] (%)	**n[a] (%)**	**n[a] (%)**	**n[a] (%)**	**n[a] (%)**	**n[a] (%)**
Calendar year at the pregnancy end						
(1979,1987]	9,995 (27%)	293,620 (26%)	3,175 (14%)	79,860 (11%)	295 (4.2%)	6,300 (2.8%)
(1987,1994]	9,820 (27%)	235,725 (20%)	7,325 (31%)	180,360 (24%)	2,110 (30%)	49,230 (22%)
(1994,2002]	8,160 (22%)	196,315 (17%)	6,105 (26%)	157,635 (21%)	2,190 (31%)	56,980 (25%)
(2002,2009]	5,365 (15%)	215,990 (19%)	4,270 (18%)	173,215 (23%)	1,575 (22%)	62,425 (28%)
(2009,2017]	3,370 (9.2%)	209,495 (18%)	2,530 (11%)	153,530 (21%)	920 (13%)	49,665 (22%)
Age at the pregnancy end, years						
<20	1,330 (3.6%)	45,875 (4.0%)	115 (0.5%)	3,025 (0.4%)	0 (<0.1%)	85 (<0.1%)
20-24	8,640 (24%)	283,905 (25%)	3,185 (14%)	80,545 (11%)	435 (6.2%)	9,005 (4.0%)
25-29	13,845 (38%)	465,615 (40%)	8,650 (37%)	270,990 (36%)	1,960 (28%)	51,050 (23%)
30-34	8,900 (24%)	258,210 (22%)	7,945 (34%)	279,750 (38%)	2,790 (39%)	99,215 (44%)
35-39	3,370 (9.2%)	82,575 (7.2%)	3,055 (13%)	96,120 (13%)	1,625 (23%)	56,765 (25%)
40+	635 (1.7%)	14,965 (1.3%)	460 (2.0%)	14,165 (1.9%)	280 (3.9%)	8,480 (3.8%)
Employment status						
Employed	25,765 (70%)	792,925 (69%)	17,105 (73%)	566,805 (76%)	4,810 (68%)	163,025 (73%)
Retirement or state pension	2,575 (7.0%)	75,390 (6.5%)	2,280 (9.7%)	54,140 (7.3%)	965 (14%)	23,630 (11%)
Unemployed	4,835 (13%)	159,775 (14%)	3,830 (16%)	114,925 (15%)	1,310 (18%)	37,060 (17%)
Missing	3,545 (9.7%)	123,060 (11%)	195 (0.8%)	8,735 (1.2%)	5 (<0.1%)	885 (0.4%)
Yearly income, quartiles						
Q1	8,910 (24%)	251,950 (22%)	4,590 (20%)	119,820 (16%)	1,550 (22%)	39,700 (18%)
Q2	6,940 (19%)	219,900 (19%)	6,615 (28%)	201,015 (27%)	2,215 (31%)	67,410 (30%)
Q3	7,795 (21%)	264,735 (23%)	6,260 (27%)	213,400 (29%)	1,785 (25%)	61,260 (27%)
Q4	9,625 (26%)	280,790 (24%)	5,770 (25%)	203,600 (27%)	1,525 (22%)	55,645 (25%)
Missing	3,445 (9.4%)	133,775 (12%)	170 (0.7%)	6,770 (0.9%)	10 (0.2%)	585 (0.3%)
Highest achieved education categories						
Basic education	10,540 (29%)	272,180 (24%)	7,425 (32%)	176,825 (24%)	2,575 (36%)	61,805 (28%)
High school or similar	12,855 (35%)	414,090 (36%)	8,995 (38%)	301,025 (40%)	2,475 (35%)	83,170 (37%)
Higher education	7,545 (21%)	265,110 (23%)	6,325 (27%)	249,795 (34%)	1,900 (27%)	76,645 (34%)
Missing	5,770 (16%)	199,765 (17%)	655 (2.8%)	16,955 (2.3%)	140 (2.0%)	2,980 (1.3%)
Smoking status recorded at 1st prenatal visit						
Smoker	4,580 (12%)	129,995 (11%)	3,815 (16%)	96,520 (13%)	1,515 (21%)	38,030 (17%)
Missing	16,760 (46%)	465,210 (40%)	8,155 (35%)	210,375 (28%)	1,650 (23%)	41,120 (18%)
Chronic conditions						
Obesity diagnosis	885 (2.4%)	35,670 (3.1%)	930 (4.0%)	39,775 (5.3%)	410 (5.8%)	15,325 (6.8%)
PCOS	465 (1.3%)	13,170 (1.1%)	420 (1.8%)	12,935 (1.7%)	175 (2.5%)	5,185 (2.3%)

	Pregnancy status at the time of the 1st childbirth of a woman		Pregnancy status at the time of the 2nd childbirth of a woman		Pregnancy status at the time of the 3rd childbirth of a woman	
Characteristic	**VB-affected, N=36,715**	**VB-unaffected, N=1,151,145**	**VB-affected, N=23,405**	**VB-unaffected, N=744,600**	**VB-affected, N=7,090**	**VB-unaffected, N=224,600**
Vaginal bleeding prevalence, %	**3.09**		**3.05**		**3.06**	
	n[a] (%)	**n[a] (%)**	**n[a] (%)**	**n[a] (%)**	**n[a] (%)**	**n[a] (%)**
Thyroid disorders	25 (<0.1%)	395 (<0.1%)	10 (<0.1%)	295 (<0.1%)	0 (<0.1%)	115 (<0.1%)
Rheumatic disorders with heart involvement	345 (0.9%)	8,635 (0.8%)	240 (1.0%)	6,565 (0.9%)	85 (1.2%)	2,135 (1.0%)
COPD	60 (0.2%)	1,465 (0.1%)	55 (0.2%)	1,165 (0.2%)	15 (0.2%)	425 (0.2%)
Chronic kidney diseases	55 (0.1%)	1,405 (0.1%)	45 (0.2%)	1,110 (0.1%)	5 (<0.1%)	520 (0.2%)
Chronic liver diseases	140 (0.4%)	4,145 (0.4%)	115 (0.5%)	3,515 (0.5%)	35 (0.5%)	1,205 (0.5%)
History of at least one termination	7,295 (20%)	149,535 (13%)	5,685 (24%)	125,770 (17%)	2,230 (31%)	49,970 (22%)
History of at least one miscarriage	8,005 (22%)	89,270 (7.8%)	6,700 (29%)	107,650 (14%)	2,395 (34%)	47,100 (21%)
Alcohol abuse history	220 (0.6%)	5,405 (0.5%)	190 (0.8%)	3,490 (0.5%)	80 (1.2%)	1,420 (0.6%)
History of any psychiatric condition diagnosis	2,605 (7.1%)	70,400 (6.1%)	1,970 (8.4%)	49,285 (6.6%)	815 (12%)	18,075 (8.0%)
Pregnancy complications						
History of hypertensive disorders of pregnancy	155 (0.4%)	4,890 (0.4%)	1,425 (6.1%)	42,275 (5.7%)	535 (7.5%)	14,860 (6.6%)
History of placenta praevia	10 (<0.1%)	195 (<0.1%)	60 (0.3%)	2,115 (0.3%)	35 (0.5%)	1,235 (0.5%)
History of abruptio placentae	20 (<0.1%)	370 (<0.1%)	220 (0.9%)	4,335 (0.6%)	145 (2.0%)	2,580 (1.1%)
Hypertensive disorders of pregnancy in pregnancy	2,425 (6.6%)	67,205 (5.8%)	535 (2.3%)	12,935 (1.7%)	130 (1.9%)	3,515 (1.6%)
Placenta praevia in pregnancy	245 (0.7%)	3,895 (0.3%)	225 (1.0%)	3,360 (0.5%)	105 (1.5%)	1,075 (0.5%)
Abruptio placentae in pregnancy	445 (1.2%)	6,405 (0.6%)	280 (1.2%)	3,590 (0.5%)	90 (1.2%)	1,190 (0.5%)

[a] All counts are clouded to the nearest 5 to prevent the identification of individuals according to Statistics Denmark requirements. Percentages ≥10% are rounded to the nearest whole number and percentages <10% are rounded to the first decimal place.
Notes: Based on the population of all identifiable pregnancies for Study III in 1979-2017. The same population is presented in other tables of this Subsection 6.3.2.1.1.
COPD, Chronic obstructive pulmonary disease

6.3.2.2. Outcomes

The PPV for epilepsy diagnosis as recorded in the DNPR and of ADHD diagnosis as recorded in the PCRR was 81%[189,204] and 87%,[205] respectively. The recording of cerebral palsy diagnoses was not validated in the DNPR. Of the children born in 1979-1982 registered in the Danish Cerebral Palsy Registry (DCPR)[206]—unavailable in study I— 81% of children was also recorded in the DNPR.[207] Of children recorded in the DNPR as having a cerebral palsy diagnosis but not appearing in the DCPR, 18% were previously excluded from the DCPR, 26.3% were not eligible due to administrative reasons, 6% had postperinatal disorders (cerebral tumours, cranial traumas, thromboembolic events, and a drowning event), 37% had an unconfirmed cerebral palsy diagnosis, and the remaining 13% met cerebral palsy criteria.[207] If cerebral palsy diagnoses did not become more accurate after 1982, approximately 34% of all such diagnoses recorded in the DNPR may be false-positive. Non-hospitalised children with clinically less severe cerebral palsy could be unrecorded in the DNPR.[207]

All-cause mortality data from the CRS are deemed very high-quality.[208] The CRS is a central administrative database utilised by other administrative and healthcare systems,[132] meaning that the errors are corrected whenever observed. Moreover, the CRS is systematically checked for inaccuracies.[132] For emigrated persons, death would be recorded if Danish authorities were notified.[132] Cause-specific mortality data is subject to non-differential misclassification. One study reported that after the autopsies were performed, a third of the causes of death differed from the ones initially reported.[149,209] Another study showed that deaths are more likely to be coded as ill-defined in Denmark than in Finland.[210] Autopsies are relatively rare in Denmark.[149,210] The cause-specific mortality as an outcome may lack specificity and may have a considerable proportion of false-positives. Furthermore, we had no data on cause-specific mortality after 2017.

The cardiovascular diagnoses in the DNPR were validated with the PPVs ranging between 71-85% for ischaemic and haemorrhagic stroke[211,212] to 92% for hypertension.[213] Overall,

cardiovascular diagnoses have high validity in the DNPR[213] and we do not expect a large proportion of false-positives for the outcomes of interest. Had the non-differential misclassification of the outcome been present, we would expect to have observed diluted associations. In study IV, cancer outcomes ascertained from the DCR are deemed to have very high validity.[150]

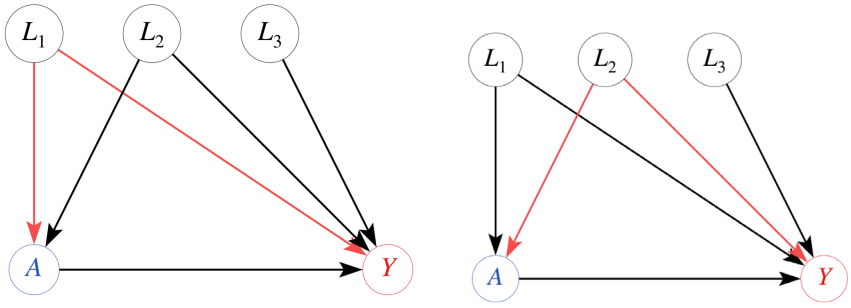
6.3.2.3. Covariables

The misclassification of confounders would lead to residual confounding and biased results in the direction of confounding. We discuss aspects of confounding bias including misclassification of confounders in the next section.

6.3.3. Confounding

Observational research is subject to confounding bias. With no exposure randomisation, the absence of confounding is an untestable assumption. The properties of a confounder are the following: 1) confounder is associated with the exposure, 2) confounder is associated with the outcome, and 3) confounder does not lie on the causal pathway between the exposure and the outcome. The assumption of no residual and unmeasured confounding is also called conditional exchangeability assumption[200] (given observed confounders, the potential outcome is independent of actually observed treatment) and ignorability assumption[214] (given measured covariables, the exposure assignment mechanism is ignorable). When the conditional exchangeability assumption holds, had the exposed been unexposed, their risk of potential outcome would have been the same (and vice versa).[159] Confounding can be conceptualised as the lack of exchangeability. The definition of a confounder according to the "backdoor criterion"[215] is the following: the set of confounders is a set of measured covariables, which close all biasing (backdoor) paths from the exposure to the outcome leading to the exposed and the unexposed being exchangeable within the levels of covariables included in the adjustment set. The notation of conditional exchangeability assumption: $Y^a \perp\!\!\!\perp A \mid L$, where Y^a is a potential outcome under exposure A and L is a covariable or a list of covariables sufficient to close all biasing backdoor paths (Figure 20; L includes L_1, L_2, L_3).

Figure 20. Simplified directed acyclic graph depicting confounding



A: exposure; Y: outcome
Adjustment sets: $\{L_1, L_2\}$ or $\{L_1, L_2, L_3\}$
Backdoor paths 1) A ←L_1→ Y 2) A ←L_2→ Y in red
DAG tool: https://causalfusion.net/app[161]

We used DAGs[160,215] to depict the confounding structure at baseline for studies I-IV (Figure 10, Figure 11). To avoid overstating that the presented causal diagram sufficiently reflects the true causal structure, we used the disjunctive confounding criterion to select confounders,[216] meaning that we included the pre-exposure covariables that were associated with the exposure, the outcome, or both and did not include instrumental variables. Irrespective of true causal structure, if a set of observed covariables is sufficient to reach conditional exchangeability, the set of covariables selected based on the disjunctive confounding criterion would also be sufficient to reach conditional exchangeability.

In study I, factors occurring during pregnancy and therefore coinciding with the placentation (maternal comorbidities starting during gestation, infections, severe stress) and factors recorded at delivery (birth weight, gestational age) could act as mediators of the total effect of *in utero* TAB on the later life neurological development of the child and were not considered confounders. However, we cannot rule out that prior infection was associated with *in utero* TAB-exposure and therefore was a confounder rather than a mediator.

In studies II-IV, we controlled for factors measured before pregnancy end, except for placental complications in pregnancies ending in delivery. Abruptio placentae, placenta praevia, and hypertensive disorders of pregnancy were deemed as later manifesting potential common

causes of VB and the outcomes and, thus, were adjusted for in the analyses of pregnancies ending in delivery.

An important strength of study I was the sibling design. In addition to shared genetic make-up, full siblings also often share living conditions and social aspects of upbringing. Comparison of full siblings within each family by design removes confounding by genetic and time-invariant confounders shared within a family. Comparison of maternal half-siblings also removes a large share of within-family time-stable confounding by design. It also allowed for increased statistical efficiency in study I due to a longer period of data availability (1979-2010) than for full siblings (1986-2010). To control all measured sources of confounding, we also adjusted within-sibling analyses for non-shared confounders used in the conventional analyses. Null association for TAB exposure *in utero* with epilepsy and ADHD in the sibling analyses may be explained by the removal of within-family confounding, or in other words, that TAB-affected and TAB-unaffected *in utero* siblings are more exchangeable than TAB-affected and TAB-unaffected children in the general population. However, we cannot rule out an alternative explanation by non-differential misclassification of TAB exposure *in utero*, which would lead to a stronger bias towards the null value in sibling comparisons than in conventional cohort analyses[152] and may partly explain the null findings for epilepsy and ADHD.

We cannot rule out that unmeasured confounding due to factors that we had no knowledge of and no data on remains. One strong confounder or multiple confounders acting in the same direction may have contributed to the observed associations between VB in pregnancy and women's outcomes in study III. For all studies, we had no information on lifestyle, including diet patterns and physical activity. Lifestyle proxies such as diagnosis of obesity and BMI in pregnancy available for study I and pregnancies ending in delivery for studies II-IV are subject to information bias. While BMI is used as an indicator of metabolic health, it does not measure adipose tissue distribution and other aspects of body composition and may be a poor proxy of metabolic health in pregnant women leading to residual confounding by pre-pregnancy

metabolic health. Capturing obesity using hospital-recorded diagnoses leaves residual confounding due to a considerable proportion of false-negatives. Similarly, we could only identify alcohol abuse by ascertaining hospital-based diagnoses leading to residual confounding due to measurement bias on this potential confounder.

In studies II-IV, the distribution of social factors was meaningfully different for women with a pregnancy affected by VB and ending in a delivery and pregnancy ending in a termination. In addition to residual confounding due to misclassification of obesity status and alcohol abuse status, the comparisons of women with VB-affected pregnancy with women having a termination are confounded by lifestyle, including smoking and BMI, since this information was not available for pregnancies not ending in delivery. Similarly, we expect unmeasured confounding by lifestyle factors to be present in comparisons of women having VB-affected pregnancy with women having a miscarriage. According to E-value,[217] one confounder or a set of confounders leading to bias in the same direction associated with the VB in pregnancy and with the outcome with the HR of 1.6-2.2 beyond measured covariables could explain away the observed associations with diabetes mellitus type 1 and type 2, and cardiovascular outcomes (Study III). Furthermore, type 1 diabetes mellitus has a distinct etiology related to autoimmune or non-autoimmune destruction of insulin-producing β-cells.[218] This may point to inflammatory pathways underlying VB or to residual confounding in Study III.

6.3.4. Other methodological considerations

In addition to the raised discussion points, there are several additional methodological challenges worth mentioning.

6.3.4.1. Well-defined exposure (consistency assumption)

Another causal assumption is consistency. When consistency holds, shifting from counterfactual outcomes notation to factually observed outcomes among those who factually had each level of exposure is warranted: $Y^{\{a=1\}} = Y\ among\ A = 1\ and\ Y^{\{a=0\}} = Y\ among\ A = 0$.[159,200] One component of the consistency assumption is the investigation of a well-defined exposure.

In studies I-IV, we could not distinguish between different biological mechanisms leading to VB in pregnancy. The estimates for the association we present capture the average effect of all potentially biologically diverse underlying mechanisms naturally occurring in the population and presenting as a threatened abortion. Put differently, VB is not a well-defined exposure,[219] and it is a clinical presentation of potentially different biologic conditions.

Although aetiologic research should not, in our opinion, be restricted to a narrow list of potentially well-defined exposures, we acknowledge that the anticipated diversity of underlying biological mechanisms of VB in pregnancy presents methodological challenges. At the same time, in clinical practice, it is also challenging—if at all possible—to accurately determine the aetiology of VB in pregnancy.

6.3.4.2. Positivity assumption

The positivity assumption states that there needs to be a non-zero probability of observing exposed and unexposed in each stratum of each of the covariables included in the adjustment set:[200] $\Pr[A = a|L = l] > 0 \; for \; all \; l$. There were several covariables with few observed VB-affected pregnancies, especially in the sensitivity analyses of sibling pairs in study I and when using data subsets starting in 2004 and subsets of first identifiable pregnancies in studies II-IV. Whenever there was a prospect of models not converging due to positivity violation, we used reduced adjustment covariables sets.

6.3.4.3. Missing data

In study I, we performed a complete case analysis, while in studies II-IV we used a separate "missing" category whenever information on civil status, employment, education, or income could not be ascertained before the index date. In data ascertained starting from 1979, there was more missingness compared with subsets of data starting in 1994 or later.

In study I, for children born in 1979-2010, up to 11% of maternal education status was missing while for children born in 1995-2010, only 0.3% had missing information on this variable; the

same pattern is true for other socioeconomic variables. In studies II-IV, the missingness on socioeconomic variables was 4-6% for education, 3% for employment, and 2-4% for income; however, it was higher than 10% (up to 19%) for civil status. The missingness on socioeconomic variables, smoking, and BMI was higher for women with pregnancies ending in a termination or miscarriage than for women with childbirths. We cannot rule out that information on women's smoking and BMI in pregnancy was missing not at random. Given the relatively low proportion of missingness, we made a pragmatic decision to not perform multiple imputation. Of note, previous research using the same data sources as this thesis showed that multiple imputation of missing values for maternal age, parity, cohabitation/civil status, residence, smoking in pregnancy, country of origin, or BMI did not change the study conclusions.[220]

6.3.4.4. Immortal time bias

In Studies I-IV of this study, at the time of exposure ascertainment at each pregnancy, we did not condition on the occurrence or the status of the subsequent pregnancies. The only exception to this was a set of sibling analyses in Study I, where we used the subset of offspring with a sibling who had discordant exposure and outcome status; however, this was a design feature and did not introduce immortal time into the study. The index date for both VB-affected and VB-unaffected pregnancies in Studies II-IV was the date of delivery, when all cohort eligibility criteria were met. By applying the principle of not conditioning on "future data", we aimed to avoid immortal time bias.[221]

6.4. External validity and future research directions

Assuming we reached sufficient internal validity, the results of studies I-IV may be generalised to other Scandinavian countries with a similar distribution of the outcomes' risk factors in children (study I) and women (studies II-IV) and with a potentially similar distribution of the underlying biological mechanisms leading to TAB.[222]

We offer five potential directions for future research. First, future studies may focus on investigating longitudinal (cumulative) effects of having a pregnancy affected by VB in multiple successive pregnancies on the long-term outcomes in women using marginal structural models or other appropriate approaches for the investigation of time-varying exposure and time-varying confounding affected by past exposure.[159] Second possibility for future studies is to investigate the mediation pathways of women's reproductive events such as TAB and spontaneous abortion and the later development of chronic conditions. Third, additional research can be conducted to investigate the effect measure modification of an association between VB and long-term morbidity in women by the level of the highest achieved education and socioeconomic position. Fourth, future research can also be directed to the search for and use of possible instrumental variables for the investigation of women's long-term health following pregnancy complications in general, including VB. Fith, threatened abortion as an outcome was rarely investigated. Future studies could investigate the association between reproductive factors (e.g., early miscarriage, subfertility) and threatened abortion in a subsequent pregnancy utilising the data from the registries of the Nordic countries or primary healthcare data from other countries.

7. Conclusion

In study I, upon controlling for time-invariant family-shared confounding with sibling design, exposure to TAB *in utero* was associated with a 2.9-fold increased relative risk of cerebral palsy but not with risks of epilepsy and ADHD. Within-family confounding explained associations with children's risks of epilepsy and ADHD found in the conventional cohort analyses. Although attenuated in a sensitivity analysis with an extended follow-up time, associations with cerebral palsy persisted in all analyses.

In study II, we found no strong evidence for the increased risks of all-cause mortality and mortality from natural and non-natural causes following VB-affected vs VB-unaffected pregnancy ending in childbirth. Comparisons of VB-affected pregnancy with termination or miscarriage showed 12-15% lower risks of all-cause and natural causes mortality and the associations with non-natural causes of death were close to the null value.

In study III, at the end of up to 25 years of follow-up, women whose pregnancy was affected by VB were at 1.2 to 1.5-fold greater risk of diabetes mellitus type 1 and type 2, and multiple cardiovascular outcomes (hypertension, ischaemic heart disease, myocardial infarction, atrial fibrillation or flutter, heart failure, and haemorrhagic and ischaemic stroke) than women with pregnancy unaffected by VB. Of note, the elevated risks of diabetes mellitus type 1 and type 2, hypertension, ischaemic heart disease, and haemorrhagic stroke were also observed in the analysis comparing having VB-affected pregnancy vs termination but not vs miscarriage.

In study IV, throughout up to 24 years of follow-up, having a VB-affected pregnancy was not associated with an increased risk of any cancer and cancers at specific sites including premenopausal breast, cervical, ovary and fallopian tube, and uterine cancer across comparisons with VB-unaffected pregnancy, termination, or miscarriage.

This work contributes to the knowledge on women's long-term health following childbirth and alternatively ending pregnancies. The results encourage the awareness of clinicians regarding

women's long-term morbidity following a VB-affected pregnancy ending in childbirth, with a focus on diabetes and cardiovascular diseases, however, no changes to the clinical practice can be recommended based solely on the findings laid out in this thesis. This thesis also provides reassuring results for the women: there was no strong evidence for the increased risk of cancer or mortality following VB-affected pregnancy vs VB-unaffected pregnancy ending in a childbirth.

8. English summary

Background: Threatened abortion (TAB) clinically manifests as a vaginal bleeding (VB) within the first 20 weeks of gestation of a viable intrauterine pregnancy without cervical dilation. It affects up to a third of clinically recognised pregnancies and is associated with adverse short-term outcomes in both the child and the mother. However, little to no data are available on the long-term health of children and women following a pregnancy with TAB.

Aims: To investigate the risk of epilepsy, cerebral palsy, and attention-deficit/hyperactivity disorder (ADHD) in children exposed vs unexposed to TAB *in utero*, and to examine the association between having a VB-affected pregnancy and women's risks of mortality, diabetes type 1 and 2, cardiovascular morbidity, and cancer as compared with having a VB-unaffected pregnancy, termination, or miscarriage.

Methods: To conduct studies I-IV, we used Danish administrative and health registries and applied a cohort design. In study I, we additionally used within-sibling comparisons. We included all singletons live-born between 1979 and 2010 to investigate the risk of epilepsy and cerebral palsy and those born between 1995 and 2010 to examine the risk of ADHD. In studies II-IV, we ascertained pregnancies ending in a childbirth using delivery records and pregnancies ending in a termination (medical and surgical abortions) or miscarriage using hospital diagnostic and procedure records from 1979-2017 for study II, 1994-2017 for study III and 1995-2017 for study IV. We conducted time-to-event analyses using Cox proportional hazards regression and computed hazard ratios with 95% confidence intervals adjusted for potential confounders, including pre-pregnancy characteristics (women's comorbidities, co-medications, and socioeconomic factors) in multivariable or inverse probability of treatment weighted models. We applied several sensitivity analyses, including, but not limited to, changing the timing of the *in utero* TAB exposure window (Study I), varying exposure and outcome definitions, and adjusting for extended covariables sets (Studies I-IV).

Results: In study I, we found no evidence for *in utero* exposure to TAB being associated with the risk of ADHD and epilepsy in children by 16 years of age; however, it was associated with a 1.4-fold increased risk of cerebral palsy by 6 years of age when analysing the cohort of all included children and a nearly 3-fold increased risk in the analysis of siblings.

In study II, we found no strong evidence for an association between VB-affected pregnancy and all-cause or cause-specific mortality among women in comparison with VB-unaffected pregnancy. Comparisons of VB-affected pregnancy with termination or miscarriage showed 12-15% lower hazard of all-cause and natural causes mortality; however, the residual confounding was a concern. There was no strong evidence for an association between VB in pregnancy and non-natural causes of mortality.

In study III, VB within 20 gestational weeks was associated with a 1.2 to 1.5-fold increased risk of diabetes mellitus type 1 and type 2 and multiple cardiovascular outcomes such as hypertension, ischaemic heart disease, atrial fibrillation or flutter, heart failure, and ischaemic and haemorrhagic stroke as compared with VB-unaffected pregnancy. The hazard of diabetes mellitus type 1 and type 2 and several cardiovascular outcomes (hypertension, ischaemic heart disease) was 1.3-fold increased for women with VB-affected pregnancy vs women with a termination. No associations were found for the investigated outcomes of women having VB-affected pregnancy vs miscarriage.

In study IV, we found no association of VB within 20 gestational weeks with women's risk of cancer across comparisons of women with VB-affected pregnancy vs VB-unaffected pregnancy, termination, or miscarriage.

Conclusions: Compared with TAB-unaffected children, TAB-affected *in utero* children had a greater risk of cerebral palsy, but not of epilepsy or ADHD. Having a VB-affected vs VB-unaffected pregnancy was associated with an increased risk of diabetes type 1 and 2 and multiple cardiovascular conditions in women at the end of up to 40 years of follow-up; however, it did not translate into increased all-cause or natural causes mortality risk. There was no strong

evidence for an association with increased mortality from non-natural causes following VB-affected pregnancy on the relative scale and the absolute mortality risk from non-natural causes was low. We found no association with cancer when comparing women with VB-affected pregnancy to women with VB-unaffected pregnancy, termination, or miscarriage.

This work contributes to the knowledge on women's reproductive events and different aspects of their own and their children's later health; it warrants awareness among clinical professionals, especially regarding women's long-term cardiovascular morbidity following a VB-affected pregnancy, but does not promote changes to the current clinical practice.

9. Dansk resumé (Danish summary)

Baggrund: En truende abort er vaginal blødning inden for de første 20 uger af en levedygtig graviditet i livmoderen, hvor livmoderhalsen ikke har udvidet sig. Tilstanden (herefter: 'tidligere vaginal blødning') forekommer i op mod en tredjedel af klinisk erkendte graviditeter og er forbundet med helbredsmæssige konsekvenser på kort sigt for både barnet og moren. Der findes dog kun meget lidt viden om, hvordan en graviditet med truende abort påvirker barnets og morens helbred på længere sigt.

Formål: 1) At undersøge risikoen for epilepsi, spastisk lammelse og attention deficit hyperactivity Disorder (ADHD) hos børn eksponeret for truende abort i livmoderen vs. børn uden eksponering for tilstanden, og 2) at undersøge sammenhængen mellem fødsler med tidligere vaginal blødning og kvinders risiko for død, diabetes type 1 og type 2, kardiovaskulær morbiditet og kræft sammenlignet med henholdsvis fødsler uden tidligere vaginal blødning, provokerede aborter og spontane aborter.

Metode: I studierne I-IV gjorde vi brug af danske administrative registre og sundhedsregistre og anvendte et kohortedesign. I studie I anvendte vi desuden et søskendesammenligningsdesign. Vi inkluderede alle enkeltfødte børn født i live og registreret i perioden 1979-2010 til at undersøge risikoen for epilepsi og spastisk lammelse og børn registreret i 1995-2010 til at undersøge risikoen for ADHD. I studierne II-IV identificerede vi gennemførte graviditeter via fødselsjournaler og afbrudte graviditeter (medicinske og kirurgiske eller spontane aborter) via diagnose- og procedurekoder i journaler for perioden 1979-2017 i studierne II og III og 1995-2017 i studie IV. Vi lavede time-to-event-analyser ved brug af Cox proportional hazards regression og beregnede hazardratioer med 95 % konfidensintervaller justeret for potentielle confoundere, herunder præ-graviditetskarakteristika (kvindernes komorbiditet, komedicinering og socioøkonomiske faktorer) in multivariable or inverse probability of treatment weighted models. Vi lavede adskillige sensitivitetsanalyser, herunder - men ikke begrænset til - ændring af tidsvinduet for eksponering for truende abort i livmoderen, forskellige eksponerings- og outcomedefitioner samt justering for et ekstra antal kovariable.

Resultater: I studie I fandt vi ingen evidens for, at eksponering for truende abort i livmoderen er associeret med en risiko for ADHD og epilepsi for børn i alderen 16 år. Derimod så vi en 1,4 gange forøget risiko for spastisk lammelse i alderen 6 år i analyserne af alle inkluderede børn og en næsten tre gange forøget risiko i analyserne af søskende.
I studie II fandt vi ingen sammenhæng mellem fødsler med tidligere vaginal blødning og død (af alle årsager) sammenlignet med fødsler uden tidligere vaginal blødning. Vi fandt ingen sammenhæng mellem vaginal blødning inden for de første 20 graviditetsuger og kvinders risiko for at dø af naturlige eller unaturlige årsager.
Studie III viste, at vaginal blødning inden for de første 20 graviditetsuger var associeret med en 1,2-1,5 gange forøget risiko for diabetes mellitus type 1 og type 2 samt flere kardiovaskulære udfald som for eksempel forhøjet blodtryk, iskæmisk hjertesygdom, atrieflimren og -flagren, hjertesvigt og slagtilfælde sammenlignet med fødsler uden tidligere vaginal blødning. Ved sammenligning med provokerede aborter fandt vi en 1.3 gange forøget risiko for type 1 eller type 2 diabetes og en række kardiovaskulære udfald (forhøjet blodtryk og iskæmisk hjertesygdom) for kvinder med vaginal blødning inden for de første 20 graviditetsuger. Ved sammenligning med spontane aborter fandt vi ingen association med udfaldene.
I studie IV fandt vi ingen sammenhæng mellem vaginal blødning inden for de første 20 graviditetsuger og kvinders risiko for kræft senere i livet ved sammenligning med fødsler uden tidligere vaginal blødning, provokerede aborter og spontane aborter.

Konklusion: Sammenlignet med børn uden eksponering for truende abort havde børn eksponeret for truende abort i livmoderen en større risiko for spastisk lammelse, men ikke større risiko for at få epilepsi eller ADHD. Efter 40 års follow-up havde kvinder, der har født efter en graviditet med vaginal blødning, en øget risiko for at udvikle diabetes type 1 eller type 2 samt en række kardiovaskulære sygdomme sammenlignet med kvinder uden vaginal blødning i de første 20 graviditetsuger. Dette førte dog ikke til en øget risiko for at dø af alle årsager eller af naturlige årsager isoleret set. Det var ingen sammenhæng mellem vaginal blødning og kvinders risiko for at dø af unaturlige årsager, og den absolutte risiko for død af unaturlige årsager var

lav. Ved sammenligning med fødsler uden tidligere vaginal blødning, provokerede aborter eller spontane aborter så vi ingen association med risiko for cancer for fødsler med tidligere vaginal blødning.

Disse studier bidrager med viden om hændelser relateret til kvinders reproduktion og forskellige aspekter af deres eget og deres børns helbred på længere sigt. Der er grundlag for, at man er mere opmærksom på især kvinders risiko for kardiovaskulær sygdom på længere sigt efter en fødsel med vaginal blødning inden for de første 20 graviditetsuger, men studierne danner ikke grundlag for ændringer i klinisk praksis.

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11. List of appendices

Appendix I Paper I

Appendix II Paper II

Appendix III Paper III

Appendix IV Paper IV

The papers have been removed from the file due to copyright issues