GENERAL DISCUSSION
This thesis focused on the safety of drug use in the female population. The first two chapters elaborated on the patterns and trends of drug utilization in pregnant women from the Netherlands. Drug risk assessments were also included based on the current pregnancy risk classification systems (the Dutch LAREB and the American Briggs classifications). The rest of this thesis dealt with adverse outcomes following drug exposure, exemplified in pregnancies resulting in birth defects, pregnancy complications (preeclampsia), and type 2 diabetes (T2DM). The underlying mechanisms proposed from literature were also discussed using insights from clinical pharmacology.

**Data sources of study populations**

Pregnant women and women constitute a special population for drug use, given their altered pharmacokinetic, pharmacodynamic and biological factors, compared with reference males [1-5]. The information of drug effects is limited in female patients due to insufficient data from clinical trials [2]. Notably, animal testing and knowledge of the therapeutic class are not always capable of predicting adverse drug reactions (ADRs) in the real world [6]. As a result, observational studies are needed to examine drug safety in female patients during the post-marketing phase of a drug [2, 7]. To add to the evidence synthesis for this specific high-risk group, this thesis based its studies on various real-world data sources.

*The population-based prescription database – IADB.nl*

Starting in 1998, the IADB.nl database is a joint effort of the Department of PharmacoTherapy, -Epidemiology & -Economics (PTEE), Groningen Institute of Pharmacy together with pharmacists from community pharmacies (https://iadb.nl/). By 2023, the IADB.nl database holds prescription records of approximately two million patients with 120 pharmacies involved, mostly in the Northern Netherlands (personal communication with Jens H. Bos, the data manager). Each record contains basic patient information (sex and date of birth) and medication information (the Anatomical Therapeutic Chemical ATC code, route of administration, dispensing date, total amount of drugs dispensed and dosage). The first data of a patient started when he/she registered with a pharmacy within the catchment of the IADB.nl database, which is irrespective of health insurance. This patient was then given a personal registration number, which allows to track his/her drug utilization over time, even when he/she receives medications from different pharmacies in the database catchment. The address registration number, which is, together with a personal registration number, assigned to all people of a family. The address information is, however, not recorded. The difference between an ‘address’ and an ‘address registration number’ is that when a family moves to another house within the catchment of the database, the address registration number stays the same. The newcomers at the old address will receive another address registration number (or take their own address registration number with them if they were already registered in the IADB.nl database) [8, 9].
The pregnancy database, a subset of the IADB.nl database, is generated by linking a child to his/her possible mother, who is 15 to 50 years older and shares the same address registration number. If there were more than one woman satisfying the criteria, the child and the possible mothers would be excluded from the pregnancy database (but are still included in the main IADB.nl database). This method could identify ~ 65% of the mothers in the main IADB.nl database with 99% accuracy as previously validated [9].

With the large and longitudinal coverage, the IADB.nl database is considered representative of the Dutch general population [9]. In addition, its ability to track patients over time and prospective recording of data enable a stable and almost complete medication profile for each patient, except for over-the-counter (OTC) drugs and drugs dispensed during hospitalization. This offers the possibility to study drug utilization on a large scale without being affected by recall bias [8, 10].

Based on the pregnancy IADB.nl database, Chapters 1-2 examined the patterns and trends of drug use in a pregnant population from the Netherlands, including a risk assessment. Meanwhile, Chapter 5 found an increased incident use of psychotropic medications in children prenatally exposed to reactive intermediate (RI)-inducing drugs. Based on the main IADB.nl database, Chapter 7 showed an increased risk of T2DM among users of antidepressants that antagonize M3 receptors. Interestingly, this risk was significantly higher in females than in male patients. Chapter 5, Chapter 7, and other publications [11, 12] have proven the usefulness of the IADB.nl database in applying multiple study designs such as cohort, case-control, case-crossover and case-sibling studies to increase validity in predictions or causality assessments.

The IADB.nl database, however, has some limitations. Firstly, we could not know whether the patients actually took the drugs of concern. If they had poor compliance, the drug exposure would be overestimated. Secondly, since the database does not record the indications, researchers must rely on a proxy to determine the outcome of interest. For instance, Chapter 5 considered the children’s initiation of psychotropic medications as a proxy for neurodevelopmental damage while Chapter 7 considered the use of oral antidiabetic medications as a proxy for T2DM. This may underestimate the incidence rate of outcomes in untreated cases. Thirdly, the database did not record patients’ risk factors such as smoking, alcohol use, BMI, obesity and so on, which could affect their susceptibility to the outcome of interest. Fourthly, regarding the pregnancy subset, because the mother-child linkage is based on the liveborn population, the association with strong teratogens might be underestimated since severely affected cases might be miscarried, terminated, or stillborn. Otherwise, unplanned pregnancies resulting in abortions may affect the estimates of drug utilization in the pregnant population in Chapter 1, Chapter 2, and Chapter 5. Fifthly, because the database does not capture OTC drugs and drugs dispensed during hospitalization, rates of exposure or outcomes (if drugs are used as a proxy for the disease of interest) might be misclassified. Finally, because
the database does not have information of pregnancy duration, in accordance with previous pregnancy-related literature we had to subtract 273 days from the child’s birthday (9 months of gestation) to estimate the conception date and thereby, the timings of exposure. Thus, misclassification of exposure according to trimesters might have occurred. However, we tried to limit it by including only singleton pregnancies, because the gestation period for multiple pregnancies is likely to be shorter. Given our attempts to control the potential bias caused by these limitations either by design or by analysis as discussed in previous chapters, the findings are considered the best available evidence.

*The European Registration of Congenital Anomalies and Twins Northern Netherlands – EUROCAT NNL*

Starting in 1981, EUROCAT NNL is a population-based birth defects registry in the Northern Netherlands, covering approximately 10% of all births in the country. Cases with major birth defects, including those associated with chromosomal and single gene disorders, are voluntarily notified to the registry by health professionals after parental consent [13, 14]. Live births, stillbirths, spontaneous abortions, and terminations are all eligible to be registered if the mother lived in the region at the time of delivery and the child has not reached the age of 16 at notification. There is no lower age limit. The registry personnel are actively involved in case verification, based on obstetric records, hospital administration data, pathology records and genetic sampling results. If new information becomes available before the child ages 16, his/her record is updated [14]. Approximately, 80% of parents gave consent to register their child in the registry; of those, about 80% completed a questionnaire asking about their health, lifestyle, drug use and pregnancy, and gave permission to retrieve their pharmacy data from three months before pregnancy till delivery. The actual use of the dispensings and OTC drugs was verified in a telephone interview with the mothers. Only actually used medications are registered and coded according to the ATC classification system [15, 16]. The information of birth defects is obtained from medical files, including pathology/genetic reports, and coded according to the International Classification of Diseases (ICD) coding system by the trained staff. For births up to 2001, ICD-9 is used; and for those from 2002 onwards, ICD-10 is used [15]. The EUROCAT NNL has been used by various case-control studies to examine birth defects associated with drugs or other maternal risk factors [15-19].

Compared with other birth defect registries [20, 21], the EUROCAT NNL offers a relatively reliable information of drug use since the exposure to prescribed medications is retrieved from the pharmacy that the mother is committed to, thus being unlikely to be affected by recall bias. However, the use of OTC drugs could have been affected because this information is retrospectively collected by interviewing the mother after her childbirth. Given that both cases and controls in *Chapter 4* were malformed, the recall bias of OTC drugs should affect them non-differentially. Thus, this should have minimal influence on our findings and may be biased only towards a null finding.
Compared with the IADB.nl database, the EUROCAT NNL involves an ascertainment of exposure and avoidance of exposure misclassification according to trimester since the actual use of drugs and pregnancy duration are verified with the mother. In the same way, maternal risk factors such as alcohol consumption, smoking, obesity, multiple gestation, co-morbidities, and folic acid supplementation are recorded [22]. Furthermore, because the EUROCAT NNL includes all birth types, premature fetal deaths from harmful drug exposure are included. For instance, in Chapter 4, stillbirths and terminations accounted for about 37% of cases with nervous system defects and 44% of all cases exposed to reactive intermediate (RI)-inducing drugs (30/68 registrations).

However, there are several drawbacks of the EUROCAT NNL. Firstly, the selection of controls is not random from the pregnant population. Chapter 4 selected registrations with other birth defects than nervous system defects as the first control group, and registrations with a genetic disorder as the second controls. This might underestimate the risk of RI-inducing drugs if there are defects left in the first control group being sensitive to the exposure as well. In contrast, genetic abnormalities in the second control group are probably not affected by drug exposure since they generally predate conception [23]. However, an underestimation of the teratogenic effect could occur because mothers of genetic offspring are often older, thus having more comorbidities and using more drugs (as shown in Chapter 1), which consequently increases her baseline level of exposure, compared with case mothers. Other studies selected cases from the EUROCAT NNL, but obtained controls from the live births in the general population (the pregnancy IADB.nl database) [15], or the live births without congenital anomalies (the Lifelines database) [18]. This approach may however overestimate the teratogenic risk if the exposure rate in the case group (all birth types) is higher than in the controls that survive to birth [24]. Therefore, compared with the selection of liveborn controls, our risk estimate in Chapter 4 was more conservative. Secondly, because the EUROCAT NNL only registers fetuses/children with major structural defects, it is not suitable to study functional defects and cases with minor insults or to study the developmental impact of exposure from the second trimester onward [4]. In this instance, the pregnancy IADB.nl database could be a better data source to investigate such outcomes, as shown in Chapter 5. Furthermore, since the IADB.nl database has elaborated on drug information, a post-hoc analysis of dose-response relationship could be performed to support the main finding. For instance, Chapter 5 noticed that a short course of exposure to RI-inducing drugs (i.e. ≤3DDDs) might not harm the fetal nervous system. This indicates a dose-response causal relationship according to the Bradford and Hill criteria for causality. Given the strengths and limitations of the databases in use, Chapters 4-5 thus complemented each other to detect both structural and functional defects associated with RI-inducing drugs.

The PHARMO Perinatal Research Network – PPRN

The Netherlands Perinatal Registry (https://www.perined.nl/) is a nationwide Dutch
registry that combines data from the professional registries of midwives (LVR-1), of general practitioners (LVR-h), of obstetricians (LVR-2), and of pediatricians (LNR), based on a validated probabilistic linkage [25, 26]. Pregnancies with a minimal gestational age of 16 weeks are registered, together with information about maternal demographics and medical conditions, details of the labour, and neonatal outcomes until 28 days after birth [27, 28]. Data from the Perinatal Registry could be used alone or combined with other datasets such as randomized controlled trials, particular hospital and questionnaire data, Statistics Netherlands, or PHARMO to determine factors that may influence perinatal outcomes as well as the impact of interventions on the obstetrical care system [27-35].

The PHARMO Database Network (https://pharmo.nl/) involves a dynamic cohort which is obtained from different data sources, including data from general practices, inpatient and outpatient pharmacies, clinical laboratories, hospitals, the cancer registry, pathology registry and perinatal registry. The period of data collection, catchment area and overlap between these sources however differ [27]. To ensure the patient privacy, the linkage and anonymization of the data are performed by the foundation ‘Stichting Informatievoorziening voor Zorg en Onderzoek’ (STIZON), which acts as a trusted third party between these data sources and the PHARMO Institute. The database contains, among others, drug-dispensing information such as type of the product, dispensing date, strength, dosage regimen, route of administration, prescriber specialty and costs [27]. The outpatients in PHARMO have been shown to be representative of the general Dutch population in terms of age and gender [36]. There might be some overlap between the PHARMO and the IADB.nl population, but it is probably small, because the IADB.nl database captures mostly the Northern Netherlands while the PHARMO catchment is most dense in the Brabant region, which is in the south of the Netherlands (personal communication).

In 2010, the PHARMO Perinatal Research Network (PPRN) was officially established, collaborating the Netherlands Perinatal Registry and PHARMO database. STIZON, again, acts as a trusted third party for the linkage between the two resources, based on the birthdates of the mother and child, the child gender, and their zip code. Although the Perinatal Registry captures virtually all deliveries in the Netherlands (~99% agreement with the municipal administration), a linkage with PHARMO could be established for more than 20% of these pregnancies because PHARMO includes data from about one quarter of the Dutch pharmacies [27, 37]. The combination of the Perinatal Registry and PHARMO has been used in several studies concerning drug utilization, drug safety and perinatal outcomes [29, 37-39].

Chapter 6 used this combination to examine the risk of preeclampsia in antidepressant users. A woman was considered to have preeclampsia if she had *de novo* gestational hypertension and a co-presence of non-infectious proteinuria. While the presence of proteinuria could only be retrieved from the Perinatal Registry, gestational hypertension was determined from both sources. The study population involved 15,878 pregnancies with gestational hypertension: 97.5% of
which had a diagnosis in the Perinatal Registry, while 11.6% were detected from the PHARMO database, based on the initiation of antihypertensives after week 20 of gestation. Such difference in detection rates could be accounted by the facts that (1) the Perinatal Registry has a restricted number of subcategories in which maternal diseases can be registered; thus, a diagnosis of gestational hypertension might not be registered in women that had multiple medical conditions [31]; (2) underreporting by the healthcare providers in the Perinatal Registry might also have a role [40]; (3) meanwhile, a low detection of gestational hypertension by the PHARMO data could be accounted by guidelines at the study period (2000-2010) that antihypertensives should be initiated when blood pressure exceeded 160/105 mmHg in pregnant women, which is much higher than the diagnostic value for hypertension (≥140/90 mmHg) [41]. Thus, combining the Perinatal Registry and PHARMO data could maximize the outcome detection, given the availability of maternal diagnosis, hospital data and medication profile.

Another strength of this combination involves an accurate estimation of the conception date, which is based on the last menstrual period or ultrasound. Thereby, factors that vary with gestational timings like drug exposure could be investigated. For instance, Chapter 6 found that the proportion with late-onset preeclampsia was doubled for women who continued tricyclic antidepressants beyond week 20 of gestation, compared with those who discontinued in the first trimester (8.6% versus 4.4%).

In addition, the combination of the Perinatal Registry and PHARMO data may limit the impact of confounding factors. The short review in the supplementary of Chapter 6 showed that maternal age, ethnicity, alcohol consumption, smoking, and endocrine disorders could modify either the risk of preeclampsia or the level of antidepressant exposure. These factors were recorded by the Perinatal Registry and could therefore be adjusted for in our study.

Finally, one advantage of the PHARMO database, compared with the IADB.nl database, is that drugs dispensed during hospitalization are recorded, thus enhancing the capture of exposure. Given a low prevalence of Dutch women being hospitalized during pregnancy (0.2%) [42], the misclassification of drug exposure due to lacking hospital data is thus minimal.

The Perinatal Registry and the PHARMO database, however, have some limitations. Firstly, like other pharmacy databases, we could not know whether the mothers actually used the drugs. If they had poor adherence due to being depressed, the actual exposure to antidepressants might be overestimated in Chapter 6. Notably, a recent study showed a high prevalence of adherence in the Dutch pregnant women, which was up to 88% for chronic medications and 100% for antidepressants [43]. We therefore believe that our estimate of exposure in Chapter 6 was valid. However, a study of OTC medications is not feasible, because the PHARMO database does not capture this information. Secondly, maternal diseases might be underreported in the Perinatal Registry due to the restricted number of subcategories for registration [31].
Similarly, some maternal characteristics such as level of education, family income, pre-pregnancy body mass index, food intake and other mental diagnoses are not captured by the Perinatal Registry. Because they were considered as risk factors for preeclampsia [44, 45], if being adjusted for, these unmeasured confounders could have moved our risk estimation in Chapter 6 closer to the null. Finally, the study of teratogenic effects of drugs might not be optimal with the Perinatal Registry and PHARMO combination due to the incomplete registration of birth defects. For instance, Fleurke-Rozema et al. compared the prevalence of cases with cleft lip (with or without cleft palate) identified from the two fetal medicine units (the Amsterdam Medical Center and the University Medical Center) with the respective value from the Perinatal Registry. It was found that in 37% of cases, this defect was not recorded in the delivery notes, and was thus not detected by the Perinatal Registry [46].

A recent publication by Houben et al., who is working as the administrative staff, mentioned that the PPRN could additionally be linked to the databases of the general practitioners (from 2003 onwards), the National Netherlands Cancer Registry, the Dutch Hospital Data Foundation and the national Pathology Registry (from 1998 onwards), thereby facilitating the retrieval of information on diagnoses, symptoms and laboratory test results [27]. This would reduce the issue of underreporting of maternal diseases in the Perinatal Registry, as mentioned previously. Furthermore, according to Houben et al., the PPRN could be used to follow subjects over time up to nearly 20 years after birth [27]. Such a study has not been published, but we expect that a multi-linkage database would make studies on long-latency effects more efficient.

**Literature review**

One challenge of studying birth defects associated with drug use is to obtain an adequate number of cases. Given that the baseline of major birth defects is about 3-4% in the general population, the prevalence of a specific defect is much lower. For instance, to detect a doubling risk of neural tube defects (1/1,000), a sample size of over 20,000 exposed pregnancies would be needed for a cohort study [23]. For a teratogen like valproic acid, which is taken by 2 per 1,000 pregnant women in the first trimester, the number of pregnancies that are required to observe an association with neural tube defects are even much larger [47].

Literature review is therefore a free and useful tool to detect signals of relatively uncommon effects associated with drug use. Jentink et al. [47, 48] reviewed literature for pregnancies exposed to carbamazepine and valproic acid, thereby synthesizing and comparing the prevalence of each specific defect in the exposed cohorts with the baseline value in the general population, obtained from the EUROCAT Registry of 14 European countries. A significant difference in the defect prevalent rates between the exposed pregnancies (from literature) and the EUROCAT population would signal a potential teratogenic effect of these drugs. Similarly, many studies have relied on literature review to examine adverse events during post-marketing
phase of drugs. For instance, the meta-analysis by Salvi et al. supports the risk of new-onset T2DM in antidepressant users, which has been inconsistently reported by observational studies [49]. Given that T2DM is a long-term effect, it is hardly captured in the context of clinical trials.

Chapter 3 was set to investigate birth defects associated with vascular disrupting drugs. Because of the very low number of pregnant users and cases, we could not study individual drugs. Rather, we grouped them by teratogenic sub-mechanisms: vasodilation, vasoconstriction and antiangiogenesis. The major pitfall of this method involves a heterogeneity from combining cohorts in which (1) the exposed mothers might differ markedly in their demographic characteristics (unmeasured) and the diseases for which drugs were indicated, and additionally (2) the definition of a birth defect (major or minor) could vary among countries/medical settings, thereby affecting the reported rates. Using a similar approach with Jentink et al. [47, 48], Chapter 3 selected the exposed pregnancies from literature, thereby synthesizing the prevalence of birth defects and comparing with the referent values of the EUROCAT population. Although we found some 'signals' of birth defects following drug exposure, the establishment of possible relations was not straightforward, because the exposed group and the reference (from the general population) are not comparable concerning data collection and composition. Therefore, Chapter 3 should be considered as an exploratory study that merits further research to determine the risk of 'signals'; for instance, esophageal atresia with antiangiogenic agents and craniosynostosis with vasoconstrictors.

Selecting study population and data collection methods to study adverse drug reactions

Due to ethical and legal issues, it is not always feasible to conduct clinical trials in women and pregnant women [2, 7]. Observational epidemiologic research is therefore important to study drug safety in such special populations. Various ways of data collection have been proposed.

Direct data collection such as pen-and-paper questionnaires is traditionally used to gather information from the subjects and/or their health care providers [50]. This method, however, has faced a decline in recruitment rates over the last decades. This is accounted by, but not limited to, the reluctance of people who have been asked to cooperate in a lot of research, the absence of an incentive, the burdensome follow-up requests, and the lack of interest when the study topic is not salient to the participants’ lives (for instance, in case-control studies, participation rates of cases who suffer from the defects are generally higher than those of controls) [51]. The questionnaire design is also a challenge since it should not be too long or too private to provoke irritation from participants but should be enough to collect necessary information and/or adjust for potential confounders; besides, the communicating language should be plain and clear. Similar issues were observed with the use of web-based questionnaires to collect information [52]. Furthermore, this method has
the possibility of selection bias that participants could differ substantially at several points from the target population. Loss of follow-up is also high for those enrolled via the internet [52]. Finally, due to the limited content of questionnaires, the direct methods can only address one or a few research questions that the questionnaires have been designed for.

Indirect data collection was used in this thesis where the study populations were obtained from available pharmacy prescription databases such as the IADB.nl (Chapters 1, 2, 5, and 7) and the PHARMO (combined with the Perinatal Registry in Chapter 6), from the birth defects registry EUROCAT NNL (Chapter 4), and from literature review (Chapter 3). As discussed previously, each source of population has its own strengths and limitations. Furthermore, the establishment of a database/registry is time consuming and demands trained staff and extensive cooperation. However, based on our experience, these sources are valuable since they can be used to address multiple research questions concerning drug use and to enable follow-up studies throughout the marketing lifecycle of a medication, which could not be done with the direct data collection methods. Given the population-based nature of these sources, they facilitate policies concerning drug utilization and public health.

**Adverse drug reactions in the view of clinical pharmacology**

Pharmacology deals with the study of drugs. Meanwhile, clinical pharmacology deals with the study of drugs in humans to promote the safe and effective use [5]. Some ADRs are predictable from pharmacology; others are rare, unpredictable or long latent, and thus can only be detected by pharmacovigilance during post-marketing phase [6]. Using available databases and registries, or reviewing literature, this thesis studied adverse reactions concerning drugs used by pregnant women and women.

As suggested by Gelder et al., there are at least six mechanisms by which drugs may harm the unborn child, including vascular disruption, oxidative stress, folate antagonism, neural crest cell disruption, endocrine disruption, and specific receptor- or enzyme-mediated teratogenesis [53]. The former two have been implicated in the mechanisms of major teratogens like thalidomide and phenytoin [54, 55]. **Chapters 3-5** focused on these mechanisms.

**Vascular disruption**

Vascular disruption caught our interest as the cardiovascular system is the first functional organ to develop in the mammalian embryo. As the embryo grows beyond a size allowing for passive diffusion of gas, nutrients, and metabolic wastes, the formation of a cardiovascular system is needed to support the development of organ rudiments [56]. This comprises two successive processes: vasculogenesis and angiogenesis. Vasculogenesis refers to the formation of nascent vessels, which involves the migration, proliferation, and assembly of endothelial cell precursors (angioblasts) into endothelial tubes, thereby creating a primary vascular plexus. During angiogenic process, preexisting vessels are remodeled, and new capillaries...
emerge. The vessels are then stabilized by recruitment of pericytes and vascular smooth muscle cells to form a mature circulation [56, 57]. These processes are tightly regulated by multiple signaling molecules (Flk-1, Ephrin-B2, EphB4, Sonic hedgehog, vascular endothelial growth factors, platelet-derived growth factor β, transforming growth factor β, angiopoietin-1,...) [56]. Depending on the chemical properties and timings of exposure, vascular disrupting agents may interfere with these signaling molecules or act directly on the vascular structure, resulting in embryonic deaths and/or a wide range of structural birth defects concerning the tissues affected [53, 57]. Data on birth defects related to vascular disrupting drugs are mostly limited to animal experiments or sporadic human cases, and have not been elucidated from cohort studies due to sample size limitation [23]. Chapter 3 was therefore set out to detect possible ‘signals’ of birth defects, accounting for different disrupting mechanisms: vasodilation, vasoconstriction, and anti-angiogenesis [53].

In the mother, vasodilators may cause a significant drop in blood pressure, thereby decreasing blood flow to the utero-placental unit and leading to hypoxic responses; meanwhile, in the fetus, dilated capillary plexus vessels are prone to rupture [58, 59]. Because maternal blood pressure is physiologically reduced in the first trimester [60], use of antihypertensives, including vasodilators, are often decreased accordingly (Chapter 1). An (inadvertent) exposure to vasodilators in the first trimester (i.e., by continuing use in those with pre-existing hypertension) could therefore have a negative impact on maternal blood pressure and the unborn child. Otherwise, vasoconstrictors may inhibit blood flow to the placenta and in the fetus, thus decreasing oxygen and nutrient supply [57, 58]. The fluctuating states of hypoxia, generated by vasodilators and vasoconstrictors, may also induce oxidative stress in the developing tissues [61]. Meanwhile, the teratogenic mechanism of antiangiogenic agents involves the inhibition of signaling molecules, resulting in death of immature endothelial cells, loss of vessel integrity and disruption of angiogenesis [57].

By comparing the prevalence of each defect in the exposed offspring (synthesized from the literature cohorts) with the respective value in the general population (obtained from the EUROCAT database of 22 European registries), Chapter 3 detected ‘signals’ of four main groups and 12 subgroups of specific birth defects in pregnancies exposed to vasodilators (complete absence of a limb), vasoconstrictors (craniosynostosis), and antiangiogenic agents (nervous system defects; neural tube defects; eye defects; anophthalmos; ear, face and neck defects; anotia; cleft palate; digestive system defects; oesophageal atresia and duodenal atresia or stenosis; renal dysplasia; congenital hydronephrosis; limb reduction; upper limb reduction; and complete absence of a limb). These results should however be interpreted with caution because most ‘signals’ had fewer than five exposed cases. Otherwise, esophageal atresia was detected in 6 exposed cases: three on both tacrolimus and mycophenolate, one on tacrolimus only, and one on mycophenolate only. Craniosynostosis was detected in five cases exposed to ergotamines and/or triptans. It is noteworthy that the cohort study [62], which contributed most exposed craniosynostosis cases (4 out of 5)
showed no risk of the drugs, using the Swedish general population for comparison. Possible explanations for this different risk estimation are that (1) Sweden has a higher baseline prevalence of craniosynostosis than other European countries (i.e., 2.7 times higher than the EUROCAT population), and that (2) different countries may have different definitions of craniosynostosis and different methods of data collection [62, 63].

Although cohort data permit evaluation of the entire spectrum of developmental outcomes, they have less power for evaluating rare defects. Therefore, Chapter 3 additionally discussed case-control studies: three concerning vasodilators (calcium channel blockers), two concerning vasoconstrictors (one on ergotamine, and one on sumatriptan), and none on antiangiogenic agents. The ‘signals’, identified from the exposed cohorts, were however not detected in any case-control studies. However, one case-control study noticed a ‘signal’ of neural tube defects with ergotamine exposure. There were several reasons for not detecting this ‘signal’ in the exposed cohorts. Firstly, this case-control study focused on the exposure during the first two months of gestation, which is the critical period for neural tube formation [64]. Secondly, ergotamine is more vasoconstrictive than triptans [65], which were mainly used in the exposed cohorts; thus, combining them into one exposure group of vasoconstrictors might have diluted the ‘signal’. However, we could not analyze ergotamine in the exposed cohorts separately, because the only study with ergotamine users pooled ergotamine and triptans together [62]. Thirdly, the size of the exposed cohorts was too small to detect the risk of neural tube defects. Given that neural tube defects occur in only 1 per 1,000 pregnancies in the general population [66], a sample size of over 20,000 exposed pregnancies would be needed to detect a doubling of risk. However, in total, we only had 3,968 vasoconstrictors-exposed pregnancies. The same issue concerns ‘signals’ of other birth defects. Larger research is therefore warranted to corroborate our findings.

**Oxidative stress**

Considering the source of oxidative stress generation, we identified drugs that are potentially bioactivated into reactive intermediates (RI). Consequently, the case-control study in Chapter 4 suggested nervous system defects as the most sensitive to RI-inducing drugs. Meanwhile, the cohort study in Chapter 5 found a significantly increased incident use of psychotropic medications, a proxy for neuronal damage, among children prenatally exposed to RI-inducing drugs. Our findings are supported by literature that found wide-spread neuronal death in the developing and adult murine brain under RI-generating conditions such as hypoxia, high glucose concentrations, and ionizing radiation [67-69]. Furthermore, human studies observed a high level of oxidative markers in adult patients with psychiatric disorders [70-72].

It should be borne in mind that the results in Chapters 4-5 do not preclude other teratogenic effects of RI-inducing drugs [53]. Yet, we reported that the nervous system
was the most vulnerable to oxidative stress among all other tissues, which is consistent with literature [55, 69]. On a biologic basis, this is accounted by the high metabolic rate of the brain, its high oxygen utilization, high content of oxidizable polyunsaturated fatty acids, high amount of metals catalyzing free radical formation (Cu, Fe), low levels of antioxidants and reduced capacity for cellular regeneration [67, 68].

Folates (folic acid and derivatives) may scavenge free radicals from oxidative stress [73] or link to the metabolism of glutathione, a cellular antioxidant [74]. Interestingly, literature has shown that periconceptional folate supplementation decreases the risk of neural tube defects, one important insult of the nervous system [75]. Furthermore, Chapters 4-5 noticed that 8 out of the 48 RI-inducing drugs possessed folate-antagonistic properties, and another five drugs (taken by the study population) could disturb folate absorption and/or metabolism. Remarkedly, folate antagonism is one of the six teratogenic mechanisms proposed by Gelder et al. [53]. Altogether, these might mediate the risk of RI-inducing drugs. Therefore, we hypothesized that folate supplementation might reduce (Chapter 4), while folate antagonism might aggravate the risk of RI-inducing drugs (Chapters 4-5). Consequently, our hypotheses were proven but the change in risk estimates was small when folate supplementation and folate antagonism were additionally accounted for.

Because RIs are not stable enough to be transported from the mother to the fetus, they must be generated within the fetus to exert oxidative stress [55]. Hence, the risk depends on the level of RI exposure, the capacity to bioactivate drugs into reactive metabolites, and the antioxidant defense of the fetus. The former, in turn, depends on maternal drug metabolism, which is affected by maternal aging and genetic variations: the more a drug is metabolized by the mother, the less it can be transferred to the fetus and thus, the less harmful it is. Otherwise, the level of RI exposure could be enhanced by maternal characteristics such as smoking, alcohol consumption and diabetes which are other sources of oxidative stress [55, 68, 76, 77]. Meanwhile, the capacity of the fetus to bioactivate drugs depends on the gestational timings since only a few of metabolizing enzymes are expressed in the first trimester such as prostaglandin H synthases, CYP1A1, CYP3A7, FMO and ADH1. As pregnancy progresses, the number of fetal enzymes increases; in other word, the generation of RIs increases [55, 78]. On the other hand, the antioxidant defense of the fetus is very weak in the early stage of organogenesis but increases significantly thereafter [61], counteracting the impact of oxidative enzymes. Since many of these factors could not be measured, our findings could have been affected. Using the EUROCAT NNL Registry, Chapter 4 limited some of their influence by adjusting for smoking, alcohol use and maternal age; we did not adjust for pre-existing diabetes because it was similarly distributed among cases and controls. Chapter 5 was based on the pregnancy IADB.nl database; thus, only maternal age and use of antidiabetics (a proxy for maternal diabetes) were captured. Therefore, a dose-response analysis was additionally performed in Chapter 5 to strengthen our hypothesis of oxidative stress mechanism. We found that the incident use of psychotropic medications increased
significantly and proportionally in children with increased exposure to RI-inducing drugs (3-14 DDDs and > 14 DDDs, compared with ≤3 DDDs).

**Adverse drug reactions with a focus on classes of action and specific transporters/receptors, concerning the pregnant users**

Risk assessment of drugs used during pregnancy is currently based on their known or suspected harm on the unborn child. To which extent that drugs may harm the mother has not been studied extensively. **Chapter 6** addressed one of such issues: the risk of preeclampsia in antidepressant users. Pregnant women with no use of antidepressants during 15 months before delivery were included as reference.

Traditionally, antidepressants are classified as selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants (TCAs), serotonin norepinephrine reuptake inhibitors (SNRIs) or monoamine oxidase inhibitors (MAOIs), based on the shared properties of the compounds on the monoamine theory of depression [79]. Yet, literature studying the risk of preeclampsia based on antidepressant classes of action has shown inconsistent results [44, 80-83]. The paper by Gentile, however, pointed out the limitation of analyzing antidepressants by pharmacological classes, because their own receptorial affinity varies widely among specific compounds [84]. Therefore, **Chapter 6** classified antidepressants by their classes, and additionally by their transporter/receptorial targets such as the serotonin and norepinephrine transporters (SERT_NET), serotonin receptors (5-HT₁B, 5-HT₂A), adrenergic receptors (α₂B_AR, α₂C_AR), histaminergic receptors (H₁, H₂) and cholinergic receptors (M₁, M₂, M₃ muscarinic, alpha-7 nicotinic), which are present in the placenta and may contribute to the pathophysiology of preeclampsia with their vasoactive and/or antiangiogenic effects [85-114].

Based on the difference in etiology, **Chapter 6** categorized preeclampsia into the early-onset (occurring before week 34 of gestation) and late-onset subtypes. The former is associated with abnormal placentation, whereas the latter is found in mothers who are highly susceptible to apoptotic trophoblast material, or of whom systemic vasculature is altered by a partly affected placenta [115]. Because the placenta is fully formed by 20 gestational weeks [116], this period was selected as the primary exposure window of antidepressants; an extended use thereafter was also studied.

**Chapter 6** did not confirm the risk with SSRIs, which was suggested Toh et al. [44], but refuted by others [81-83]. Given that Avalos et al. [45] showed a stronger association for ‘any’ use of SSRIs (including combinations) compared with the SSRI monotherapy, and that De Ocampo et al. [83] found an elevated risk with exposure to ≥2 antidepressant classes, a synergistic mechanism involving the serotonin transporters and an additional pathway could have contributed to the risk of preeclampsia. Our study, however, could not examine the impact of the antidepressant combinations due to the low numbers of exposed cases, given the
Dutch guideline that prefers antidepressant monotherapy in pregnant users [117].

Chapter 6 could not confirm the risk with SNRIs, which was proposed by Palmsten and Bernard et al. [80, 81, 118], but refuted by De Ocampo and Avalos et al. [45, 83]. A possible explanation is that we, De Ocampo and Avalos et al. excluded women with pre-existing hypertension, while the other authors did not. It should be noticed that Palmsten et al. found no increased risk of preeclampsia in women who started SNRIs after the first trimester [81].

Chapter 6 confirmed the risk with TCAs, which is reported by Palmsten et al. [80, 81]. Yet, we could not know whether this finding is accounted by their therapeutic effect [79]. Rather, TCAs block a wide range of transporters and receptors, which could synergistically work on the risk of preeclampsia. As evidenced in Chapter 6, the risk associated with the antagonism at α₂c adrenergic, H₁, H₂, M₁, M₂, M₃ and alpha-7 nicotinic receptors was nullified when TCA users were excluded.

No studies have found the risk with MAOIs due to the limited number of exposed pregnancies.

Interestingly, Chapter 6 found the risk of early-onset preeclampsia among those with extended use of 5-HT₂A antagonists, excluding TCA users. This was supported by the biologic basis about the deleterious effect of 5-HT₂A antagonism. Physiologically, peripheral serotonin is elevated during pregnancy, stimulating 5-HT₂A receptors which in turn upregulate local estrogen production in the placenta, thereby affecting trophoblast function, angiogenesis, and placental development [86]. In a reciprocal manner, rising levels of estrogens and progesterone during pregnancy increase the density of 5-HT₂A receptors, activating downstream pathways of placental cell lines to enhance cell viability and cell cycle progression [86, 88, 89]. A dysregulation in this co-ordination by targeting 5-HT₂A receptors could therefore trigger abnormal placentation and the development of early-onset preeclampsia. Alternatively, the antagonism at 5-HT₂A receptors may cause vasodilation, depending on the vessels that they are located, thus enabling a local rather than systemic effect [119, 120]. Given the presence of 5-HT₂A receptors in the uteroplacental bed, vasodilation may cause a hyperoxic status which, if happening prior to weeks 10-12 weeks, may result in loss of placental mass [86, 115].

Notably, among six preeclamptic cases exposed to the 5-HT₂A antagonism, five had an additional inhibition of the serotonin reuptake transporter (SERT): three took fluoxetine (which is also an SSRI), one took the combination of mirtazapine and paroxetine (an SSRI), one used trazodone (which also inhibits SERT moderately). Therefore, it is possible that SERT inhibition synergistically increases the damage of 5-HT₂A antagonism on the placenta [119, 121], and thus the risk of preeclampsia.

Importantly, based on the abundant information of the Perinatal Registry and PHARMO linkage, Chapter 6 excluded users of antiangiogenic agents and adjusted for maternal characteristics (age, ethnicity, alcohol consumption, smoking during...
pregnancy, concurrent use of vasoconstrictors and endocrine disorders), which could modify the risk of preeclampsia and/or the metabolism of antidepressants [44, 122-125]. However, maternal depression, for which antidepressants are mainly indicated, could have confounded our results. A sensitivity analysis was thereby performed in Chapter 6, comparing users of TCAs and 5-HT$_2$A antagonists with SSRI users. Briefly, the increased risks with TCAs and 5-HT$_2$A antagonists were determined.

**Adverse drug reactions with a focus on target receptors, concerning the female population**

An understanding of structure-activity relationships, shared by members of a given drug class, is considered helpful in predicting the efficacy and adversity of this class. Indeed, this view has been incorporated into regulatory action in the form of class labeling [23]. The Naranjo Scale also considers ‘an adverse reaction to a structurally related drug in the past’ as an increase in probability of adversity for the medication under investigation [126]. The case with glutethimide/thalidomide [127] as well as our illustration in Chapter 6 however showed that this might not hold for teratogenesis and some other ADRs.

In corroboration, Chapter 7 differentiated the risk of new-onset T2DM among antidepressants, based on their antagonism at M$_3$ muscarinic receptors. The biologic basis comes from observations that short-term inactivation of M$_3$ receptors in the brain and pancreas decreases fasting plasma insulin and glucose-stimulated insulin response, whereas chronic exposure could lead to hyperinsulinemia and hepatic insulin resistance due to a compensatory upregulation of M$_3$ receptors over time [128].

A case-control study was conducted in Chapter 7, using a subset cohort from the IADB.nl database who initiated antidepressants between ages 20 and 40 years and who did not receive any antidiabetic prescriptions at baseline. Compared with no use of antidepressants during the last two years, exposure to M$_3$-antagonistic antidepressants significantly increased the risk of new-onset T2DM, even when antipsychotics users were excluded. A dose-response relationship was detected at $>365$ DDDs. Interestingly, Chapter 7 noticed a sex difference, showing a significantly elevated risk of T2DM in females exposed to M$_3$-antagonistic antidepressants (by 63%), but not in male users. Conversely, antidepressants that do not antagonize M$_3$ receptors showed no association with T2DM at any stratified analyses (age, sex, co-morbidity, and dose-response).

The imbalance of sex distribution in clinical trials has limited analyses concerning sex differences in drug effects [7]. Post-marketing surveillance is thus useful to detect this issue. Chapter 7 were consistent with literature that recommended a higher rate of ADRs among female users [2, 3]. Although the risk of T2DM concerning M$_3$ receptors is biologically based [128] and was evidenced in our study population, the association with female sex warrants further investigation so that our finding can contribute to
treatment decisions for depressed female patients who are at risk of glucose intolerance.

**Drug utilization is dynamic in the ‘pregnant’ world**

Drug utilization varies markedly across pregnancy trimester and over the years. Firstly, it is affected by common physiological and pathological changes during pregnancy [60, 129-131]. Using the Dutch pharmacy IADB.nl database, Chapter 1 showed that use of drugs for acid-related disorders, laxatives and drugs for anemia increased as pregnancy progressed. Meanwhile, antiemetics and propulsives peaked in the first trimester when hyperemesis gravidarum is most prevalent. Physiologically, blood pressure decreases in early pregnancy but rises thereafter due to increased cardiac output and vascular resistance [60]. This corresponded to changes in dispensing rates of antihypertensives. In pathological conditions such as preeclampsia, thrombosis, and gestational diabetes [60, 132, 133], use of antihypertensives, antithrombotic agents and insulins could increase beyond their preconceptional values. Such varying patterns of therapeutic classes were similarly observed in our study, as well as those conducted in other countries [38, 134-141].

Secondly, drug utilization is affected by treatment guidelines and drugs-related policies. For instance, meclozine is the first, and metoclopramide is the second choice for hyperemesis gravidarum in the Dutch guideline [142]. Accordingly, Chapter 1 found them the most popular antiemetics prescribed to the Dutch pregnant women with an upward trend over the years. Elsewhere, the preferred agents were prochlorperazine, promethazine, and cyclizine in the UK [143]; doxylamine, metoclopramide, promethazine, and dominantly ondansetron in the US [144]; and doxylamine/pyridoxine in Canada [137]. Our finding of the reduced use of antidepressants during pregnancy could be accounted by the Dutch guideline to wean or adjust antidepressants to the lowest dosage of effectiveness in pregnant women [117]. Similar practices were reported in New Zealand, the US, France and Finland [136-138, 140, 141]. Notably, Chapter 1 showed an abrupt cut-off of gonadotrophins and clomifene to almost nil after the new Dutch IVF policy that limits reimbursement [145].

Thirdly, risk potentials may have a role in drug utilization. Chapter 2 found that the number of pregnancies exposed to potentially harmful medications declined during the first and second trimesters. This indicated a conservative step in prescribing during the sensitive periods of structural and functional malformations [4]. Additionally, we found a preference for oxazepam and temazepam over other benzodiazepines due to their short half-life, thus lowering fetal risk [146]. We also found a downward trend of paroxetine in the Netherlands, Canada, the US and Ireland [137, 144, 147], coinciding with its reports of fetal heart anomalies that emerged since 2005 [148, 149]. Simultaneously, Chapter 2 noticed an upward trend for other safer SSRIs such as citalopram and sertraline, which was similarly observed in the US [144]. Another example is shown in Chapter 1 that the dispensing rate of omeprazole and other proton pump inhibitors in the Dutch pregnant population has doubled over
the last decade. Correspondingly, proton pump inhibitors are considered ‘safe’/‘low-risk’ by the Dutch LAREB and the American Briggs classifications, but previously it was assigned with FDA category C that ‘risk cannot be ruled out’ [129].

Finally, effectiveness may also determine drug utilization. Chapter 2 showed that utilization rate of nitrofurantoin doubled over 2005-2018, whereas that of amoxicillin, which is considered ‘most safe’ in pregnancy [36], decreased by a quarter. This is consistent with the trend in the Dutch general population that nitrofurantoin is effective but amoxicillin has increased resistance rates in urinary tract infection (the most common infection in pregnant women) [150].

The roles of mediating factors in adverse drug reactions

Patient characteristics may affect their susceptibility to ADRs [151]. Female sex is a risk factor. It has been shown that at a given dose, a drug tends to result in higher unbound concentration, longer half-life, or hypersensitivity in women than in men [2]. Chapter 7 is an example where we found a higher risk of T2DM in females exposed to M₃-antagonistic antidepressants, compared with male users. This finding is similar to that reported by Khoza et al. [152]; however, the types of antidepressants were not specified in their sex comparison.

Age is the second risk factor for ADRs. Chapters 1-2 concerned drug use in the pregnant population, thereby suggesting a potential risk to pregnancies born from mothers of advanced age, who had increased exposure to polypharmacy and potentially harmful medications, as compared with younger mothers. Otherwise, Chapter 7 categorized antidepressant users by their age at T2DM onset (<45 and ≥45 years), thereby showing a significant association with M₃-antagonistic antidepressants in the younger age group; meanwhile, no association was detected in the older. This is consistent with Wu et al. [153]. Given that literature has suggested an increased incidence of T2DM in people over 45 years old due to a weakening of the antioxidant defense system [154, 155], the high baseline risk of T2DM in this population might have diluted the association with M₃-antagonistic antidepressants.

Comorbidity is the third risk factor. Chapter 7 found no increased risk of T2DM in antidepressant users who had concurrent cardiovascular diseases and dyslipidemia. Meanwhile, the risk was markedly elevated for those without such co-morbidities. This could be accounted by the fact that these co-morbidities may share a common genetic pathway with T2DM [156] or result from the use of antidepressants [157]. Like aging, co-morbidities might increase the baseline risk in affected patients, thus diluting the impact of M₃-antagonistic antidepressants. Therefore, pharmacovigilance studies should account for these potential confounders in evaluating ADRs.

Interestingly, literature notices an association between aging and chronic comorbidities requiring treatment [158-161]. Consistently, Chapters 1-2 showed a substantial prevalence of chronic drug use (a proxy for chronic comorbidities) in mothers of advanced age before conception (p<0.001). As pregnancy progressed, we
observed that polypharmacy and use of potentially harmful medications were also more common in mothers with a chronic history than in those without. Additionally, among the former, polypharmacy and harmful medication use was positively associated with aging.

Though not directly measuring the impact of ethnicity, alcohol consumption, smoking and co-medications (vasoconstrictors, antiangiogenic agents), the supplementary of Chapter 6 has a nice review of how these factors, together with age and co-morbidities (endocrine disorders), could affect the level of drug exposure (antidepressants) and/or the outcome of interest (preeclampsia). Chapter 6 therefore adjusted for these factors in the risk calculation.

Finally, given the availability of data sources, factors including, but not limited to, level of education, family income, and food intake/supplements were not captured in this thesis, and thus might have affected our findings [44, 45]. Further research should be prompted.

**Future perspectives**

In this thesis, we hypothesized an ADR based on existing biologic evidence and thereby examined it in observational human studies. By grouping drugs of the same detrimental mechanism together, we could gain more statistical power and preserve the characteristic of the proposed mechanism. The problem with this method is that we created a heterogenous exposure group because each drug might have its own mechanistic contribution to the adverse event. For instance, methotrexate and cyclosporine commonly causes vascular disruption; besides, the former could induce folate antagonism, while the latter could generate oxidative stress [53, 162-164]. When the numbers of exposed cases allowed, we tried to examine drugs individually. The supplementary of Chapter 5 and Chapter 7 showed elevated risk estimates with most drugs, which seems to be a generalized effect. In addition, the dose-response relationships in Chapter 5 and Chapter 7 have strengthened our suggestive mechanisms. Therefore, we propose that clinical pharmacologists consider studying ADRs based on their potentially harmful mechanisms.

As discussed previously in Chapter 7, patients with certain factors like advanced age and co-morbidities might have a higher baseline of risk than those without. Hence, the estimate of drugs-related risk could be mitigated in these patients. We propose that subgroup analyses or adjustment for potential confounders should be considered, when appropriate, in pharmacovigilance studies. Otherwise, unmeasured factors could bias risk estimates either closer or further from the null.

Chapter 7 analyzed the cumulative dosing regimens, showing that use of M₃-antagonistic antidepressants at >365 DDDs had the strongest impact on T2DM development. Besides, Chapter 6 and the study by Palmsten et al. [81] had some discussions about the risk of preeclampsia that was (1) highest in women who continued antidepressants beyond week 20 of gestation, (2) slightly reduced in those
who discontinued earlier, and (3) negligible in those who initiated antidepressants after the first 20 weeks of gestation. Together, these indicate the potential of a long-latency effect that might not be captured during the clinical trial phase. In agreement with Santoro et al. [165], we propose that continuous ADR monitoring is needed throughout the life cycle of a medicinal product to promote and protect public health.

Illustrations in Chapters 1-2 show that drug utilization is dynamic in the ‘pregnant’ world. Given the various impact of drugs-related policies, knowledge of drug efficacy and safety, the birth of new medications and updated guidelines, drug utilization is dynamic in the non-pregnant as well [166-168]. We propose that periodic analysis of drug use data should be made to rationally allocate intervention strategies and post-marketing surveillance, targeting novel and other pharmaceutical products that are likely to be used by pregnant women and other special populations.

Finally, due to the limited exposed cases and insufficient information of dosing regimens in Chapters 3-4, we could neither perform a dose-response relationship nor analyze the teratogenic risk for individual drugs. This may also happen to newly approved medications in which the number of exposed patients is slowly accumulated. The same issue is in Chapter 6 that the association between 5-HT2A antagonistic antidepressants and early-onset preeclampsia was based on only six exposed cases. Given a high rate for suicide attempts in depressed women [169], pharmacotherapy might be necessary during pregnancy. Switching to an antidepressant without 5-HT2A antagonism could be considered in preconception counseling. To contribute to treatment decisions, larger population-based studies combining various data sources are needed to corroborate our results.

**Conclusions**

To conclude this thesis, the female population showed an increased risk of ADRs, including the potential harm on the unborn child and maternal health when they get pregnant, as well as the higher susceptibility to the drug effects, compared with male users. Furthermore, the dosing regimen and patient characteristics like aging and comorbidity might contribute to this risk significantly. Since this thesis was based on the availability of data sources, we could not preclude the contribution of other unmeasured factors. Further studies to identify such factors are needed to facilitate intervention strategies for drug use. Another important feature of this thesis was that drugs were grouped according to their potential harmful mechanisms, in addition to their pharmacological classes. Our results with antidepressants suggest that clinical pharmacologists be aware of drugs with complicated effects, given their affinity toward multiple biological targets and the long latency for certain ADRs to emerge. A differentiation between therapeutic and adverse actions of these drugs would contribute to treatment decisions in the population of concern.
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