Use of corticosteroids in pregnancy

With special focus on the relation to congenital malformations in offspring and miscarriage

PhD thesis

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Preface

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Chapter 1. Background

1.1 Introduction

Congenital malformations have been known through the human history. Through centuries a congenital malformation was viewed as a punishment for sins. Elements of this perception persist today as guilt, and as parents search for the cause of the malformation, their attention often focuses on the drugs used in pregnancy.[1]

Until 70 years ago, it was believed that the placenta protected the fetus aginst all noxious agents.[1] This belief was first shattered in 1941 by the recognition that maternal rubella infection in pregnancy produced a distinctive pattern of malformations.[2] In December 1961, McBride described a case series of children born with major limb reduction (phocomelia) among women who had used thalidomide during their pregnancy.[3] Because phocomelia is an uncommon congenital malformation, the finding strongly suggested a causal link with thalidomide. However, thousands of infants over many years were born with this congenital malformation before the causal link was confirmed.[4] The thalidomide catastrophe showed the teratogenic potential of antenatal drug exposure.[3,5]

Drug therapy during pregnancy involves specific pharmacological problems. Any drug or chemical substance administered to a pregnant woman may cross the placenta, and the fetus is often unable to metabolize the drug in the same way as the pregnant woman.[6] The fetus is vulnerable to exposure during all steps through the reproductive process, e.g. the brain continues its development during the breast-feeding period.[7] However, the first trimester is the most vulnerable period with respect to structural malformations because most fetal organs are formed during gestational weeks 5 to 12.[7] Often, women are unaware of their pregnancy in its early weeks making it difficult to prevent harmful exposures in this period.[8]

A special group of pregnant women is the women with medical conditions that necessitate drug use in pregnancy. Asthma, which is one of the most common medical conditions among pregnant women, was estimated to affect approximately 4-8% of all pregnancies in the USA between 1997 and 2001.[9] The prevalence of asthma increased two-fold among pregnant women from 2.9% in 1976-1980 to 5.8% in 1988-1994.[9] Types of medical conditions affecting

pregnancy can be identified by hospital contacts.[10] In the USA, the overall reported rate of antenatal hospitalization was 10.1 per 100 deliveries in a managed-care population of over 46,000 pregnant women.[11] About one-third of these hospitalizations were for non-obstetrical conditions such as pulmonary, infectious, and gastrointestinal diseases. In a recent Australian study including 55,002 women who had their first birth in 2005-2006, 2,4% of the women had preexisting asthma/chronic obstructive pulmonary disease (COPD), 1.6% had preexisting psychiatric disorders, 1.0% had preexisting hypertension, 0.8% had a preexisting autoimmune disease, and 0.6% had preexisting diabetes.[12] Other medical conditions that commonly affect women of childbearing age are inflammatory bowel disease[13] and rheumatoid arthritis.[14] Inflammatory bowel diseases are common in North America, the UK, and Scandinavia with annual prevalence rates per 100,000 population reported as 40-100 for ulcerative colitis and 4-6 for Crohn's disease.[15] Approximately 50% of patients with inflammatory bowel diseases are less than 35 years of age at the time of diagnosis and 25% conceive for the first time after their diagnosis.[13] Furthermore, inflammatory bowel disease among women in Denmark has increased two-fold from 1978-2002.[16] Rheumatoid arthritis, a chronic systemic autoimmune inflammatory disease, most often affects women.[17] The prevalence of rheumatoid arthritis was 0.2% among pregnant women giving first-time singleton birth in Denmark from 1994 to 2006.[14]

Prescribed drug use can be another marker of chronic and acute morbidity in pregnant women as the prescribed drug use can be a surrogate for the status of the chronic disease.[18] Increase in prevalence of chronic diseases among pregnant women would be expected to be followed by a concurrent increase of drugs prescribed for treatment of these medical conditions.

In this thesis, we focus on use of corticosteroids, which are commonly used to treat asthma, inflammatory bowel disease, and rheumatoid arthritis as well as other medical conditions.[19,20] We provide an overview of utilization of corticosteroids in pregnancy, with special focus on associations with congenital malformations in offspring and miscarriage.

1.2. Considerations regarding drug utilization in pregnancy

In 1977, drug utilization was defined by the World Health Organization as the "marketing, distribution, prescription, and use of drugs in a society, with special emphasis on the resulting

medical, social, and economic consequences".[21] A narrower definition includes only "the prescribing, dispensing, and ingesting of drugs".[22] Both definitions imply that drug utilization is defined not only by pharmacological factors, but also by demographic, social and economic forces. Secular trends in the characteristics of pregnant women could thus affect drug utilization in pregnancy. Notably, in developed countries there is now a trend for women to delay childbearing until a relatively late reproductive age.[23] In Denmark, the prevalence of first-time mothers older than 30 years increased from 29% in 1997 to 41% in 2007.[24] An increasing proportion of older first-time mothers could increase the overall morbidity of pregnant women as a group. For example, the prevalences of diabetes, hypertension, and rheumatoid arthritis all increase with increasing age[14,25] and this could lead to a trend of increasing prevalence of drug use in pregnant women over time.[26]

As a consequence of the thalidomide catastrophe, every drug was feared to be a potential new thalidomide. However, during the 50 years following the thalidomide catastrophe, only 50-60 drugs of more than 1,000 drugs available at the marked proved to be teratogenic and are contraindicated in women who are or may become pregnant.[6] Anxiety in relation to drug use during pregnancy may result in discontinuation of necessary drug treatment. An untreated medical condition can put both mother and fetus at risk.[26] For example, untreated asthma has been associated with an increased risk of maternal morbidity, e.g. exacerbations,[27] untreated diabetes has been associated with an increased risk of fetal death,[28] and untreated urinary tract infections have been associated with an increased risk of preterm birth.[29]

The associated challenges for physicians treating pregnant women with a chronic medical disease include the need for treatment optimization before pregnancy, for selecting the lowest effective dose during pregnancy, and installing arrangements such as prenatal testing, ultrasonic follow-up, or consulting with an obstetric specialist.[30] This is done to protect the vulnerable fetus from possible embryo- and fetotoxic drug effects and to avoid that an untreated disease will harm the pregnant women and/or the fetus.[8]

To guide drug use in pregnancy, the Food and Drug Administration (FDA) of the USA classified drugs into five major categories A, B, C, D, and X according to potential fetal risk[31] (Appendix 1). Selected drugs with proven teratogenic effects (FDA category D and X) are listed in Table

1. The drug review was based on the reference guide by Briggs et al.,[6] which described all marketed drugs in the USA in relation to fetal and neonatal risk.

However, such drug risk classification is rather crude, since data from well conducted epidemiological studies are lacking for many substances.[8] As a result, medical doctors, pharmaceutical personal, and others in the health sector frequently face a dilemma when guiding pregnant women about risks and benefits of drug utilization in pregnancy.[1]

Table 1. Selected drugs with proven teratogenic effects; adapted from Briggs et al¤.[6]

Group of drugs#	Indication	Teratogenic effect	FDA Classification*
Androgens (testosterone)	Hormone replacement therapy in male hypogonadal disorders secondary to various causes. Not relevant for pregnancy unless used for anabolic purpose (doping).	Genital malformations.	Category X.
Angiotensin-converting-enzyme inhibitors (captopril, enalpril, quinapril).	Hypertension.	Fetal hypocalvaria, renal malformations.	Category D in 2nd and 3rd trimester, otherwise category C.
Anti-epileptic drugs (carbamazepine, phenobarbital, phenytoin, valproic acid).	Epilepsy.	Various major and minor malformations reported. Neural tube defects.	Category D.
Benzodiazepines (diazepam, triazolam).	Insomnia.	Various major malformations reported.	Category D.
Ergotamine.	Migraine.	Fetal toxicity or teratogenicity (because of maternal and/or fetal vascular disruption).	Category X.
Estrogens (estradiol).	Hormone replacement therapy.	Cardiovascular malformations, eye and ear malformations, Down Syndrome, hypospadias.	Category X.
Lithium (lithium carbonate, lithium citrate).	Manic episodes of manic-depressive illness.	Cardiovascular defects.	Category D.
Methothrexate.	Psoriasis, rheumatoid arthritis, malignant diseases.	Methotrexate embryopathy, fetal aminopterin-methotrexate syndrome.	Category X.
Misoprostol.	Used to prevent gastric ulcers induced by NSAIDs.	Moebius syndrome.	Category X.
Oral contraceptives.	Contraceptives.	Genital malformations.	Category X.
Quinine.	Treatment of malaria.	CNS malformations, limb malformations.	Category D.
Ribavirin.	Chronic hepatitis C.	Teratogenicity or embryo lethality at doses well below the recommended human dose in all animal species tested. Malformations observed included malformations of the skull, palate, eye, jaw, limbs, skeleton, and	Category X.

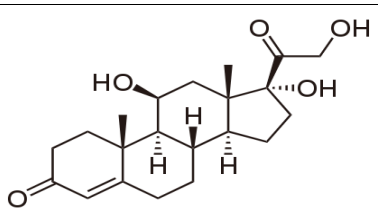
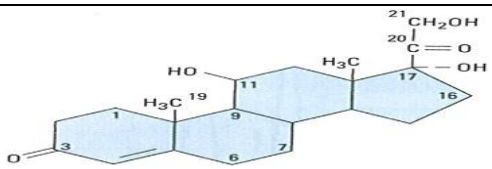
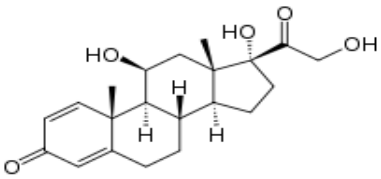
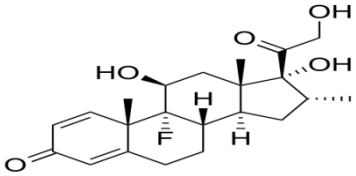
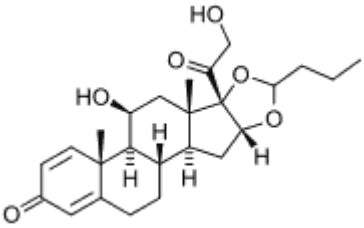
Group of drugs[#]	Indication	Teratogenic effect	FDA Classification[*]
		gastrointestinal tract.	
Statins (atorvastatin, fluvastatin, lovastatin, pravastatin, simvastatin).	Hypercholesterolemia.	CNS, cardiovascular, and limb deficiencies.	Category X.
Systemic retinoids (acitretin, isotretinoin).	Severe psoriasis, severe acne.	CNS, craniofacial, cardiovascular malformations.	Category X.
Tetracycline .	Infectious disease.	Malformations on teeth and bone.	Category X in 2nd and 3rd trimester, otherwise category D.
Thalidomide.	Acute treatment of leprosy, myelomatosis.	Phocomelia, internal organ malformations.	Category X.
Warfarin.	Thromboembolic disease.	Fetal warfarin syndrome.	Category D. (Category X according to manufacturer).
#All drugs listed are distributed in Denmark (www.medicin.dk). *Classification of risk factor is based on the U.S. Food and Drug Administration (FDA) categories according to potential fetal risk[31] listed in Appendix 1.			
¤*Drugs in pregnancy and lactation: a reference guide to fetal and neonatal risk* by Briggs et al.[6] was used to identify the FDA category for every drug listed. Identification of potentially harmful drugs was supplemented with information from other studies listing drugs according to the D and X classification.[26,32]			

1.3 Corticosteroid hormones

Corticosteroids are a class of steroid hormones. Cortisol, the naturally occurring corticosteroid, is synthesized and released by the adrenal cortex and is tightly regulated by the central nervous system, which is very sensitive to negative feedback by the circulating cortisol and exogenous (synthetic) corticosteroids.[33] Cortisol exerts a diverse range of physiologic effects, including regulation of intermediary metabolism, cardiovascular function, growth, and immunity. Corticosteroids therefore influence the function of most cells in the body. Their primary functions are to raise blood sugar through gluconeogenesis; to suppress the immune system; and to participate in the metabolism of lipids, proteins and carbohydrates.[33]

There are various synthetic forms of corticosteroids (Table 2). The actions of the synthetic corticosteroids are similar to those of cortisol:[33] they bind to the same intracellular receptor proteins, although most of the synthetic corticosteroids bind more powerfully to the receptors, e.g. prednisolone (potency 5:1 in relation to cortisol) and dexamethasone (potency 30:1 in relation to cortisol).[33]

Table 2. Selected natural and synthetic corticosteroids. Adapted from Chrousos.[33]

Agent	Forms available	Chemical structure
Cortisol. (Naturally occurring corticosteroid).		
Hydrocortisone. (Short-to medium-acting corticosteroid).	Oral, injection, topical.	
Prednisolone. (Active metabolite of prednisone. Short-to medium acting).	Oral, injection.	
Dexamethasone. (Long-acting corticosteroid).	Oral, injection, topical.	
Budesonide (Analog of prednisolone).	Inhaled, oral.	

During pregnancy, changes in the cardiovascular, renal, adrenal, immune, and gastrointestinal systems affect the pharmacokinetics of drugs.[34] For example, maternal tissue perfusion is increased during pregnancy, including perfusion of the bronchial mucosa. Therefore systemic absorption of inhaled corticosteroids may be enhanced during pregnancy,[35] potentially causing higher levels of corticosteroids within the maternal circulation. Cortisol crosses placenta, although in reduced concentration. Plasma cortisol concentrations in paired maternal and fetal venous samples (n=43) tested at 13-35 weeks of gestation showed that up to 90% of maternal cortisol was metabolized while passing through the placenta.[36] However, because the fetal concentration of cortisol is much lower than maternal levels, even a

contribution of 10-20% from the mother could still double fetal concentrations and thus have substantial impact on fetal cortisol levels.[36]

1.4 Embryogenesis

A complex sequence of events leads from conception to birth of a healthy infant.[37] Normal development over the first weeks of fetal life depends on precise timing of cell differentiation and migration, leading to formation of organ systems and subsequent growth and development.[37] A simplified time line of the reproductive process along with the problems that can arise during the process are pictured in Figure 1. The adverse events are further described in Table 3.

Figure 1. The time line for adverse events that can arise from conception to birth. Adapted from Savitz.[37]

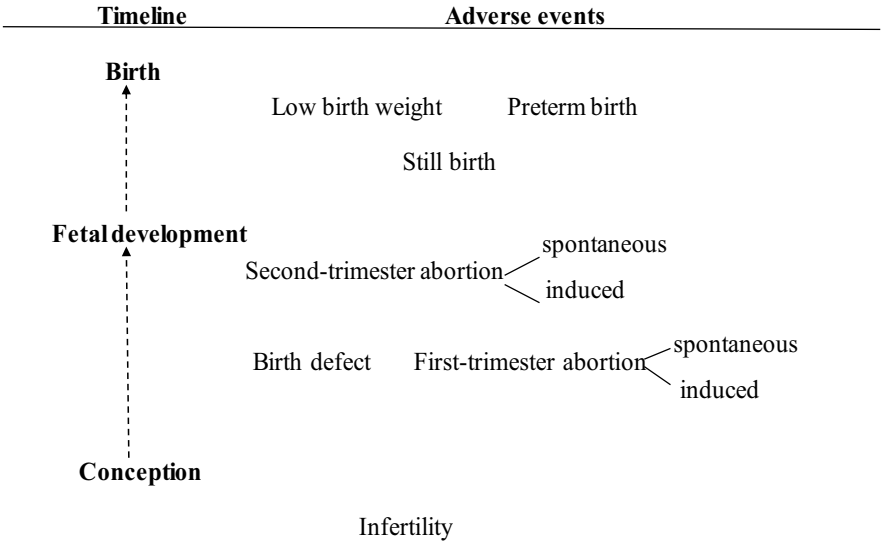


Table 3. Description of the adverse events that can arise from conception to birth. Based on definitions described in Williams Obstetrics.[10]

Adverse event that can arise from conception to birth	**Definition**
Low birth weight	Birth weight less than 2500 grams.
Preterm birth	Birth before 37 completed gestational weeks.
Stillbirth	The delivery of a fetus that has died before birth defined as the absence of signs of life at birth occurring at or beyond gestational week 22nd.
Spontaneous abortion (miscarriage)	Spontaneous end of a pregnancy at a stage where the embryo or fetus is incapable of surviving independently. From conception until 22nd gestational weeks.
Induced abortion	Termination of pregnancy by the removal or expulsion from the uterus of a fetus or embryo before birth.
Birth defect (congenital malformation)	Structural or functional defects that is congenital in origin.
Infertility	The biological inability of a person to contribute to conception.

Congenital malformations occur among 3-5% of live-born infants.[37] However, the prevalence of congenital malformation depends upon definition and diagnostic routines. Each individual type of malformation is rare, with the most common malformations (e.g., ventricular septal defects and neural tube defects) having prevalences in the order of 5-10 per 1,000 live births.[38,39]

Speculations that corticosteroids were teratogenic arose in 1951 because of the findings that treatment of pregnant mice with corticosteroids caused oral clefts in the offspring.[40] The development of the mammalian secondary palate is a complex process, and cortisol, along

with other hormones or growth factors, is required for normal growth and differentiation of the palate's epithelial and mescenchymal cells.[41] It was hypothesized that high levels of cortisol may reduce the collagen content of connective tissue by inhibiting the collagen synthesis. This may disrupt cell-to-cell and tissue-to-tissue interactions and affect the interaction between epithelium and mesenchyme in the palate, and thereby disrupt normal palatal development. Further concern grew that the teratogenic effect of corticosteroids could lead to even more life-threatening malformations mainly because corticosteroids affect almost every cell in the body[42] but also in relation to the higher potency of the synthetic corticosteroids.[43]

Miscarriage is the most common adverse event of early pregnancy occurring in approximately 20% of pregnancies.[37] The exact mechanisms and mediators causing a miscarriage are complex and not well understood. An abnormal maternal immune response has been assumed to act as an initiator of miscarriage. Evidence from murine and human pregnancy studies points to a strong association between maternal Th2-type immunity and successful pregnancy, whereas Th1-type immune reactivity is associated with pregnancy loss.[44] Well-established risk factors for miscarriage include fetal chromosome abnormalities,[45] advanced maternal age,[46,47] and history of infertility.[48] Studies of congenital malformations usually focus on the prevalence of malformations at birth and pregnancies ending as a miscarriage are often not addressed.[1,49] Consequently, effects of drugs that always cause miscarriage by causing malformations incompatible with life will remain undetected in studies that do not address miscarriage as an outcome. For example, if use of corticosteroids is related to an increased risk of malformation-induced miscarriage, the risk for congenital malformations detected at birth among women who used corticosteroids would be underestimated.

1.5 Summary of existing literature

To review the literature of utilization of corticosteroids in pregnancy and its association with congenital malformations in offspring and miscarriage, we searched the PubMed database. We limited the search to include only studies in humans, in English language, and that had been added to PubMed over the past 15 years. In addition we identified studies through communication with other researchers and by reviewing the reference lists of relevant articles. For the identification of studies of drug utilization during pregnancy, we used the following MeSH terms "drug utilization" and "pregnancy" (yielded 224 articles). To identify

studies of congenital malformations in offspring and use of corticosteroids we used "congenital abnormalities" or "cleft palate", "glucocorticoids", and "pregnancy" (yielded 82 articles). Finally, we used the following MeSH terms "spontaneous abortion" and "glucocorticoids" (yielded 13 articles) to identify studies that addressed the association between miscarriage and use of corticosteroids.

We used the following criteria to select the literature: (1) we selected only studies that had the same outcomes as in this thesis (i.e., drug utilization in pregnancy; congenital malformations in offspring; oral clefts in offspring; and miscarriage); (2) for studies that addressed congenital malformations and miscarriage, we selected only studies that reported inhaled or oral corticosteroid use; (3) for studies that addressed congenital malformations and miscarriage, we selected only studies that reported corticosteroid use in early pregnancy; and (4) in case more than one study was conducted based on the same data sources as other studies and with overlapping study periods, we only included the most comprehensive study.

This yielded nine utilization studies,[50-58] seven prevalence studies,[59-65] and five case-control studies.[19,66-69] We summarized the selected studies that met our criteria in Tables 4 and 5.

Table 4. Studies of drug utilization during pregnancy

Author, country, study period	Data sources	Study population	Drugs identified	Prevalence of drug use during pregnancy (lactation period not included)	Most commonly used drugs during pregnancy (lactation period not included)	Use of corticosteroids (lactation period not included)
Data collection from Nordic countries						
Stephansson *et al.*[58] Sweden. 2007.	The Swedish Medical Birth Register and the Swedish Prescribed Drug Register.	102,995 pregnant women (singleton births).	Prescribed drugs dispensed 3 months prior to pregnancy until 3 months after delivery. OTC drugs and drugs used during hospital admission not included.	57.6%	Anti-infective use, 10.8%; respiratory drug use, 10.6%; neurological drug use, 5.6%.	0.57% of women redeemed a prescription of systemic corticosteroid preparation (ATC code H02) in first trimester. Inhaled corticosteroid use not reported separately.
Engeland *et all*[53] Norway. 2004-2006.	The Medical Birth Registry of Norway and the Norwegian Prescription Database	106,329 pregnancies (singleton births).	Prescribed drugs dispensed 3 months prior to pregnancy until 3 months after delivery. Drugs dispensed to institutions and hospitals not included.	57.0%	Anti-infective use, 11.6%; respiratory drug use, 7.7%; gynecological drug use, 5.3%.	Inhaled and oral corticosteroid use not reported separately.
Malm *et al*[56] Finland. 1999.	Maternal Grants Register of the Social Insurance Institution in Finland	43,470 pregnant women.	All reimbursed drugs dispensed 12 months before pregnancy until 3 months after pregnancy. OTC drugs and non-	46.2%	Systemic antibiotic use, 24.1%; gynecological anti-infectives, 8.3%; nasal preparations, 5.0%.	Inhaled and oral corticosteroid use not reported separately.

Author, country, study period	Data sources	Study population	Drugs identified	Prevalence of drug use during pregnancy (lactation period not included)	Most commonly used drugs during pregnancy (lactation period not included)	Use of corticosteroids (lactation period not included)
			reimbursed drugs not included, unless these drugs were prescribed for chronic diseases.			
Olesen *et al.*[57] Denmark. 1991-1996.	The Danish Medical Birth Registry and the North Jutland Prescription Database (now part of the Aarhus University Prescription Database).	15,756 primiparous women.	All prescribed reimbursed drugs dispensed from 12 weeks before conception until 12 weeks post-partum. Drugs dispensed in-hospital, non-reimbursed drugs, and OTC drugs not included.	44.2%	Penicillin, 59.7%; gynecologic anti-infectives, 29.0%; sulphonamide, 17.7%.	The prescription proportion per 1000 women of oral corticosteroids (ATC code H02A) was 1.8 in first trimester. Inhaled corticosteroid use not reported separately.
Data collection from non-Nordic countries						
Irvine *et al.*[54] Scotland. 2007.	The Scottish Maternity Record and the Medicines Monitoring Unit in Tayside, Scotland.	3937 pregnant women.	Prescribed drugs dispensed 3 months prior to conception until delivery.	85.2%	Antibiotics, 35.0%; oral iron, 34.3%; folic acid preparations, 30.0%.	0.5% of women used oral corticosteroids in first trimester. Inhaled corticosteroid use not reported separately.
Bakker et al.[51] Netherlands. 1994-2003.	InterAction database.	5412 pregnant women (liveborn singelton births).	Prescribed drugs dispensed from 2 years before until 3 months after pregnancy. OTC drugs and	79.1%	The prevalence not reported.	Prescription rate per 100 women in first trimester who used systemic corticosteroids (ATC code H02) was 0.4.

Author, country, study period	Data sources	Study population	Drugs identified	Prevalence of drug use during pregnancy (lactation period not included)	Most commonly used drugs during pregnancy (lactation period not included)	Use of corticosteroids (lactation period not included)
			drugs dispensed during hospitalizations not included.			Inhaled corticosteroid use not reported separately.
Egen-Lappe *et al.*[52] Germany. June 2000-May 2001.	German statutory sickness fund.	41,293 pregnant women.	Reimbursed prescription drugs dispensed 450 days before and 180 days after childbirth.	96.4%	Prevalence of drug use other than vitamin or mineral supplement; 85%.	Inhaled and oral corticosteroid use not reported separately.
Andrade *et al.*[50] USA (Washington state, Massachusetts, Minnesota, Michigan, Colorado, Georgia, Oregon). 1996-2000.	Eight health maintenance organizations involved in the Health Maintenance Research Network Center for Education and Research on Therapeutics.	152,531 deliveries.	Prescription drugs dispensed within 1 year before the date of delivery.	82.0%	Vitamin or mineral supplement, 54%; anti-infective drugs, 40%; respiratory drugs, 19%.	0.7% of women used oral or injectable corticosteroids in first trimester and the prevalence of use during entire pregnancy was 1.7%. Inhaled corticosteroid use not reported separately.
Lacroix *et al.*[55] France. 1996.	Records of the Caisse Primaire d'Assurance Maladie de la Haute-Garonne (French Health Insurance System).	1000 pregnant women.	Prescribed drugs dispensed during pregnancy.	99.0%	Iron, 75%; drugs for alimentary system, 69%; dermatological drugs, 63%.	Approximately 10% of women used systemic corticosteroids during pregnancy. Inhaled corticosteroid use not reported separately.

Author, country, study period	Data sources	Study population	Drugs identified	Prevalence of drug use during pregnancy (lactation period not included)	Most commonly used drugs during pregnancy (lactation period not included)	Use of corticosteroids (lactation period not included)
Abbreviations; OTC, over-the-counter						

Table 5. Studies of corticosteroid use in early pregnancy and risk of congenital malformations overall in offspring, oral clefts in offspring, and miscarriage.

Author, country, study period	Data sources, study population	Study design	Exposure	Outcome of interest		
				Relative risk estimates* (95% confidence interval);n of exposed infants		
				Congenital malformations overall	Oral clefts	Miscarriage
Källen[61] Sweden. 1995-2004.	Swedish Medical Birth Registry. 892,362 pregnancies.[a]	Prevalence study	Inhaled	1.1 (1.0-1.2); 627[e]	All Clefts: 1.4 (1.0; 1.9); 48	
Alexander[59] Canada. 1991-1993.	Nova Scotia Atlee Perinatal Database, Halifax County, Canada, Grace Maternity Hospital. 14,526 pregnancies.	Prevalence study	Inhaled	0.8 (0.4-1.7); 8[e]		
Gur[60] Israel. 1988-2001.	The Israeli Teratogen Information Service. 1101 pregnancies.	Prevalence study	Systemic[b]	2.0 (0.9-4.4)[#]; 10[f]		1.7 (1.1-2.5)[#]; 36[i]
Park-Wyllie[62] *Canada. 1985-1995.*	Canadian Motherisk cohort.	Prevalence study	Systemic[c]	2.1 (0.5-9.6); 4[g]		1.0 (0.5-2.1)[#]; 13[j]

Author, country, study period	Data sources, study population	Study design	Exposure	Outcome of interest Relative risk estimates* (95% confidence interval);n of exposed infants Congenital malformations overall	Oral clefts	Miscarriage
	372 pregnancies.					
Schatz[63] USA. Jun 1978-Dec 1989.	Kaiser-Permanente Prospective Study of Asthma During Pregnancy. 1502 pregnancies.	Prevalence study	Inhaled, intranasal, and oral	1.4 (0.9-2.5)[#]; 14[h]		
Tata[65] England and Wales. Jan. 1988-Nov. 2004.	The Health Improvement Network. 281,019 pregnancies.	Prevalence study	Inhaled			1.2 (1.2-1.3)[i]
Silverman[64] Trial including 32 countries. Oct. 1996-Jan. 1998.	START (inhaled Steroid Treatment As Regular Therapy) trial. 313 pregnancies.	Prevalence study	Inhaled			1.3 (0.6-2.5)[#]; 23[i]
Carmichael[19] USA. Oct. 1997-Dec. 2002.	American National Birth Defects Prevention Study. 1769 infants with oral clefts. 4143 control infants.	Case-control	Inhaled Systemic[c]		CLP: 1.5 (0.9-2.5); 19 CP: 0.7 (0.3-1.8); 5 CLP: 2.1 (0.9-4.7); 9 CP: 0.8 (0.2-3.6); 2	

Author, country, study period	Data sources, study population	Study design	Exposure	Outcome of interest Relative risk estimates* (95% confidence interval);n of exposed infants Congenital malformations overall	Oral clefts	Miscarriage
Pradat[68] Australia, France, Italy, Israel, Japan, the Netherlands, South America. 1990-2002.	Malformation Drug Exposure Surveillance Project – MADRE. 11,150 malformed infants. 23,517 control infants.	Case-control	Inhaled Systemic[d]		All clefts: 0.6 (0.2-1.7); 4 CLP: 0.7 (0.2-2.2); 3 CP: 0.6 (0.1-5.1); 1 All clefts: 1.3 (0.7-2.2); 15 CLP: 1.8 (1.0-3.1); 13 CP: 0.3 (0.04-1.5);1	
Carmichael[66] USA. 1987-1988.	The California Birth Defects Monitoring Program. 1299 malformed infants. 734 control infants.	Case-control	Oral		CLP: 4.3 (1.1-17.2); 6 CP: 5.3 (1.1; 26.5); 3	
Rodriguez-Pinilla[69] Spain. Apr. 1976-Dec. 1995	Spanish Collaborative Study of Congenital Malformations – ECEMC. 24,038 malformed infants. 23,517 control infants.	Case-control	Oral		All clefts: 5.2 (1.5-17.1); 5	
Czeizel[67] Hungary. 1980-1994.	The Hungarian Congenital Abnormality Registry – HCCSCA. 20,830 malformed infants. 35,727 control infants.	Case-control	Oral		All clefts: 1.3 (0.8-2.0); 1	

Abbreviations; CLP, cleft lip with or without cleft palate; CP, cleft palate.

Study population definition: [a]the study population defined in Källen et al., 2007, "Use of anti-asthmatic drugs during pregnancy. 1. Maternal characteristics, pregnancy and

Author, country, study period	Data sources, study population	Study design	Exposure	Outcome of interest		
				Relative risk estimates* (95% confidence interval);n of exposed infants		
				Congenital malformations overall	Oral clefts	Miscarriage
delivery complications".[70]						
Exposure definitions regarding systemic use: [b]oral, intramuscular, and intravenous preparations; [c]oral and intravenous preparations; [d]Oral and injection according to the ATC-classification H02A.						
Outcome definition regarding congenital malformations overall: [e]not categorized; [f]non-genetic major congenital malformations; [g]major and minor malformations according to Heinonen et al.[71]; [h]major malformations.						
Outcome definition regarding miscarriage: [i]not defined; [j]miscarriage before 26 gestational week.						
*the risk estimates are given as prevalence odds ratios for the prevalence studies and odds ratio for the case-control studies.						
# risk estimates calculated using the Episheet software (version 2011, by Kenneth J. Rothman).						

1.5.1 Studies on drug utilization in pregnancy

The most recent review of drug utilization among pregnant women in Denmark (from 1991 to 1996) reported that 44.2% of all women used prescribed drugs in pregnancy and 0.2% used corticosteroids in the first trimester.[57]

The prevalence of prescribed drug use during pregnancy refers to the number of women who used prescribed drugs at some point during the pregnancy divided by all pregnant women in the study population. Prevalence of drug use in pregnancy is most often measured within pregnancy periods (first, second, and third trimester). Most studies also include a preconception period[50-54,56-58] and some studies included a lactation period.[51-53,56-58] However, the length of the preconception period varied among studies. Two studies reported drug use up to one year[50,56] or two years[51,52] before delivery whereas four studies reported drug use three months before conception[53,54,57,58] and one study did not report the preconception drug use.[55] Six studies included a lactation period of three months[51-53,56-58] whereas three studies did not include a lactation period.[50,54,55] These differences in pregnancy periods between the studies also contribute to the variation in the observed prevalence of drug use.

The prevalence of drug use was in the same order of magnitude (46.2%-57.6%) in the other Nordic countries,[53,56,58] as expected given their similar health care and record-keeping practices.[72] Compared with the Nordic countries, all non-Nordic countries reported higher prevalence of drug use during pregnancy (79%-99%).[50-52,54,55] The differences in drug utilization patterns between Nordic and non-Nordic countries may be explained by differences in reporting of use of over-the-counter drugs, differences in prescribing and reimbursement patterns, differences in record-keeping, or differences in socioeconomic or health characteristics of the underlying populations.[22]

Six of the identified drug utilization studies[50,51,54,55,57,58] addressed corticosteroid utilization in early pregnancy. The reported prevalences of corticosteroid use in the first trimester ranged from 0.2%[57] to 0.7%.[50]

Differences over time in either corticosteroid use or total drug use during pregnancy were not described in the identified drug utilization studies.[50-58] Furthermore, information on whether and to what extent changes of the characteristics of pregnant women influence drug use in pregnancy over time is lacking in the existing literature.

1.5.2 Studies on corticosteroid use and risk of congenital malformations in offspring

We identified five prevalence studies of congenital malformations in offspring following early-pregnancy use of corticosteroids.[59-63] Because of spontaneous fetal loss (miscarriage, extrauterine pregnancy, and stillbirth) and induced abortions, the prevalence of congenital malformations at birth differs from the incidence.[73] Therefore prevalence is the measure of occurrence of congenital malformations at birth.[49] The prevalence odds is the ratio of two probabilities: the probability of an event divided by 1- the probability of that event.[74] The identified studies are consistent with both presence and absence of an association between congenital malformations in offspring and use of corticosteroids. Prevalence odds ratios (POR) ranged from 0.8 (95% confidence interval (CI), 0.4-1.7)[59] to 2.1 (95% CI, 0.5-9.6)[62] (Table 5). The largest prevalence study included 892,362 pregnant women, of whom 12,478 used corticosteroids during pregnancy and reported a POR for congenital malformations overall of 1.1 (95% CI, 1.0-1.2) comparing users and non-users of inhaled corticosteroids during pregnancy.[61] The association of corticosteroid use in early pregnancy and oral clefts in offspring was evaluated in five case-control settings[19,66-69] and the reported odds ratios (OR) ranged from 0.6 (95% CI, 0.2-1.7)[68] to 5.2 (95% CI, 1.5-17.1).[69]

The evidence about an association between use of corticosteroids in early pregnancy and risk of congenital malformations is inconclusive. Factors such as route of administration of corticosteroids and the classification of malformations differed among the existing studies, which complicated comparisons. Furthermore, there may be limitations inherent in study design. In the case-control studies that reported an increased risk of oral clefts with use of oral corticosteroids, early pregnancy exposure information was based on retrospective data collection by means of interviews or questionnaires,[19,66,68,69] with the risk of differential recall of drug use.[73] The Hungarian Case-Control Surveillance System of Congenital Abnormalities (HCCSSCA), which was established in 1980, contains information of 22,843 cases of congenital malformations captured between 1980-1996.[75] Data of exposure during pregnancy were collected through a questionnaire that women filled in after the outcome of the birth was known, and differential recall bias could thus be present. One study examined impact of recall bias and misclassification in the HCCSSCA by comparing self-reported drug intake with medically notified intake for specific disease.[76] Differential recall was found to frequently cause spurious associations, with biased ORs up to a factor of 1.9.[76] Furthermore, two studies

were based on teratogenic information system reporting,[60,62] in which self-referral bias cannot be ruled out. Self-referral bias may threaten validity, because the reasons for contacting the teratogenic information system may be associated with the outcome under study[77] and this could bias estimates away from the null.[73] Finally, most studies on the issue were imprecise.[19,59,60,62,63,66-69]

1.5.3 Studies on corticosteroid use and risk of miscarriage

Use of corticosteroids has been reported to increase the risk of miscarriage, with relative risk estimates ranging from 1.2 to 1.7,[60,64,65] although one study found no difference in odds of miscarriage among 184 corticosteroid users compared with 188 women exposed to either topical retinoic acid or oral astemizole (OR, 1.0; 95% CI, 0.5-2.1)[62] (Table 5). The largest prevalence study included almost 300,000 pregnancies from the Health Improvement Network in England and Wales of whom 8,849 used inhaled corticosteroids and they reported an OR for miscarriage of 1.2 (95% CI, 1.2-1.3).[65] The two other prevalence studies were smaller.[60,64] Gur et al. identified women from the Israeli Teratogen Information Service and identified 311 women who reported to use corticosteroids and 790 who did not report use of corticosteroids.[60] Silverman et al. identified 196 women who used inhaled corticosteroids and 117 non-users in a population of asthma women who participated in the Inhaled Steroid Treatment As Regular Therapy trial.[64] Furthermore, only one study[65] controlled for potential confounding (age, smoking, and body mass index).

The datasets used in previous studies lack information regarding gestational age at miscarriage[60,62,64,65] and therefore the exposure timing relevant to embryonic development cannot be accurately determined. Consequently, inferences about the teratogenic potential of corticosteroids are difficult. Other limitations include risk of overestimation of the teratogenic potential of corticosteroids.[60,62] because of self-referral bias.[73] Three studies are based on self-reported drug use,[60,62,65] which may be an inaccurate measurement of drug use and it could cause non-differential misclassification with bias towards the null.[73]

1.6 Considerations when planning an observational study of corticosteroid use in pregnancy

Pharmacoepidemiological evidence from observational studies is central in establishing evidence of safety of drug use in pregnancy because randomized controlled trials rarely

include women of childbearing age, mainly because of ethical concerns about potential teratogenicity of the drug under study.[1] However, the construction of a study to examine corticosteroid use in pregnancy with special focus on congenital malformations in offspring and miscarriage is challenging. Such studies, as any observational studies, do not benefit from random allocation, strict clinical definitions, and blinding.[78]

1.6.1 Data sources

Danish public registers and medical databases provide unique opportunities for conducting pharmacoepidemiological studies.[79] Although data are collected for administrative purposes and not for research, the fact that data already exist eliminates the need for primary data collection, which is often time consuming and expensive. Furthermore, data that cover large populations contribute to high precision of risk estimates and enable studies of rare exposures and outcomes.[79]

1.6.2 Study design

Drug utilization studies are descriptive studies, either quantitative (estimate drug utilization in populations) or qualitative (link drug utilization data to reason for drug prescribing).[22] They provide information on patterns of drug use in a given population and may identify disadvantageous drug utilization patterns. However, they include no follow-up measures and often the indication for drug prescription is incomplete or even lacking.[22]

Spontaneous notifications from case reports (events observed in single patients) and case series (collection of patients all of whom have a single exposure and whose clinical outcomes are then evaluated and described) play an important role in the surveillance of adverse effects of drugs.[80] A case series contains no control group, so the background rates of events (in the absence of exposure) cannot be evaluated for comparison. For this reason, case series are not very useful in determining causation, instead they provide clinical description of a disease or of patients who receive an exposure[81] and could thus be the first signal of a teratogenic drug, as in the thalidomide case.[3]

Cohort studies rely on data in which exposure information refers to an earlier time than that of disease occurrence.[82] Measuring the exposure before the outcome has occurred will reduce differential misclassification of exposure.[83] In an ideal cohort study of reproductive outcomes, the women would be followed from conception to the appearance of the outcome of interest.

In reality, loss to follow-up is present because of adverse reproductive outcome such as spontaneous fetal loss (extrauterine pregnancy, miscarriage, and stillbirth) and induced abortions. Therefore, the prevalence of congenital malformations at birth differs from the incidence.[73] A cross-sectional study involves a cross-sectional sampling to obtain the study cohort (pregnant women) and then assesses corticosteroid exposure and reproductive outcomes of interest in the members of that cohort.[80,84] Cross-sectional studies cannot measure disease incidence and therefore these studies are often referred to as prevalence studies.[82]

Pregnancy loss is a common event, occurring in over 20% of pregnancies but at early stages of gestation the pregnancy loss is difficult to identify with accuracy.[37] Still, women with a registered miscarriage can be included as cases in a case-control study and compared with women without a miscarriage to compare previous corticosteroid exposure.[80]

1.6.3 Confounding factors

Confounding implies that the effect of the study exposure is mixed with – or masked by – the effect of another variable, leading to bias.[77] Predictors of drug use by a pregnant woman that are independent risk factors for a given adverse birth outcome, e.g. congenital malformations in offspring or miscarriage, can confound the association between the drug and the adverse birth outcome under study.[73] Examples of potential confounding factors include maternal age, geography, race, and socioeconomic status.[1] Co-medication is another important confounding factor. For instance, non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to treat rheumatic diseases such as rheumatoid arthritis[85] for which corticosteroid use is also indicated and use of NSAIDs has also been associated with an increased risk of miscarriage.[86]

Unknown confounding factors cannot be controlled in the analysis except indirectly if they are associated with a factor that is measured and can be controlled. In randomized studies, successful randomization balances out both known and unknown confounder whereas in observational studies the usual methods to control confounding (standardization, matching, stratification, restriction, and regression modeling) do not remove confounding by unmeasured factors.[83] One way to deal with unmeasured confounding is by external adjustment.[87] Then the effect of the potential confounding on the observed effect estimate is

considered under an "array of informed assumptions" about the association between confounder, exposure, and outcome.[87]

A special case of unmeasured confounding is residual confounding which occurs when controlling for a set of variables used to measure a confounding factor does not completely remove confounding by these measured factors.[73] This may occur if the variable is misclassified owing to poor measurement or due to inadequate categorization.

In pharmacoepidemiological studies, it is difficult to separate the effect of a specific drug of interest from the effect of the underlying disease that indicated the treatment (confounding by indication).[88] To reduce confounding by indication one could stratify data by the underlying indication for corticosteroid treatment or study women with, e.g. asthma, who do not receive corticosteroid treatment. One could also examine the effect of corticosteroid exposure in a period of pregnancy, e.g. second or third trimester, not expected to be relevant for the development of the congenital malformation. However, these approaches may introduce other obstacles, since use of different medications for the same indication may vary according to severity or etiology of disease, both of which may influence the outcome.[89,90] Other analytic techniques (e.g. propensity score methods) can seek to counter the effects of confounding by indication[91] but the bias is still difficult to avoid and difficult to address properly without randomization.[88]

1.7 Conclusions leading to the present study

Drug use in pregnancy is unavoidable. Any effect – harmful, neutral, or protective – has important implications for pregnant women and their infants, explaining the great concern surrounding the use of drugs in pregnancy.

Corticosteroids are very potent anti-inflammatory and immunosuppressive drugs, and these drugs are necessary for some women in pregnancy. Common indications for corticosteroid treatment among women of childbearing age are asthma, inflammatory bowel diseases, and rheumatoid arthritis and the prevalence of these diseases has increased in pregnant women in recent years indicating that use of corticosteroids in pregnancy may have changed. Yet, evidence about use of corticosteroids in pregnancy is limited. Corticosteroids have been suspected to be teratogenic, although the existing evidence about teratogenicity of

corticosteroids is inconclusive and has limitations, including lack of data on gestational age and insufficient size to examine specific malformations.

Chapter 2. Aims of the thesis

The overall aim of this thesis was to provide an evaluation of corticosteroid use in pregnancy. The description of drug utilization in pregnancy and the evaluation of the teratogenic potential of corticosteroids were addressed in three studies:

Study 1 – *drug utilization in pregnancy*

- To analyze the use of corticosteroids over time among pregnant women in Denmark.
- To analyze the patterns of all prescribed drugs in relation to maternal age and by type of drug.

Studies 2 and 3 – *evaluation of the teratogenic potential of corticosteroids*

- To investigate use of corticosteroids in early pregnancy and its association with:
    - Congenital malformations overall and oral clefts in offspring (**Study 2**)
    - Miscarriage (**Study 3**)

Chapter 3. Materials and methods

The studies of this thesis were based on data from medical databases of northern Denmark (the Central and North Regions of Denmark), which comprises about 33% (1.8 million people) of the entire Danish population. Data were linked through the Civil Registration System using the unique 10-digit personal identifier (the CPR number).[92] The CPR number is assigned to all Danish residents at birth. It is used in all Danish registries and allows data linkage (Figure 2).

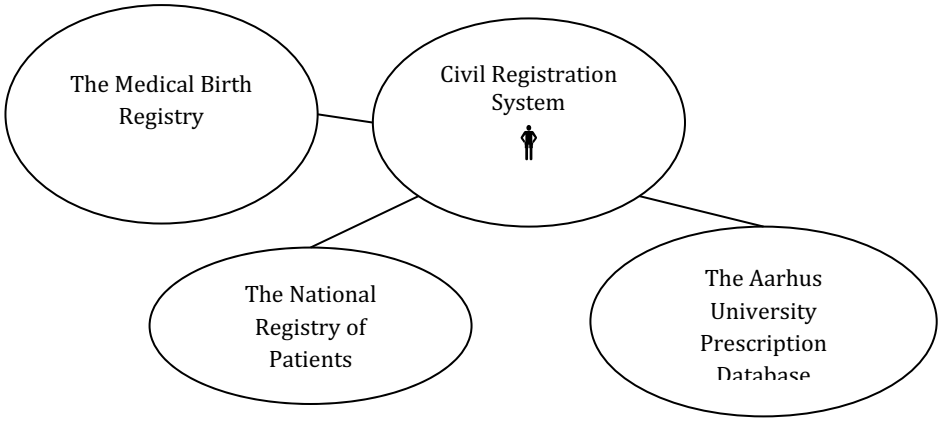


Figure 2. Data sources for **Studies 1-3**

3.1 Data sources

Below is a detailed description of the data sources used in this thesis. All relevant diagnostic codes and drug codes are given in Appendix 2.

3.1.1. The Medical Birth Registry

The Medical Birth Registry was used to identify the mothers and their newborns (**Studies 1-3**). The Medical Birth Registry contains computerized records of all births in Denmark since 1973.[93] Each record includes data on characteristics of the mother (including age, citizenship, residence, marital status, parity, and self-reported smoking status) and the newborn (including vital status at birth, sex, birth weight, and gestational age).

Gestational age is estimated mainly based on ultrasound[94] and is recorded in days.[95] The conception date was calculated as birth date minus gestational age in days plus 14 days.

3.1.2. The National Registry of Patients

The National Registry of Patients was used to identify congenital malformations (**Study 2**), miscarriages (**Study 3**), and important covariates (**Studies 2-3**). The National Registry of Patients was established in 1977 and records visits to all somatic hospitals in Denmark, including dates of admission and discharge, diagnosis codes, and surgical procedures.[96] Contacts to emergency rooms and outpatient clinics have been registered since 1995. All coding is conducted by medical doctors according to the International Classification of Diseases, eighth revision (ICD-8) until the end of 1993 and tenth revision (ICD-10) thereafter.

3.1.3 The Aarhus University Prescription Database

The Aarhus University Prescription Database was used to obtain information on drug use in pregnancy (**Studies 1-3**). The Aarhus University Prescription Database tracks prescriptions for reimbursed drugs redeemed at the regions' outpatient pharmacies.[97] The pharmacies use electronic accounting systems to secure reimbursement from the National Health Service. Denmark's tax-supported health care system partially refunds the costs of most prescribed drugs.[98] The type of drug is coded using the Anatomic Therapeutic Chemical (ATC) classification system (Appendix 3).[99] The Aarhus University Prescription Database does not track in-hospital medical treatment. Also, non-reimbursed drugs (e.g., over-the-counter preparations, prescription sedatives, hypnotics, or oral contraceptives) are not recorded unless they are approved for reimbursement, e.g., to treat a chronic condition. Reimbursed drugs include inhaled and oral corticosteroids, which are available by prescription only. To secure full prescription record for each pregnancy it was required that the women resided in one of the two regions from 30 days before conception through delivery (**Studies 1-2**) or the women resided in one of the two regions for a minimum of one year before the index date (**Study 3**).

3.2 Study design

3.2.1 Study 1 – a drug utilization study

We identified all primiparous women who delivered their first live- or stillborn child at ≥ 22 gestational week from 1 January 1999 to 31 December 2009. We defined drug use as a record of at least one prescription dispensation recorded from 30 days before conception until delivery. We evaluated the prevalence of prescriptive drug use over time and by pregnancy

periods (immediate preconception: 1-30 days before estimated conception; 1st trimester: gestational week 1-12; 2nd trimester: gestational week 13-28; and 3rd trimester: gestational week 29 to delivery) according to maternal age and according to different drug categories corresponding to the ATC classification system.

3.2.2 Study 2 – a prevalence study

The study population consisted of primiparous women giving birth from 1 January, 1999 to 31 December, 2009 in northern Denmark. We restricted the study population to primiparous women to remove the effects of an adverse outcome in a previous pregnancy that could influence a woman's drug use in a new pregnancy.[100,101] The outcome of interest was oral clefts and congenital malformations overall in offspring. Because not all congenital malformations are apparent at delivery,[49] we included congenital malformation diagnoses registered during the infants' first year of life. Diagnoses of congenital dislocation of the hip and undescended testes were excluded due to their expected low validity[102] and infants with known chromosome disorders were excluded. Oral clefts were defined as cleft lip with or without cleft palate or isolated cleft palate.[103]

We defined use of corticosteroids in early pregnancy as a record of at least one prescription for inhaled or oral corticosteroids from 30 days before estimated conception to the end of the first trimester (until gestational week 12). Use of corticosteroids in late pregnancy was defined as a record of at least one prescription for inhaled or oral corticosteroids redeemed from gestational week 13 until delivery and no use of corticosteroids in early pregnancy. We categorized use of corticosteroid as inhaled corticosteroids, oral corticosteroids, and concomitant use of inhaled and oral corticosteroids. We defined non-users (the reference) as women who did not use inhaled or oral corticosteroids at any time from 30 days before estimated conception until delivery.

3.2.3 Study 3 – a case-control study

Cases and controls were identified in northern Denmark from 1 January1997 to 31 December 2009. Cases were all women who during that period had a first-time recorded miscarriage before 22nd gestational week and no previously recorded birth. The admission date of miscarriage was the index date. Controls were defined as women with a first live birth during the study period and no previous recorded miscarriage. For each case, we selected 10 controls

matched on year of conception. We also matched the exposure information on gestational age. Thus, for each individual control woman the index date was set as the date when they had the same gestational age as their corresponding case at time of admission.

We grouped the women into the following exposure categories (Table 6): current use (most recent prescription of inhaled or oral corticosteroids filled within 60 days before the index date); recent use (most recent prescription filled within 61 - 180 days before the index date); former use (most recent prescription of inhaled or oral corticosteroids filled > 180 days before the index date); new use (the first prescription of inhaled or oral corticosteroid use within 60 days before the index-date); and never use (no prescription of inhaled or oral corticosteroids identified in the Aarhus University Prescription Database).

Table 6. Exposure categories of corticosteroid (CS) use. The parentheses illustrate that CS use is possible in the given period but not a necessity to be defined into the given exposure category.

Exposure category	← 181 days	180 days ← 61 days	60 days ← Index date
Current use	(CS use)	(CS use)	CS use
Recent use	(CS use)	CS use	No CS use
Former use	CS use	No CS use	No CS use
New use	No CS use	No CS use	CS use
Never use	No CS use	No CS use	No CS use

Based on the length of pregnancy at miscarriage, we categorized miscarriage into; early miscarriage (miscarriage occurring before gestational week 13) and late miscarriage (miscarriages from gestational week 13 until week 22).

3.2.4 Data on covariates (Study 2-3)

We obtained information about maternal diagnoses of asthma, rheumatoid arthritis, and inflammatory bowel disease recorded from 1977 until delivery, as corticosteroids are used in

medical treatment of these diseases commonly occurring among women of childbearing age.[9,14,16] Underlying diseases may themselves be risk factors for adverse events such as congenital malformations in offspring and miscarriage.[14,63,104] We further obtained information of women's hospital diagnoses of diabetes and prescription history of anti-diabetics because diabetes has been associated with an increased risk of congenital malformations in the offspring[105] and with miscarriages.[106] Furthermore, use of corticosteroids may induce diabetes.[107] We also obtained information of women's hospital diagnoses of epilepsy and prescription history of anti-epileptic drugs because use of antiepileptic drugs has been associated with an increased risk of congenital malformations in the offspring and with miscarriage.[108] Finally, we obtained information of women's prescription history for NSAIDs because use of these drugs has been associated with an increased risk of miscarriage.[86]

For **Study 2,** we included the disease information recorded from 1977 until delivery. For **Study 3,** we included the disease information recorded from 1977 until the index date.

Smoking has been associated with an increased risk of miscarriage[109,110] (**Study 3**). The National Registry of Patients contains no information on smoking status. Instead we used smoking information reported in the Medical Birth Registry. For cases, we collected smoking status from the first registration in the Medical Birth Registry following the miscarriage and used that information as a proxy measure of smoking status at the time of miscarriage. For controls, we used information on smoking recorded during the pregnancy.

3.3 Ethics

The studies were approved by the Danish Data Protection Agency (journal number: 2003-41-3103). The studies were conducted in accordance with the rules of the Danish Data Protection Board, University of Aarhus, and with "Good Epidemiological Practice".[111]

3.4 Statistical analyses

A detailed description of the statistical methods used in each study is provided below.

S**tudy 1:** Prevalence of drug use among primiparous women was computed in categories corresponding to the main anatomical group of the ATC classification system as we selected those main anatomical groups whose prevalence of use exceeded 4%. These groups, listed in

the order of decreasing prevalence of use were: anti-infective drugs for systemic use; gynecological drugs; dermatological drugs; drugs for respiratory diseases; drugs for alimentary tract and metabolism; and neurological drugs. We then stratified according to maternal age at delivery and smoking during pregnancy. Additionally, we examined the prevalence of corticosteroid use (inhaled and oral preparations) over time.

We computed the age-standardized prevalence of drug use for each calendar year (1999-2009), with the age distribution in year 1999 as the standard. We constructed a general linear model of age- and smoking-adjusted prevalence ratios (PR) for drug use with corresponding 95% confidence intervals using 1999 as the reference year. We used a Chi-square test to test for the presence of a trend across years.

We described the patterns of drug use by pregnancy periods (immediate preconception, 1-30 days before estimated conception; 1st trimester, gestational week 1-12; 2nd trimester, gestational week 13-28; and 3rd trimester, gestational week 29 to delivery). We then compared the prevalence of drug use in 2008-2009 with that in 1999-2000 (the reference) by estimating pregnancy period-specific PRs, adjusted for maternal age at delivery and smoking in pregnancy.

Study 2: We computed prevalence of congenital malformations overall and oral clefts in offspring by exposure status. We used logistic regression to estimate PORs with associated 95% CI as we compared women who used corticosteroids in early pregnancy with non-users. In an additional model we adjusted for age, smoking, and diabetes. We then carried out additional analyses excluding the women who filled only a single prescription of corticosteroids in early pregnancy in order to illuminate whether number of prescriptions did affect the results. We also considered the prevalence of different sub groups of congenital malformations as categorized by EUROCAT (European surveillance of congenital anomalies) definitions.[112]

Study 3: We cross-tabulated women's demographic and health characteristics according to case/control status. We used conditional logistic regression, adjusted for age, past medical history of diabetes and epilepsy, and use of NSAIDs, to estimate ORs with 95% CIs as estimates of an association between corticosteroid use and risk of miscarriage, separately for

oral and inhaled drugs. We then examined the association between steroid use and miscarriage according to gestational age.

Finally, we conducted a series of sensitivity analyses. First, we examined whether variation in exposure definition affected study results. Although few corticosteroid prescriptions in Denmark are expected to last more than 60 days (definition of current use in this study), we examined the impact of extending the definition of current and new use from 60 days to 90 days before the index date. Second, we examined the impact on the results of previous obstetric history, in another sensitivity analysis, as we recalculated ORs while excluding cases and controls with a history of induced abortion. Third, we examined the impact of smoking as a confounder. We stratified the corticosteroid users according to their smoking status and recalculated the analyses.

All analyses for **Study 1** and **Study 2** were performed using Stata software 10.0 (www.stata.com). The analyses for **Study 3** were performed using SAS® software (version 9.2; SAS Institute, Cary, NC, USA).

Chapter 4. Results

A brief summary of the main results is described below.

4.1 Study 1: Use of prescribed drugs among primiparous women: an 11-year population-based study in Denmark

During the period 1999-2009, 85,710 primiparous women delivered 88,003 live- or stillborn children in northern Denmark. Mean age at delivery was 28 years (range 13-52 years); the proportion of primiparous women aged 30 years and older increased from 29.0% in 1999 to 35.8% in 2009. Overall, 151,221 prescriptions were redeemed by 47,982 (56.0%) primiparous women. Primiparous women who redeemed prescriptions, redeemed on average 3.2 prescriptions and 2.3% of the women redeemed more than 10 prescriptions. The age-standardized prevalence of overall drug use increased from 54.7% in 1999 to 61.2% in 2009, corresponding to a PR of 1.13 (95% CI, 1.10-1.16). Throughout the study period, women of 35 years or older had a higher prevalence of overall drug use than women in other age-groups. Anti-infective drugs were the most prevalent drugs used by primiparous women over the study period and the prevalence of use increased throughout the study period (25.5% in 1999; 36.3% in 2009; PR, 1.44; 95% CI, 1.38-1.51).

In total, 2,167 (2.5%) women redeemed a prescription of corticosteroids. Compared with all primiparous women, those with corticosteroid drug use were less likely to be smokers and more likely to be 30 years or older. The prevalences of low birth weight and preterm birth were higher in women who used corticosteroids (low birth weight, 6.2%; preterm birth, 7.4%) than in all primiparous women (low birth weight, 4.8%; preterm birth, 6.7%) (Table 7).

Table 7. Characteristics of primiparous women who redeemed at least one prescription of corticosteroids in northern Denmark from 1999-2009.

	All primiparous women (n=85,710)	**Corticosteroid use (n=2,167)**	**Inhaled corticosteroid use (n=1,836)**	**Oral corticosteroid use (n=366)**
Age at delivery, years				
<25	18,170 (21.2)	375 (17.3)	321(17.5)	58 (15.9)
25-29	39,221 (45.8)	973 (44.9)	837 (45.6)	152 (41.5)
30-34	21,540 (25.1)	597 (27.6)	493 (26.9)	112 (30.6)
≥35	6,779 (7.9)	222 (10.2)	185 (10.0)	44 (12.0)
Smoking during pregnancy[a]	15,046 (17.6)	330 (15.2)	275 (15.0)	65 (17.8)
Single births	83,405 (97.3)	2,106 (97.2)	1,796 (97.8)	346 (94.5)
Twin births	2,256 (2.6)	60 (2.6)	39 (2.1)	20 (5.5)
Triplet births	49 (0.1)	1 (0.1)	1 (0.1)	0 (0)
Low birth weight[b] (<2500 g)	3,975 (4.8)[c]	130 (6.2)[c]	101 (5.6)[c]	33 (9.5)[c]
Preterm birth (< 37 weeks)	5,550 (6.7)[c]	155 (7.4)[c]	116 (6.5)[c]	43 (12.4)[c]
Stillbirth (=> 22 weeks)	362 (0.4)[c]	13 (0.6)[c]	12 (0.7)[c]	1 (0.3)[c]

[a]1,826 missing values (2.1%), [b]494 missing values (0.6%), [c]singleton pregnancies only

Table 8 shows the age-standardized prevalence of corticosteroid drug use by calendar year and PR adjusted for age and smoking. The age-standardized prevalence of corticosteroid drug use increased from 1.8% in 1999 to 3.3% in 2009, PR 1.73 (95% CI, 1.42-2.11). Women aged 35 years and older had a higher prevalence of corticosteroid drug use (3.3%) than women in

the other age groups: 2.8% of women between 30 to 34 years; 2.5% of women aged 25 to 30; and 2.1% of women younger than 25 years.

Table 8. Age-standardized prevalence (P) and prevalence ratios (PR) adjusted for age and smoking of corticosteroid drug use among primiparous women in northern Denmark 1999-2009.

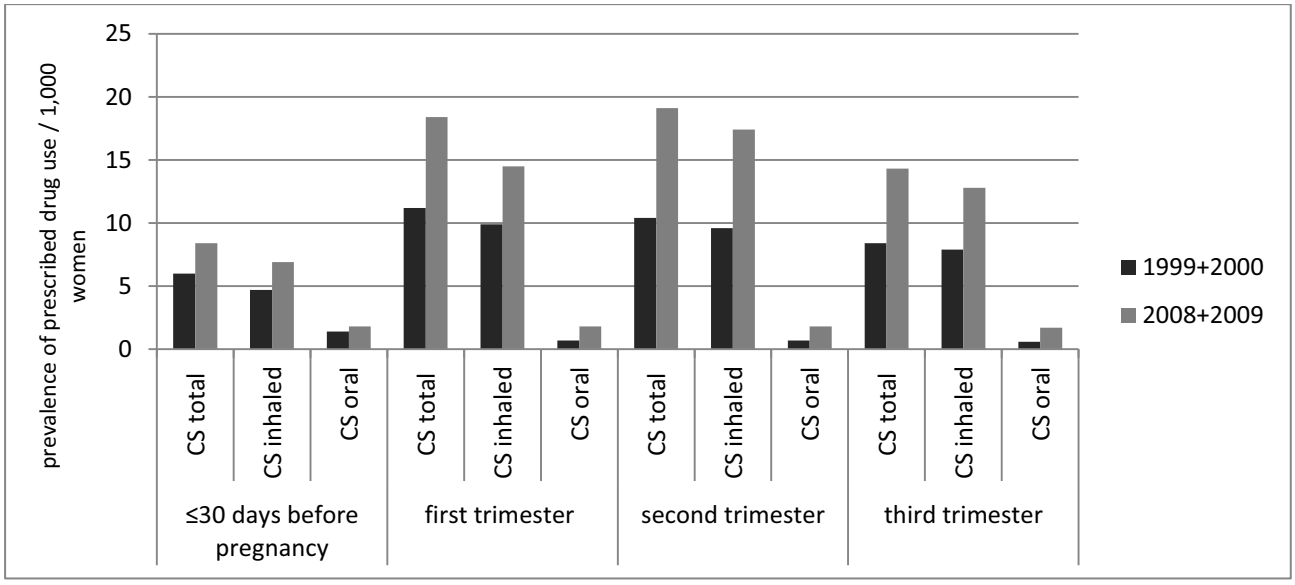
	Calendar year of delivery											
	1999 (n=7,949)	**2000** (n=8,134)	**2001** (n=8,031)	**2002** (n=7,812)	**2003** (n=8,197)	**2004** (n=7,956)	**2005** (n=7,883)	**2006** (n=7,906)	**2007** (n=7,745)	**2008** (n=5,997)	**2009** (n=8,100)	**test for trend**
total drug use												
n	4,346	4,223	4,132	4,302	4,434	4,445	4,434	4,486	4,596	3,608	4,976	
P	54.7	52.0	51.6	55.1	54.3	56.0	56.3	56.8	59.3	60.1	61.2	*p<0.001*
PR (95% CI)	1	0.95 (0.92;0.97)	0.94 (0.91;0.97)	1.00 (0.98;1.03)	0.99 (0.96;1.02)	1.02 (0.99;1.05)	1.03 (1.00;1.06)	1.04 (1.01;1.07)	1.09 (1.06;1.12)	1.11 (1.07;1.14)	1.13 (1.10;1.16)	
total CS use												
n	151	148	173	178	198	231	209	208	219	175	277	
P	1.9	1.8	2.1	2.3	2.4	2.9	2.6	2.5	2.8	2.9	3.3	*p<0.001*
PR (95% CI)	1	0.94 (0.75;1.19)	1.11 (0.89;1.38)	1.18 (0.95;1.47)	1.24 (1.00;1.53)	1.47 (1.20;1.80)	1.37 (1.11;1.68)	1.33 (1.08;1.64)	1.46 (1.18;1.79)	1.51 (1.22;1.87)	1.73 (1.42;2.11)	
inhaled CS use												
n	131	126	144	143	168	211	179	178	187	145	224	*p<0.001*
P	1.7	1.5	1.8	1.8	2.0	2.6	2.3	2.2	2.4	2.4	2.7	
PR (95% CI)	1	0.93 (0.73;1.19)	1.06 (0.84;1.35)	1.10 (0.86;1.39)	1.22 (0.97;1.53)	1.56 (1.25;1.94)	1.35 (1.08;1.69)	1.33 (1.06;1.67)	1.44 (1.15;1.80)	1.46 (1.16;1.85)	1.64 (1.32;2.03)	
oral CS use												
n	22	27	34	34	28	21	33	33	40	38	56	
P	0.3	0.3	0.4	0.4	0.3	0.3	0.4	0.4	0.5	0.6	0.6	*p=0.001*
PR (95% CI)	1	1.15 (0.65;2.02)	1.47 (0.86;2.52)	1.55 (0.91;2.64)	1.17 (0.67;2.05)	0.84 (0.46;1.56)	1.48 (0.86;2.54)	1.34 (0.78;2.33)	1.79 (1.06;3.02)	2.13 (1.26;3.63)	2.27 (1.38;3.73)	

Abbreviations: ATC=Anatomical Therapeutic Chemical classification of drugs; n=number, P= prevalence, PR=prevalence ratio; CI=confidence interval.

Prevalences are standardized to age distribution in 1999; prevalence ratios are adjusted for age and smoking during pregnancy.

The prevalence of pregnancy period specific drug use of corticosteroids increased from 1999-2000 to 2008-2009 as illustrated in Figure 3. First-trimester use of corticosteroids increased from 1.1% in 1999-2000 to 1.8% in 2008-2009, PR 1.65 (95% CI 1.36-1.99); second trimester use increased nearly two-fold from 1.0% in 1999 to 1.9% in 2009, PR 1.84 (95% CI 1.52-2.23); and third trimester use increased from 0.8% in 1999 to 1.4% in 2009, PR 1.70 (95% CI 1.37-2.11). We calculated prevalence and PRs standardized according to age and adjusted for smoking for each pregnancy period; however, this did not change the estimates notably (data not shown).

Figure 3. Prevalence (per 1000 women) of pregnancy period specific corticosteroid (CS) drug use among primiparous women in 1999-2000 and 2008-2009.



4.2 Study 2: Use of corticosteroids in early pregnancy is not associated with risk of congenital malformations in the offspring

We identified a total of 83,043 primiparous women. In total, 1,449 women (1.7%) used corticosteroids in early pregnancy. Among corticosteroid users, 491 (33.9%) women had a hospital diagnosis of asthma, rheumatoid arthritis or inflammatory bowel disease compared with 2.4% among the non-users.

The prevalence of congenital malformations was 4.3% among both users and non-users of corticosteroids (unadjusted POR, 1.02; 95% CI, 0.79-1.32). Adjustment for maternal age, smoking, and diabetes did not change this estimate notably (data not shown). The prevalence of congenital malformations did not differ between users of inhaled or users of oral corticosteroids (Table 9). We identified one woman who had an infant with an oral cleft (0.08%) among the 1,223 users of inhaled corticosteroids compared with 145 (0.2%) among the 80,950 non-users. The unadjusted POR was 0.47 (95% CI, 0.07-3.34). When we excluded women who filled one prescription of inhaled and oral corticosteroids in early pregnancy, we identified 30 women (3.5%) who gave birth to a malformed infant among inhaled corticosteroid users (unadjusted POR, 0.80; 95%CI, 0.56-1.16) and 7 women (6.7%) who gave birth to a malformed infant among oral corticosteroids users (unadjusted POR, 1.62; 95%CI, 0.75-3.50).

The prevalence of congenital malformations divided into subgroups among users and non-users of corticosteroids are presented in Table 10.

Table 9. Prevalence and unadjusted prevalence odds ratios (POR) for congenital malformations according to corticosteroid use in early pregnancy among primiparous women in northern Denmark 1999-2009.

	No use of inhaled or oral corticosteroids at any time during pregnancy	**Use of inhaled or oral corticosteroids in early pregnancy**	**Use of inhaled corticosteroids in early pregnancy**	**Use of oral corticosteroids in early pregnancy**
Number of women	80,950	1,449	1,223	226
Congenital malformations				
number	3,446	63	53	10
prevalence (%)	4.3	4.3	4.3	4.4
POR (95% CI)	*Reference*	1.02 (0.79-1.32)	1.02 (0.77-1.34)	1.04 (0.55-1.96)
Oral clefts				
number	145	1	1	0
prevalence (%)	0.2	0.07	0.08	-
POR (95% CI)	*Reference*	0.39 (0.05-2.75)	0.47 (0.07-3.34)	-

Abbreviations: POR, prevalence odds ratio; CI, confidence interval.

Table 10. Prevalence of congenital malformations in offspring categorized in subgroups as defined by EUROCAT according to used of corticosteroids in early pregnancy among primiparous women in northern Denmark 1999-2009.

Outcome	Use of corticosteroids in early pregnancy (n=1,449)	Use of inhaled corticosteroids in early pregnancy (n=1,223)	Use of oral corticosteroids in early pregnancy (n=226)	No use of inhaled or oral corticosteroids at any time in pregnancy (n=80,950)
Congenital malformations overall, number (%)	63 (4.3)	53 (4.3)	10 (4.4)	3,446 (4.3)
Sub-groups, number (%)				
Nervous system	3 (0.2)	2 (0.2)	1 (0.4)	120 (0.2)
Eye	1(0.1)	1 (0.1)	-	66 (0.1)
Ear, face and neck	-	-	-	19 (0.02)
Congenital heart disease	21 (1.5)	19 (1.6)	2 (0.9)	822 (1.0)
Respiratory	1 (0.1)	1 (0.1)	-	98 (0.1)
Orofacial clefts	1 (0.1)	1 (0.1)	-	145 (0.2)
Digestive system	2 (0.1)	2 (0.2)	-	143 (0.2)
Abdominal wall defects	-	-	-	33 (0.04)
Urinary	5 (0.4)	5 (0.4)	-	193 (0.2)
Genital	2 (0.1)	1 (0.1)	1 (0.4)	208 (0.3)
Limb	13 (0.9)	11 (0.9)	2 (0.9)	564 (0.7)
Musculo-skeletal	2 (0.1)	1 (0.1)	1 (0.4)	97 (0.1)
Other malformations	2 (0.1)	1 (0.1)	1 (0.4)	103 (0.1)

4.3 Study 3: Risk of miscarriage and use of corticosteroid hormones: a population-based case-control study

We identified 10,974 cases of miscarriage and 109,740 controls giving live birth. Cases were more likely than controls to be 30 years or older on the index date (34.1% vs. 26.9%). Overall, 1,381 (12.5%) of cases and 19,762 (17.9%) of controls were reported to be smokers. Information on smoking was missing for 3,352 (30.4%) cases and 2,546 (2.3%) controls. Cases and controls were similar with respect to prevalence of asthma, rheumatoid arthritis, inflammatory bowel diseases, diabetes, and epilepsy.

For inhaled corticosteroids, the adjusted OR of miscarriage was 1.20 (95% CI: 1.01-1.44) for current use and 1.05 (95% CI: 0.96-1.15) for former use. Current, recent, and new use of oral corticosteroids did not differ among cases and controls. For current use of oral corticosteroids, the adjusted OR for miscarriage was 0.78 (95% CI: 0.53-1.15). For former use, the adjusted OR was 1.07 (95% CI: 0.97-1.18) (Table 11).

A total of 9,735 (88.7%) early miscarriages and 1,239 (11.3%) late miscarriages were identified. Among women with early miscarriage, 129 (1.3%) were current users of inhaled corticosteroids whereas 11 (0.9%) of women with late miscarriage were current users of inhaled corticosteroids. Table 12 shows the ORs for early and late miscarriage in relation to use and timing of inhaled or oral corticosteroids. The adjusted OR for an early miscarriage associated with current use of inhaled corticosteroids was 1.22 (95% CI: 1.01-1.49) and that for a late miscarriage was 1.06 (95% CI: 0.56-1.99). Among women with early miscarriage we identified 27 (0.3%) current users of oral corticosteroids. Among women with late miscarriage we identifies one (0.1%) current user of oral corticosteroids.

After extending the definition of current use to 90 days before the index date, prevalence of current use of inhaled corticosteroids was 1.5% among cases and 1.3% among controls (adjusted OR = 1.09; 95% CI: 0.92-1.28). Prevalence of newly-defined current use of oral corticosteroids was 0.3% among cases and 0.4% among controls (adjusted OR = 0.74; 95% CI: 0.52-1.05).

After excluding 1,585 cases (14.4%) and 13,197 controls (12.0%) with a record of induced abortion, the analysis did not change in ways to affect interpretation.

Among women who smoked, the prevalence of current use of inhaled corticosteroids did not differ between cases and controls (1.0% respectively). When adding smoking to the adjusted analyses, we did not observe any substantial change in the estimates (adjusted OR including smoking for miscarriage with current use of inhaled corticosteroids = 1.16; 95% CI 0.92-1.44, adjusted OR for miscarriage including smoking with current use of oral corticosteroids = 0.99; 95% CI 0.62-1.57).

Table 11. Use of corticosteroids and miscarriage among women in northern Denmark, 1997-2009.

Corticosteroid use	**Case/control** ratio	**Unadjusted OR** (95% CI)	**Adjusted OR*** (95% CI)
Corticosteroids overall			
Current use	165/1,447	1.15 (0.97-1.35)	1.11 (0.95-1.31)
Recent use	118/1,286	0.92 (0.76-1.12)	0.92 (0.76-1.11)
Former use	976/9,213	1.07 (1.00-1.14)	1.07 (0.99-1.14)
Never use	9,768/98,291	*reference*	*reference*
New use	19/245	0.78 (0.49-1.25)	0.75 (0.47-1.19)
Inhaled corticosteroids			
Current use	140/1,143	1.23 (1.03-1.47)	1.20 (1.01-1.44)
Recent use	87/907	0.95 (0.76-1.19)	0.94 (0.75-1.17)
Former use	575/5,496	1.05 (0.96-1.15)	1.05 (0.96-1.15)
Never use	10,172/102,194	*reference*	*reference*
New use	9/95	0.92 (0.46-1.82)	0.86 (0.43-1.72)
Oral corticosteroids			
Current use	28/341	0.82 (0.56-1.21)	0.78 (0.53-1.15)
Recent use	35/411	0.85 (0.60-1.21)	0.85 (0.60-1.20)
Former use	474/4,387	1.08 (0.98-1.19)	1.07 (0.97-1.18)
Never use	10,437/104,601	*reference*	*reference*
New use	10/171	0.60 (0.32-1.14)	0.57 (0.30-1.07)

*Adjusted for age at the index date, history of diabetes and epilepsy, and use of NSAIDS 12 weeks before the index date.

OR: odds ratio; CI: confidence interval.

Table 12. Use of oral and inhaled corticosteroids prescription stratified by early miscarriage (gestational week 1-12) or late miscarriage (gestational week 13-21); northern Denmark, 1997-2009.

Corticosteroid use	Early miscarriage			Late miscarriage		
	Case/control Number	**Crude OR** (95% CI)	**Adjusted OR** (95% CI)	**Case/control** Number	**Crude OR** (95% CI)	**Adjusted OR** (95% CI)
Inhaled corticosteroids						
Current	129/1,047	1.24(1.03-1.49)	1.22(1.01-1.46)	11/96	1.09(0.58-2.04)	1.06(0.56-1.99)
Recent	74/786	0.93 (0.73-1.18)	0.92 (0.72-1.17)	13/121	1.07(0.60-1.91)	1.06 (0.59-1.89)
Former	515/4,867	1.06(0.97-1.17)	1.07(0.97-1.17)	60/629	0.95 (0.72-1.25)	0.97(0.74-1.27)
New	9/88	0.99 (0.50-1.97)	0.93(0.47-1.86)	0/7	-	-
Never	9,017/90,650	*reference*	*reference*	1,155/11,544	*reference*	*reference*
Oral corticosteroids						
Current	27/318	0.86(0.58-1.27)	0.81(0.55-1.20)	1/23	0.41(0.06-3.06)	-
Recent	32/367	0.87 (0.61-1.25)	0.87 (0.60-1.25)	3/44	0.70(0.22-2.26)	0.68 (0.21-2.20)
Former	431/3,884	1.11(1.01-1.23)	1.10(0.99-1.22)	43/503	0.85(0.62-1.17)	0.83(0.61-1.14)
New	10/164	0.63 (0.33-1.19)	0.59(0.31-1.12)	0/7	-	-
Never	9,245/92,781	*reference*	*reference*	1,192/11,820	*reference*	*reference*

* adjusted for age at index date, history of diabetes and epilepsy, and use of NSAIDS 12 weeks before the index date.

OR, odds ratio; CI, confidence interval.

Chapter 5. Discussion of study results

When interpreting the results of the thesis, it is necessary first to consider whether an apparent association is real or could be an artifact because of bias or chance. If these problems are considered unlikely, one must consider whether the association occurs indirectly though another (confounding) factor before reading the results as evidence of causality (Figure 4).[113]

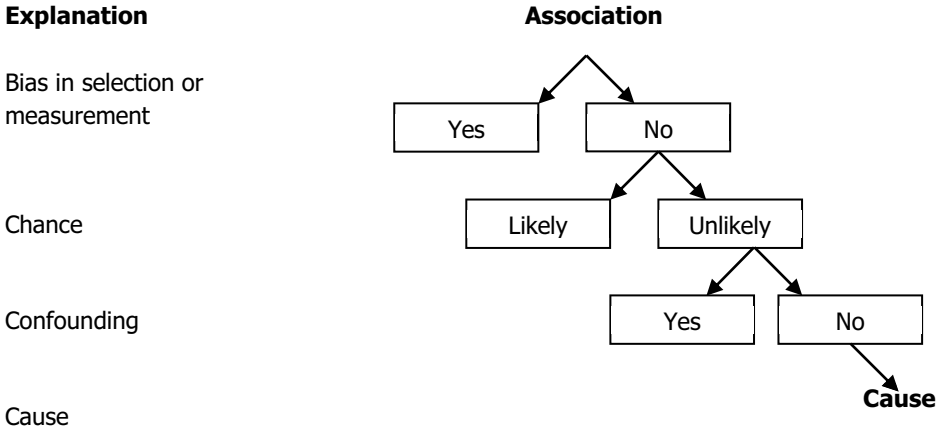


Figure 4. Association and cause adapted from Fletcher & Fletcher.[113]

In the following sections, we will first discuss the study results and methodological considerations separately for each study. Then, at the end of the chapter, an overall discussion of confounding and chance will be presented for all studies.

5.1 Drug utilization in pregnancy (Study 1)

5.1.1 Main findings

More than half of pregnant primiparous women in northern Denmark used prescribed drugs at some point during their pregnancy. Use of prescribed reimbursed drugs increased modestly (6.5% in absolute terms) from 1999 to 2009. Prevalence of corticosteroid use nearly doubled over the observation period, but the absolute prevalence remained low. The overall prevalence of drug use increased with age; however, increasing age of primiparous women did not explain the overall increase in prevalence of drug use over time.

5.1.2 Main findings in relation to the existing literature

We extended the existing literature on drug utilization in pregnant women[50-58] and provided information of drug utilization during pregnancy over time. We further provided information on use of corticosteroids in pregnancy over time. The observed modest increase in drug use among women giving first birth could be a reflection of a general population trend of increased drug use. In 2005, the Danish Institut of Public Health reported that use of prescribed drugs increased 24% as measured in defined daily doses (DDD) from 2001 to 2005. The increasing prevalence of corticosteroid use could reflect an increasing prevalence of diseases in pregnant women, e.g. asthma[9] and inflammatory bowel disease[16] for which corticosteroid drug therapy is needed.

Although the increasing age of primiparous women did not explain the overall increase in prevalence of drug use over time, the prevalence of drug use increased with age. Our findings that women aged 35 years or older had a slightly higher prevalence of overall drug use than women in younger age groups are similar to findings from an Irish study involving 61,252 women giving birth in Dublin from 2000-2007.[100]

5.1.3 Methodological considerations

We measured drug exposure through automated reimbursement and routine electronic record-keeping, which enabled us to avoid recall bias, and to estimate drug utilization systematically.[114] The Aarhus University Prescription Database lacks information on dispensation of over-the-counter drugs (such as vitamins), in-hospital treatment (such as in-hospital antibiotics), or sales of non-reimbursed prescribed drugs (such as sedatives, hypnotics, or oral contraceptives).[97] Consequently, our estimated overall prevalence of drug use during pregnancy is likely an underestimate.[115]

On the other hand, we do not know if women actually took their prescribed medication and low compliance would lead us to overestimate the drug use. A validation study found a high degree of agreement between self-reported drug intake and that recorded in the Aarhus University Prescription Database among non-pregnant women.[116] The agreement between filled prescriptions and self-reported drug intake among pregnant women could be more complex, mainly because of fear of a potential teratogenic effect of the drug.[1] However, a series of Hungarian validation studies on drug use in pregnancy showed that only a small

group of pregnant women (2.4%) did not use prescribed drugs due to the suspected teratogenic risk.[117] Olesen *et al*. compared prescription data from the former North Jutland Prescription Database (now part of the Aarhus University Prescription Database) with information on drug intake provided by pregnant women (n=2,041) to the Danish National Birth Cohort in order to estimate the probability of pregnant women reporting drug intake to the Danish National Birth Cohort after a filled prescription.[118] Reported drug use was based on a questionnaire about the past three months drug use and for some women also a telephone interview at gestational week 12-15. Overall, 43% of the filled prescription drugs were reported to be used. However, agreement between the prescription registry and self-reported drug intake differed according to drug type, for example drugs for chronic diseases (e.g. insulin, thyroid hormones, and anti-epileptic drugs) were always reported to be taken, whereas agreement for drugs used for local or short-term treatment (e.g. anti-infectives, antacids, NSAIDs, and gynecologic drugs) was low. For intake of systemic corticosteroids, only 20% (95% CI, 0-55%) of the dispensed drugs were reported to be used but this estimate was based on only five women. There was no estimate on inhaled corticosteroid use. Moreover, the accuracy of that validation study was hampered by the fact that the actual time window for reported drug intake was not known for all participating women. A recent Danish prevalence study investigated the adherence to medical treatment among women with ulcerative colitis (n=115) prior to and/or during pregnancy from 2000-2005.[119] Overall, 58 women stated to be in medical treatment prior to and / or during pregnancy, among whom 50 had fulfilled a prescription on relevant medication according to the Prescription Database. This yielded a positive predictive value of self-reported drug use of 86.2% (95% CI 74.6–93.9).

In conclusion, we find it likely that the pregnant women took their prescribed corticosteroid drugs, because women with chronic diseases in general take their recommended therapy as pictured in the presented validation studies.[118,119]

5.2 Corticosteroid use and risk of congenital malformations in offspring (Study 2)

5.2.1 Main findings

Use of corticosteroids in early pregnancy was not associated with an increased risk of congenital malformations overall or oral clefts in offspring, though the estimates were imprecise.

5.2.2 Main findings in relation to the existing literature

Our findings do not corroborate the previous studies that reported increased risk of congenital malformations overall[60,62] or oral clefts in offspring[19,66,68,69] following corticosteroid exposure; however our estimates are imprecise. It is a main limitation of our study that we identified a low number of events. Even with large databases available, we identified only one woman who used corticosteroids in early pregnancy and who gave birth to an infant with oral cleft.

Congenital malformations cannot be considered as a single homogenous outcome because the mechanisms of the malformation vary according to the embryologic tissue of origin, the gestational occurrence, and the mechanism of development.[1] Thus, no single drug have been proven to be associated with an increased risk of all congenital malformations.[49] We found no specific pattern of congenital malformations in the offspring of exposed women which could indicate that corticosteroids are not teratogenic.

5.2.3 Methodological considerations

We did not capture data on women with early pregnancy loss (miscarriage and induced abortions). Thus, we did not capture congenital malformations that might have been present in lost embryos. If the prevalence of early pregnancy loss differed between corticosteroid users and non-users, selection bias could be present.[73] Selection bias could thus explain the lack of an association between corticosteroids and congenital malformations reported in **Study 2** and in three of the other studies presented in the literature review.[59,61,68] However, in **Study 3**, we found only a slight increase in risk of miscarriage in women who used corticosteroids which suggest that selection bias due to miscarriage may be a minor issue in studies of birth outcome.

The National Registry of Patients is considered a valid tool for epidemiological research of congenital malformations. A previous study assessed the predictive value of a registration of a congenital malformation diagnosis in the National Registry of Patients through a review of a sample of medical records. The positive predictive value of a congenital malformation diagnosis, defined as the number of infants correctly diagnosed with a congenital malformation in the registry divided by the total number of infants recorded with a congenital malformation in the registry, was estimated to be 88%.[102] Recently, a validation study showed

that the positive predictive value of congenital cardiac malformation diagnoses registered in the Danish National Patient Registry compared with the clinical record of each individual showed an agreement of data of more than 90%.[120] If imperfect classification of congenital malformation exists, this may lead us to underestimate the prevalence of congenital malformations.[77] However, since congenital malformations are rather rare we expect few false-negative records of birth defects (high specificity) and therefore relative estimates of effect will be unbiased, provided no other bias is at work.[77]

Ascertainment of all congenital malformations at birth is not possible because not all congenital malformations are present at delivery. Many heart defects or hypospadia, for example, do not manifest themselves until after initial discharge from hospital[49] and therefore we include congenital malformations registered during the first year of life. However, some malformations may go undiagnosed until adulthood, for example some heart defects or abnormalities of neurologic development[49] and misclassification could therefore still be at work. Such misclassification is assumed to be non-differential between corticosteroid exposed and unexposed and may nullify the observed estimate of effect, if an effect exists.

Non-differential misclassification because of stockpiling could also cause an underestimation of the corticosteroid intake.[115] Inhaled and oral corticosteroids are not sold over-the-counter in Denmark where most corticosteroids are indicated for chronic diseases (www.medicin.dk). Such use could be administered by multiple refills and these women would not be identified as users in our study. This could potentially produce the null result; if, however, the true effect is null, non-differential misclassification is irrelevant. [83]

5.3 Corticosteroid use and risk of miscarriage (Study 3)

5.3.1 Main findings

Use of inhaled corticosteroids was associated with a slightly increased risk of miscarriage. Use of oral corticosteroids did not seem to be associated with an increased risk of miscarriage. Use of corticosteroids was associated with a slightly increased risk of early miscarriage but not with late miscarriage.

5.3.2 Main findings in relation to the existing literature

Our findings corroborate two previous studies which reported a slightly increased risk of miscarriage with inhaled corticosteroid use[64,65] and one previous study that found no

increased risk of miscarriage with oral corticosteroid use.[62] We extend the previous literature[60,62,64,65] as we included information about gestational age at miscarriage. This allowed us to select controls at a gestational age where they were eligible to become cases and to ascertain corticosteroid use in the same preceding period for both cases and controls. Data on gestational age also allowed differentiation between early and late miscarriage, which may have different etiologies. We found that current use of inhaled corticosteroids was associated with a slightly increased risk of early miscarriage but not with late miscarriage. This could reflect that exposure in early pregnancy influences the fetus' environment and therefore increases the risk of early pregnancy loss.

On the other hand, we observed an association for miscarriage with use of inhaled corticosteroids, which are used in asthma treatment, and no association with oral corticosteroids which suggests that the underlying asthma may play a role. A prevalence study using data from the Kaiser-Permanente Prospective Study of Asthma during Pregnancy reported an increased risk of miscarriage of 1.57 (95% CI 1.02-2.41) among 1,044 pregnant women with asthma compared with 860 pregnant women without asthma.[63] A large prevalence study based on The Health Improvement Network in England and Wales of almost 300,000 pregnancies, reported a higher risk of miscarriage (OR, 1.28; 95% CI, 1.15-1.43) among asthmatic women who experienced one or more exacerbation in the year before pregnancy compared with non-asthmatic women.[65] Although not fully clarified, the proposed biological mechanisms for the increased risk of miscarriage in women with asthma are related to maternal hypoxia during asthma exacerbations.[121,122] Also, the abnormal smooth muscle activity in the uterus are related to similar mechanisms of airway smooth muscle contraction in asthma.[121,122] An observed association between an asthma medication and adverse pregnancy outcome could therefore be confounding by indication.[88] Yet, we found no major difference in the prevalence of asthma diagnoses between cases and controls.

5.3.3 Methodological considerations

The positive predictive value of miscarriages recorded in the National Registry of Patients has been estimated to be 97%[123] which indicates a high specificity for this diagnosis. Thus, the National Registry of Patients is a valid source for identifying cases of miscarriage.

In a case-control study, it is important that the controls are sampled to represent the distribution of exposure in the underlying source population. We selected our controls among women who had given birth, because these women would have become cases had they suffered a miscarriage. On the other hand, under the current case-control study design with a prevalent outcome the OR may overestimate the underlying risk or rate ratios.[37,124]

The source population could also have included women whose pregnancies ended in an induced abortion or an extrauterine pregnancy. We may have underestimated the level of exposure in the source population, if induced abortion or extrauterine pregnancies are related to use of corticosteroids.[73] We have no reason to believe that induced abortions or extrauterine pregnancy should not be represented randomly among women and we observed a similar history of recorded induced abortions among cases and controls.

In order to evaluate the teratogenicity of corticosteroids we addressed the issue of selection bias due to spontaneous abortion of fetuses with malformations by examining the association between use of corticosteroids and miscarriage. However, many cases of early miscarriages occur without the woman ever having known she was pregnant. These women are not examined by a physician and thereby not recorded in hospital registries. A previous Danish study assessed the occurrence of miscarriage by comparing interview data with data from the National Registry of Patients.[124] It was estimated that 25% of the miscarriages reported by the women were not registered in the National Registry of Patients. The missing cases were probably early, non-hospitalized miscarriages.[124] If women in treatment with corticosteroids more often than other women in early pregnancy were referred to examination by a physician in case of early miscarriage symptoms, this may have caused a selection bias resulting in a bias away from the null.[49]

5.4 Confounding

We were able to control for confounding by age (**Studies 1-3**), presence of diabetes (**Studies 2-3**), and presence of epilepsy (**Study 3**). We also controlled for self-reported smoking (**Studies 1-3**). We had to use a proxy measure of smoking status at the time of miscarriage (**Study 3**) because data was not available from the National Registry of Patients. Therefore we collected smoking status from the first registration in the Medical Birth Registry following the miscarriage. Adjusting for these potential confounding factors did not change the estimates

notably. However, in **Study 3** the adjustment for smoking was incomplete because 30% of cases did not have a later birth registration in the Medical Birth Registry.

The estimates could also be affected by residual confounding. However, the quality of the data in the Medical Birth Registry is reportedly good.[125] Moreover, in all three studies, the relative estimates were virtually unchanged after adjustment for e.g. age, which speaks against substantial residual confounding.

Unmeasured confounding cannot entirely be ruled out. We lack information on socio-economic factors in the health registries and this hinders our ability to control for confounding in relation to income, education, or occupation, which may be related to drug use during pregnancy,[100,126] including use of FDA risk category D or X drugs.[101] Because of the null result of **Study 2**, any confounding would need to be by factors associated with maternal use of corticosteroids and also with a reduced risk of congenital malformations overall. No such factor has been identified, to the best of our knowledge.

5.4.1 Confounding by indication

We had information about the underlying diseases (**Studies 2-3**). However, among women who used inhaled corticosteroids **(Study 2)** only about 30% had a record of asthma episode requiring a hospital contact. A validation of asthma diagnoses in the National Registry of Patients against independently confirmed diagnoses of asthma also showed that only 44% of asthma patients had a hospital contact because of the disease.[127] This reflects the practice of treating most asthma episodes in primary care. Although it may be difficult to separate the effect of corticosteroids from the effect of the underlying disease that indicated the treatment, confounding by indication is unlikely in **Study 2** because of the null result. In **Study 3**, we found no difference in prevalence of underlying disease among cases and control.

5.5 Chance

Chance (random error) is the component of overall error that cannot be predicted, but can be quantified using statistical distributions.[128] Although our study populations were large compared with most other studies, the number of congenital malformations available for analyses was small (**Study 2**) and we had only few women who used inhaled or oral corticosteroids in each strata (**Studies 2-3**). Thus, the wide confidence intervals of several of our estimates complicate their interpretation. Therefore the existing literature has still not

been able to provide sample sizes large enough to provide evidence of whether or not corticosteroids are teratogenic.

In **Study 2** our main outcome was congenital malformations, which are rare outcomes, and because we restricted our analysis to primiparous women, we actually halved our potential population. We restricted to primiparous women, because an adverse outcome in a previous pregnancy were thought to influence a woman's drug use in a new pregnancy. However, we could have stratified or adjusted for parity instead.

Chapter 6. Main conclusions

Based on the results obtained and our considerations of potential bias and confounding, the following conclusions can be drawn from the thesis:

Study 1: More than half of all pregnant women use prescribed drugs. Drug utilization in pregnancy increased slightly from 1999 to 2009. Use of corticosteroids increased nearly two-fold. Increasing age of primiparous women did not seem to increase drug utilization over time.

Study 2-3: Use of corticosteroids did not seem to increase the risk of congenital malformations, but the estimates are imprecise. Use of inhaled corticosteroids was associated with a slightly increased risk of miscarriage. Selection bias due to miscarriage seems to be only a minor issue in the evaluation of the teratogenic effect of corticosteroids.

Chapter 7. Perspectives

This thesis adds to the knowledge of drug utilization of corticosteroids in pregnancy and provides more evidence to the ongoing discussion of whether there is a teratogenic potential of these drugs or not. However, this thesis also raises important methodological issues such as sample size considerations, indication for prescribing, and unmeasured confounding that are important to consider when planning future pharmacoepidemiological studies of drug use in pregnancy.

1) Sample size considerations.

   Despite that corticosteroids are commonly used drugs, they are infrequently used during pregnancy. We showed that this infrequent use combined with low prevalence of oral clefts produced only one exposed case in our population of more than 83,000 pregnancies. Therefore, nationwide studies or preferable, even larger studies based on an international collaboration could be the solution to enable sample sizes large enough to provide a larger precision of the estimates.[72] However, to detect even a common congenital malformation a population of at least half a million women are needed and as many as 5 million are required to detect rare events.[129] A Nordic Pharmacoepidemiological Network (NorPEN) has been established in order to facilitate knowledge exchange, research and training across the Nordic countries (www.nhv.se/norpen). Nordic prescription databases cover populations up to about 25 million inhabitants[72] and a collaboration could provide sample sizes large enough to detect even rare teratogenic effects of corticosteroids. Although, differences in methodology, coverage, validity, and access to data between the Nordic countries are challenging, a Nordic monitoring system to evaluate safety of drugs taken during pregnancy would be a major step forward to achieve more detailed information of exposure-outcome associations.

2) Indication for prescribing.

   The indication for prescribing the corticosteroids is an important confounding factor and this methodological problem remains an elusive task in pharmacoepidemiology. All available study designs can to some extend control for confounding by indication in the analysis stage, but it requires that valid and complete information is obtained and translated into standardized and measurable criteria for the indication of prescribing the

drug. These data are not available in the Aarhus University Prescription Database.[97] It would be a major step forward if reliable data on indication for prescribing was registered in this prescription registry. Another approach to solve this issue could be to review medical charts from hospital admissions in order to define the actual indication for drug therapy. Finally, use of a case-time-control design may eliminate some of the confounding by indication. In a case-time-control study, we use cases and controls of a conventional case-control study as their own referents and then we could eliminate some of the biasing effect.[130] The case-time-control approach provides an unbiased estimated of the OR in the presence of confounding by indication, even though indication for drug use or severity of disease is not measured, because of the within-subject analysis. However, if the congenital malformation under study is believed to be caused by fluctuation in the underlying disease, rather than the treatment, confounding by indication is still an issue in this design.[130]

3) Unmeasured confounding.
   An important challenge for future pharmacoepidemiological studies of drug use in pregnancy is to get at better understanding of risk factors for adverse birth outcome such as congenital malformations in offspring and miscarriage. We did have information of potential confounding factors like maternal age, maternal diseases, use of other drugs, and smoking status although we had to use a proxy measure of smoking status at the time of miscarriage (Study 3). Data of smoking status at time of miscarriage could be obtained by reviewing medical charts from the hospital.

As outlined in this thesis, it is challenging to interpret data from the existing studies of whether an association of corticosteroid use and risk of congenital malformations in offspring or miscarriage is present or not. As we are ignorant of most biologic mechanisms by which congenital malformations in offspring occur it is difficult to determine when a finding may be biologically plausible.[49] Furthermore, drug use in pregnancy is not tested by randomized trials.[1] Therefore, pharmacoepidemiological studies are the best tool available at present to illuminate the teratogenic potential of corticosteroid drugs.

The pharmacological breakthrough of glibenclamide (a drug to treat gestational diabetes) which is transferred from the fetal to the maternal circulation against its concentration

gradient,[131] gives hope for future design of a perfect corticosteroid drug. This complicated process may be due to an interaction of high protein binding, short elimination half-life, and the role of specific placental transporters. The role of these transporters, which leads corticosteroids to one unique target, could be a key to unworried corticosteroid treatment in pregnancy in the future.

Chapter 8. Summary

Drug use in pregnancy can potentially harm the fetus. Because pregnant women are typically excluded from randomized studies of drugs, evidence about drug utilization and safety in pregnant women comes primarily from surveillance. Drug use in pregnancy is unavoidable and of special concern are women with medical conditions that necessitate drug use during pregnancy. Asthma, inflammatory bowel disease, and rheumatoid arthritis are some of the most common medical condition affecting women of childbearing age and prevalence of these diseases has increased in recent years. Corticosteroids are very potent drugs that have anti-inflammatory and immunesuppressive effects and they are used in the treatment of these medical diseases. A number of human studies have examined the association of first-trimester use of corticosteroids and risk of congenital malformations and miscarriage but the existing evidence about teratogenicity of corticosteroids is inconclusive and has limitations.

We conducted three epidemiologic studies based on data from the Medical Birth Registry, the National Registry of Patients, the Aarhus University Prescription Database, and the Civil Registration System in order to describe prescribed drug use and corticosteroid use in pregnancy **(Study 1).** We further examined corticosteroid use in relation to congenital malformations in offspring **(Study 2)** and miscarriage **(Study 3)**.

In **Study 1**, we found that more than half of pregnant primiparous women in northern Denmark used prescription drugs. Use of prescribed drugs increased modestly (6.5% in absolute terms) from 1999 to 2009. The prevalence of corticosteroid use nearly doubled over the observation period, but the absolute prevalence remained low. The prescription database lacks information on dispensation of e.g. over-the-counter drugs and in-hospital treatment, so our estimated drug use is most likely an underestimate of the true prevalence. On the other hand, as we had no data on the intake of the medication, we have likely overestimated the actual use of some of the drugs.

In **Study 2**, we found that use of corticosteroids in early pregnancy was not associated with an increased risk of congenital malformations in the offspring, but the estimates were imprecise because the number of congenital malformations available for analyses was small. We did not capture data on women with early pregnancy loss (miscarriage and induced abortions). Selection bias could thus explain the lack of an association between corticosteroids and

congenital malformations. However, results of **Study 3** suggested that selection bias due to miscarriage is a minor issue in studies of adverse birth outcomes.

In **study 3**, use of inhaled corticosteroids was associated with a slightly increased risk of miscarriage. Use of oral corticosteroids did not seem to be associated with an increased risk of miscarriage. We observed an association between corticosteroid exposure and first trimester miscarriages but not for miscarriages at a later stage in pregnancy. Adjusting for smoking, which has also been associated with increased risk of miscarriage, did not change the estimates notably.

The studies in this thesis have shown that large medical databases can provide data to carry out pharmacoepidemiological studies of drug exposure in pregnancy. Provided that such studies are properly conducted, the results may contribute to the ongoing discussion of the teratogenic potential of corticosteroids.

Chapter 9. Dansk resumé

Brug af medicin under graviditeten kan påvirke fosteret uhensigtsmæssigt men af etiske årsager er det ikke muligt at undersøge medicinens påvirkning under graviditeten ved hjælp af kliniske forsøg. Kroniske sygdomme som astma, inflammatoriske tarmsygdomme og visse gigtformer er hyppige hos kvinder i den fødedygtige alder. Disse sygdomme kan kræve medicinsk behandling under en eventuel graviditet og forekomsten er stigende. Binyrebark-hormon (kortikosteroid) bruges bl.a. i behandlingen af inflammatoriske lidelser. Flere internationale studier har imidlertid rejst mistanke om, at brug af binyrebark-hormon under graviditeten kan medføre øget risiko for medfødte misdannelser og ufrivillig abort, men dette er fortsat uafklaret. Via indsamlede oplysninger fra registre (det Medicinske Fødselsregister, Landspatientregisteret, Receptdatabasen ved Århus Universitet og CPR-registeret) i Region Midt og Region Nordjylland, etablerede vi tre studier. Formålet var at undersøge forbruget af receptpligtig medicin samt forbruget af binyrebark-hormon blandt gravide kvinder (**Studie 1**) samt undersøge forekomsten af medfødte misdannelser (**Studie 2**) og ufrivillig abort (**Studie 3**) blandt brugerne af binyrebark-hormon sammenlignet med ikke-brugere.

Studie 1 (deskriptivt studie): Mere end halvdelen af alle gravide kvinder brugte receptpligtig medicin og forbruget steg moderat (6.5% i absolutte tal) fra 1999 til 2009. Prævalensen af binyrebark-hormon forbruget blev fordoblet henover studieperioden, omend forbruget stadig var lavt blandt gravide (2.5%).

Studie 2 (prævalens studie): Brug af binyrebark-hormon i den tidlige graviditet syntes ikke at medføre en øget risiko for udvikling af misdannelser omend vores statistiske præcision var lav. I studiet havde vi ingen adgang til data omkring tidlig foster død (ufrivillig abort og provokeret abort) som kunne være forårsaget af medfødte misdannelser. Selektions bias kunne således forklare, at vi ikke fandt en association mellem brug af binyrebark-hormon og udvikling af misdannelser. Denne potentielle fejlkilde blev undersøgt i det følgende studie

Studie 3 (case-kontrol studie): Brug af binyrebark-hormon i inhalationsform syntes at være forbundet med en lille øget risiko for ufrivillig abort. Denne tendens var især gældende for ufrivillig abort i første trimester. Omvendt fandt vi ikke samme tendens for oralt brug af binyrebark-hormon. Rygning synes ikke at påvirke udfaldet. Desværre havde vi ikke komplet adgang til rygeoplysninger på alle cases, idet omkring 30% af disse data manglede.

Sammenfattende fandt vi at farmakoepidemiologiske studier baseret på eksisterende registre kan bidrage med væsentlig viden om konsekvenserne af medicinforbrug under graviditeten såfremt fejlkilder i form af bias og confounding tages i betragtning.

Appendix 1

Risk classification from the U.S. Food and Drug Administration Classification.[9]

Category A: Controlled studies in women fail to demonstrate a risk to the fetus in the first trimester (and there is no evidence of a risk in later trimesters), and the possibility of fetal harm appears remote.

Category B: Either animal-reproduction studies have not demonstrated a fetal risk but there are no controlled studies in pregnant women or animal-reproduction studies that have shown an adverse effect (other than a decrease in fertility) that was not confirmed in controlled studies in women in the first trimester (and there is no evidence of a risk in later trimesters).

Category C: Either studies in animals have revealed adverse effects on the fetus (teratogenic or embryocidal or other) and there are no controlled studies in women or studies in women and animals are not available. Drugs should be given only if the potential benefit justifies the potential risk to the fetus.

Category D: There is positive evidence of human fetal risk, but the benefits from use in pregnant women may be acceptable despite the risk (e.g., if the drug is needed in a life-threatening situation or for a serious disease for which safer drugs cannot be used or are ineffective).

Category X: Studies in animals or human beings have demonstrated fetal abnormalities or there is evidence of fetal risk based on human experience or both, and the risk of the use of the drug in pregnant women clearly outweighs any possible benefit. The drug is contraindicated in women who are or may become pregnant.

Appendix 2

Codes used in study 1-3 according to the International Classification of Diseases (ICD) and Anatomical Therapeutic Chemical (ATC) classification in order to identify relevant hospital diagnoses from the National Registry of Patients and to identify use of prescribed drugs from the Aarhus University Prescription Database.

	ICD-8	ICD-10	ATC-codes
Corticosteroids			
Inhaled corticosteroids			R03BA01, R03BA02, R03BA05, R03BA07, R03AK06, R03AK07
Oral corticosteroids			H02AB04, H02AB06, H02AB07, H02AB09
Study 1			
Anti-infective drugs for systemic use			J
Gynecological drugs			G
Dermatological drugs			D
Drugs for respiratory diseases			R
Drugs for alimentary tract and metabolism			A
Neurological drugs			N
Study 2			
Congenital malformations		Q00-Q99	
Undescended testis		Q53	
Congenital dislocation of the hip		Q65.0-Q65.6	
Congenital chromosomal defects		Q90-Q99	

Oral clefts		Q35-Q37	
Study 3			
Miscarriage	643	O02-O03	
Induced abortion	640, 641, 642	O04	
Covariates (study 2 and study 3)			
Asthma	493	J45-J46	
Rheumatoid arthritis	712.19, 712.39, 712.59	M05-M06	
Inflammatory bowel disease	563.00, 563.01, 563.10, 569.02	K51-K50	
Diabetes	250	E10-E14	
Epilepsy	345	G40	
Non-steroid anti-inflammatory drugs (NSAIDs)			M01A
Anti-diabetica			A10
Anti-epiletics			N03A

Appendix 3

The Anatomic Therapeutic Chemical (ATC) classification system.[99] A system of five hierarchical levels: a main anatomical group, two therapeutic subgroups, a chemical-therapeutic subgroup, and a chemical substance subgroup. Coding structures for budesonide are provided as an example of the building of the system.

ATC Classification (R03BA02)

R Respiratory system

(First level, main anatomical group)

03 Preparations for obstructive lung disease

(Second level, main therapeutic group)

B Other preparations for obstructive lung disease, inhalation

(Third level, therapeutic subgroup)

A Glucocorticoids

(Fourth level, chemical therapeutic subgroup)

02 Budesonide

(Fifth level, chemical substance)

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Studies 1-3

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ORIGINAL RESEARCH

Use of prescribed drugs among primiparous women: an 11-year population-based study in Denmark

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Purpose: To describe patterns of prescribed drug use over time among primiparous women in Denmark.

Methods: Through the Danish Medical Birth Registry, we identified all primiparous women giving live birth or stillbirth at ≥ 22 gestational weeks in northern Denmark, from 1999 to 2009. From the Aarhus University Prescription Database we obtained information on the women's prescriptions for reimbursed drugs filled from 30 days before conception until delivery.

Results: Among 85,710 primiparous women, 47,982 (56.0%) redeemed at least one prescription from 30 days before conception until delivery. Women aged 35 years and older had the highest overall prevalence of prescription drug use (61.1%). Age-standardized prevalence of drug use was 54.7% in 1999 and 61.2% in 2009, prevalence ratio (PR) of 1.13 (95% confidence interval 1.10; 1.16), adjusted for age and smoking.

Conclusion: Over the 11-year period from 1999 to 2009, we found a modest increase in overall use of drugs by primiparous women in Denmark. This increase was not, however, explained by an increasing proportion of older first-time mothers. We noted changes in patterns of use of anti-infective drugs and antidepressants.

Keywords: drug utilization, epidemiology, pregnancy

Introduction

Reported prevalence of drug use during pregnancy in Western countries ranges from 44% to 99%, and many pregnant women use several different drugs.[1–7] Because pregnant women are typically excluded from randomized studies of drugs, evidence about drug utilization and safety in pregnant women comes primarily from surveillance.[8,9]

Despite lack of data on safety, drug therapy during pregnancy is sometimes required to treat maternal conditions.[10] Women in developed countries are delaying childbearing into later reproductive years:[11] in Denmark, the prevalence of first-time mothers older than 30 years has increased from 29% in 1997 to 41% in 2007.[12] Temporal changes in demographic, social, or clinical characteristics of pregnant women as well as modifications in treatment guidelines may affect patterns of drug utilization in pregnancy.[10,13]

Previous studies have mainly reported period prevalence of drug use among pregnant women, and have examined use according to trimester of pregnancy.[1–7] Little data exist on temporal changes in drug use during pregnancy.[10,14] In this population-based study, we examined changes in patterns of prescribed drug use from 1999 to 2009 among Danish primiparous women.

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Methods

Study population

In the Danish Medical Birth Registry, we identified all primiparous women (ie, women delivering their first live- or stillborn child at ≥22 weeks' gestation)[15] from 1 January 1999 to 31 December 2009 in the Central and the North Denmark Regions, which together comprise about 33% of the total Danish population (1.8 million people). The Medical Birth Registry has recorded all births in Denmark since 1973 and contains data on characteristics of the mother (including age, residence, parity, and self-reported smoking status) and the newborn (including vital status at birth, sex, gestational age, and birth weight).[16] The information on gestational age is based on ultrasound and is recorded in full completed weeks (through 1996) and in fractional weeks (based on days) thereafter.[17] We calculated the conception date as birth date minus gestational age in days plus 14 days.

Identification of prescribed drugs

We obtained information on drug use in pregnancy using the Aarhus University Prescription Database, which tracks prescriptions for reimbursed drugs redeemed at the regions' outpatient pharmacies.[18] The pharmacies use electronic accounting systems to secure reimbursement from the National Health Service. Denmark's tax-supported health care system partially refunds the costs of most prescribed drugs.[19] To secure full prescription records for each pregnancy in the study population, we restricted our study to women who were residents of the two regions from 30 days before conception until delivery and who were therefore assumed to have redeemed their prescriptions in the regions' outpatient pharmacies.

We defined drug use as a record of at least one prescription dispensation recorded in the Aarhus University Prescription Database from 30 days before conception until delivery. For all prescriptions, we noted the woman's personal identifier, date of reimbursement, and type of medication, coded using the Anatomical Therapeutic Chemical (ATC) classification. The Aarhus University Prescription Database does not track in-hospital medicinal treatment. Nonreimbursed drugs (over-the-counter [OTC] preparations, prescription sedatives, hypnotics, or oral contraceptives) are not recorded unless they are approved for reimbursement, eg, to treat a chronic condition.[18]

Data linkage

Data were linked using the unique 10-digit personal identifier ("CPR number"), assigned to all Danish residents at birth by the Civil Registration System since 1968.[20] The CPR number, which encodes date of birth and sex, is used in all public records. Maternal CPR number is a variable on the newborn's Medical Birth Registry entry, enabling unambiguous linkage to the maternal prescription record. Furthermore, the Civil Registration System contains a variable encoding residence.

Statistical analyses

We computed prevalence of drug use among primiparous women according to maternal age at delivery (<25 years, 25–29 years, 30–34 years, and ≥35 years), smoking during pregnancy (yes/no), and categories corresponding to the major anatomical ATC groups.[21] We further analyzed six major anatomical ATC groups with prevalence of use in pregnancy exceeding 4%. These groups, listed in the order of decreasing prevalence of use, were: anti-infective drugs for systemic use (ATC group J), gynecological drugs (ATC group G), dermatological drugs (ATC group D), drugs for respiratory diseases (ATC group R), drugs for alimentary tract and metabolism (ATC group A), and neurological drugs (ATC group N). In 1998, clinical guidelines were introduced in Denmark for treatment of asymptomatic urinary tract infections in pregnancy.[22,23] For anti-infective drugs, we therefore specifically examined prevalence of drug use indicated for urinary tract infections (UTIs) (sulfamethizole (J01EB02), pivmecillinam (J01CA08), and nitrofurantoin (J01XE01)), while examining use of penicillin (phenoxymethylpenicillin (J01CE02), pivampicillin (J01CA02), and amoxicillin (J01CA04)) for comparison. After observing an increasing trend in use of neurological drugs throughout the study period, we did a post-hoc analysis to examine change over time in prevalence of drug use in specific subgroups: antidepressants (N06A), anti-epileptics (N03), and opioids (N02A).

We computed age-standardized prevalence of drug use in each calendar year (1999–2009), with age distribution in year 1999 as the standard. Further, we estimated age- and smoking-adjusted prevalence ratios (PRs) for drug use with corresponding 95% confidence intervals (CIs), using 1999 as the referent year. Furthermore, we tested for presence of a trend across years using the Chi-square test for trend.

We examined patterns of drug use over time within four gestational periods: immediate pre-conception (1–30 days before estimated conception), first trimester (gestational week 1–12), second trimester (gestational week 13–28), and third trimester (gestational week 29 to delivery). We compared pre-conception and trimester-specific

Table 1 Characteristics of primiparous women in Northern Denmark 1999–2009

	All primiparous women (n = 85,710)	Primiparous women who redeemed at least one prescription during pregnancy (n = 47,982)
Age at delivery, years		
<25	18,170 (21.2)	10,637 (22.2)
25–29	39,221 (45.8)	20,824 (43.4)
30–34	21,540 (25.1)	12,382 (25.8)
≥35	6779 (7.9)	4139 (8.6)
Smoking during pregnancy[a]	15,046 (17.6)	9014 (18.8)
Single births	83,405 (97.3)	46,348 (96.6)
Twin births	2256 (2.6)	1593 (3.3)
Triplet births	49 (0.1)	41 (0.1)
Low birth weight[b] (<2500 g)	3975 (4.8)[c]	2309 (5.0)[c]
Preterm birth (<37 weeks)	5550 (6.7)[c]	3217 (6.9)[c]
Stillbirth (≥22 weeks)	362 (0.4)[c]	221 (0.5)[c]

Notes: [a]1826 missing values (2.1%); [b]494 missing values (0.6%); [c]Singleton pregnancies only.

prevalence of drug use in 1999–2000 (the first two years of observation) with that in 2008–2009 (the last two years of observation). Using years 1999–2000 as the reference, we estimated gestational-period specific prevalence ratios for drug use, adjusted for age at delivery and smoking in pregnancy.

All analyses were performed using Stata software 10.0 (College Station, TX). The study was approved by the Danish Data Protection Agency (journal number: 2003-41-3103).

Results

During the study period, we identified 85,710 primiparous women, delivering 88,003 live- or stillborn children. Mean age at delivery was 28 years (range 13–52 years); the proportion of first-time mothers aged 30 years and older increased from 29.0% in 1999 to 35.8% in 2009. Compared with all primiparous women, those with prescription drug use were more likely to be smokers, to have multiple births, and to be older. Prevalence of preterm birth and low birth weight differed slightly among groups (Table 1).

Overall, 47,982 (56.0%) of primiparous women redeemed at least one prescription for a reimbursed drug from 30 days before conception until delivery. Women who redeemed prescriptions, redeemed, on average, 3.2 prescriptions (2.3% of these redeemed >10 prescriptions). Women who used anti-infective drugs redeemed on average 1.6 prescriptions (lowest prescription rate per woman), whereas women who used neurological drugs on average redeemed 4.0 prescriptions (highest prescription rate per woman).

The age-standardized prevalence of drug use increased from 54.7% in 1999 to 61.2% in 2009, PR 1.13 (95% confidence intervals [CI]: 1.10; 1.16); the prevalence decreased slightly in the first two years of observation.The overall prevalence of drug use was 58.5% among women younger

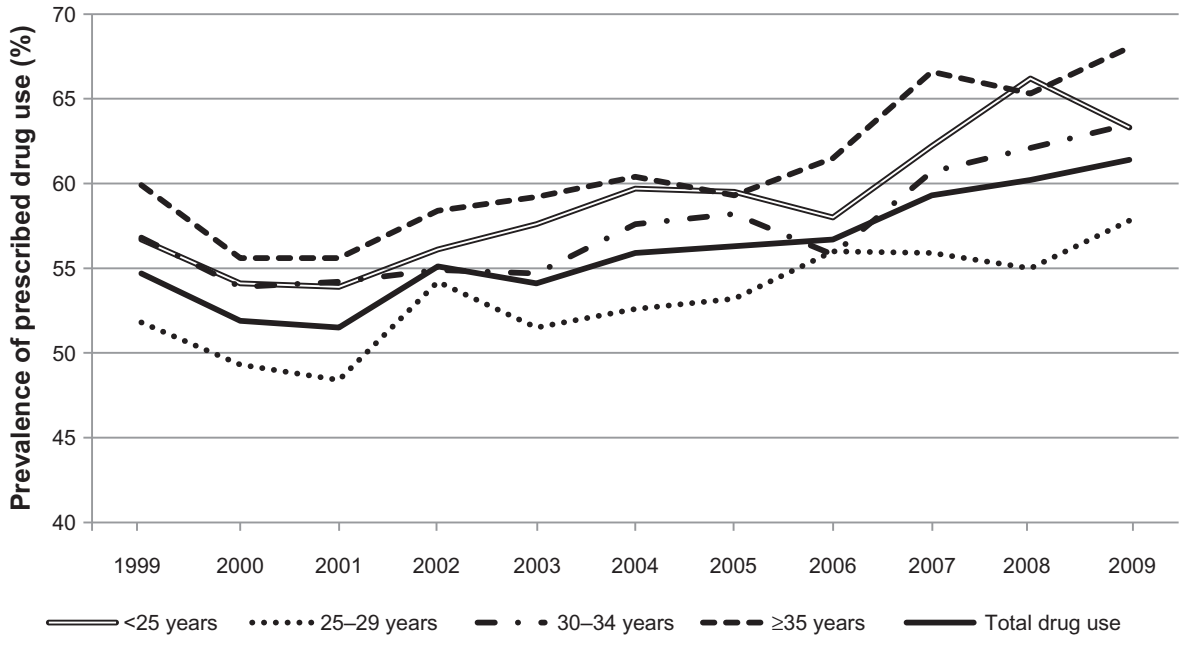

Figure 1 Prevalence of prescribed drug use according to age among primiparous women. Northern Denmark 1999–2009.

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Table 2 Age-standardized prevalence of prescribed drug use and prevalence ratios for overall and ATC-specific drug use among primiparous women in Northern Denmark 1999–2009

ATC group	Calendar year of delivery											
	1999 (n = 7949)	2000 (n = 8134)	2001 (n = 8031)	2002 (n = 7812)	2003 (n = 8197)	2004 (n = 7956)	2005 (n = 7883)	2006 (n = 7906)	2007 (n = 7745)	2008 (n = 5997)	2009 (n = 8100)	*P*-value for linear trend
All ATC groups												
n	4346	4223	4132	4302	4434	4445	4434	4486	4596	3608	4976	$P < 0.001$
P	54.7	52.0	51.6	55.1	54.3	56.0	56.3	56.8	59.3	60.1	61.2	
PR (95% CI)	1	0.95 (0.92;0.97)	0.94 (0.91;0.97)	1.00 (0.98;1.03)	0.99 (0.96;1.02)	1.02 (0.99;1.05)	1.03 (1.00;1.06)	1.04 (1.01;1.07)	1.09 (1.06;1.12)	1.11 (1.07;1.14)	1.13 (1.10;1.16)	
ATC group A												
n	498	395	305	350	392	392	371	433	518	369	560	$P < 0.001$
P	6.3	4.9	3.8	4.5	4.8	5.0	4.7	5.4	6.6	6.1	6.8	
PR (95% CI)	1	0.77 (0.68;0.88)	0.62 (0.54;0.71)	0.71 (0.62;0.82)	0.76 (0.67;0.87)	0.80 (0.70;0.91)	0.75 (0.65;0.85)	0.88 (0.77;1.00)	1.07 (0.95;1.20)	0.99 (0.87;1.13)	1.10 (0.98;1.24)	
ATC group D												
n	1058	1040	867	930	889	876	785	844	777	604	766	$P < 0.001$
P	13.3	12.8	10.8	11.9	10.9	11.0	9.9	10.8	10.1	10.1	9.5	
PR (95% CI)	1	0.95 (0.88;1.03)	0.81 (0.75;0.89)	0.90 (0.83;0.98)	0.81 (0.75;0.89)	0.83 (0.76;0.90)	0.75 (0.69;0.82)	0.81 (0.74;0.88)	0.76 (0.70;0.83)	0.76 (0.69;0.83)	0.71 (0.65;0.78)	
ATC group G												
n	1112	1165	1002	1017	1036	970	1013	914	1,126	981	1,270	$P = 0.001$
P	14.0	14.3	12.5	12.9	12.5	12.1	12.6	11.3	14.0	15.7	14.9	
PR (95% CI)	1	1.02 (0.94;1.10)	0.89 (0.82;0.97)	0.92 (0.85;0.99)	0.87 (0.81;0.95)	0.85 (0.78;0.92)	0.90 (0.83;0.97)	0.81 (0.74;0.88)	1.01 (0.93;1.09)	1.16 (1.07;1.26)	1.10 (1.02;1.18)	
ATC group J												
n	2030	1977	2123	2265	2311	2409	2520	2576	2592	2055	2902	$P < 0.001$
P	25.5	24.4	26.6	29.2	28.8	30.8	32.5	33.0	34.0	34.7	36.3	
PR (95% CI)	1	0.95 (0.90;1.01)	1.04 (0.99;1.10)	1.15 (1.09;1.21)	1.13 (1.07;1.19)	1.21 (1.16;1.28)	1.29 (1.22;1.35)	1.31 (1.25;1.38)	1.34 (1.28;1.41)	1.38 (1.31;1.45)	1.44 (1.38;1.51)	
ATC group N												
n	183	208	219	283	314	330	336	377	463	356	529	$P < 0.001$
P	2.3	2.6	2.7	3.6	3.8	4.2	4.3	4.7	5.9	5.9	6.5	
PR (95% CI)	1	1.11 (0.91;1.35)	1.21 (0.99;1.47)	1.62 (1.34;1.95)	1.69 (1.41;2.03)	1.87 (1.56;2.23)	1.93 (1.61;2.31)	2.18 (1.82;2.59)	2.74 (2.31;3.25)	2.67 (2.24;3.19)	2.97 (2.52;3.52)	
ATC group R												
n	752	725	765	811	799	766	795	739	826	554	794	$P = 0.270$
P	9.5	8.9	9.5	10.3	9.7	9.5	10.0	9.2	10.6	9.2	9.7	
PR (95% CI)	1	0.93 (0.84;1.03)	1.00 (0.91;1.10)	1.08 (0.99;1.19)	1.01 (0.92;1.11)	0.99 (0.90;1.09)	1.05 (0.95;1.15)	0.97 (0.88;1.07)	1.11 (1.01;1.22)	0.96 (0.87;1.07)	1.02 (0.93;1.12)	

Note: Prevalences are standardized to age distribution in 1999; prevalence ratios are adjusted for age and smoking during pregnancy. ATC group A, drugs for alimentary tract and metabolism; ATC group D, dermatological drugs; ATC group G, gynecological drugs; ATC group J, anti-infective drugs for systemic use; ATC group N, neurological drugs; ATC group R, drugs for the respiratory system.
Abbreviations: ATC, Anatomical Therapeutic Chemical classification of drugs; n, number; P, prevalence; PR, prevalence ratio; CI, confidence interval.

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than 25 years, 53.1% among women between 25 and 29 years, 57.5% among women aged 30–34 years, and 61.1% among women aged 35 years and older. Throughout the study period, women aged 25–29 years had a lower prevalence of drug use than women in other age groups (Figure 1). Drug use over time in each age group was tested for trend ($P < 0.001$ for linear trend in all age groups).

Table 2 shows prevalence of drug use stratified by calendar year and ATC group and PRs of drug use adjusted for age and smoking. Anti-infective drugs were the most prevalent drugs used the by the primiparous mothers as measured by one or more dispensed prescriptions. The age-standardized prevalence of use of anti-infective drugs increased from 25.5% in 1999 to 36.3% in 2009, PR 1.44 (95% CI: 1.38; 1.51). Women younger than 25 years had a higher prevalence of anti-infective drug use (37.3%) compared with women in all other age groups: 28.1% of women between 25 and 29 years; 28.3% of women aged 30–34; and 27.8% of women aged 35 years and older. There were 14,469 (16.9%) women redeeming one or more prescriptions for UTI antibiotic drugs and 11,761 (13.7%) women redeeming one or more prescriptions for penicillin. The prevalence of UTI-specific drug use more than doubled (10.9% in 1999; 22.9% in 2009, PR 2.15 [95% CI: 1.99; 2.31]) and the increasing prevalence was observed in all age groups (data not shown). We also observed an increase over time in the prevalence of penicillin use (13.3% in 1999; 14.1% in 2009, PR 1.10 (95% CI: 1.02; 1.19)).

The age-standardized prevalence of neurological drug use increased nearly three-fold (2.3% in 1999; 6.5% in 2009, PR 2.97 [95% CI: 2.52; 3.52]) (Table 2). At any time during pregnancy, 1872 (2.2%) women used antidepressants, 582 (0.7%) used opioids, and 451 (0.5%) used anti-epileptics. Prevalence of antidepressant use increased nearly six-fold (0.8% in 1999; 4.1% in 2009, PR 5.95 [95% CI: 4.51; 7.85]).

Prevalences of gestational-period specific drug use in 1999–2000 and 2008–2009 are shown in Figure 2. Over time, prevalence of immediate pre-conception and trimester-specific use of anti-infective drugs increased. Prevalence of trimester-specific use of neurological drugs also changed over time. For example, first-trimester use increased more that three-fold from 1.4% in 1999–2000 to 4.1% in 2008–2009, PR 3.19 (95% CI: 2.73;3.74); second trimester use increased more that four-fold from 0.8% in 1999 to 3.4% in 2009, PR 4.30 (95% CI: 3.54;5.22); and third trimester use increased from 0.7% in 1999 to 2.3% in 2009, PR 3.54 (95% CI: 2.85;4.40). Prevalence of immediate pre-conception use of gynecological drugs more than doubled from 1999–2000 to 2008–2009 (3.9% in 1999–2000; 9.3% in 2008–2009, PR 2.18 (95% CI: 2.00; 2.39), whereas third-trimester use almost halved from 3.2% in 1999–2000 to 1.8% in 2008–2009, PR 0.57 (95% CI: 0.49; 0.66).

Discussion

Use of prescribed reimbursed drugs increased modestly (6.5% in absolute terms) from 1999 to 2009 in this population of almost 86,000 primiparous women. From 2001 to 2005, the Danish Institute of Public Health reported a 24% increase of prescribed drug use measured in defined daily doses (DDDs) among the general Danish population.[24] The

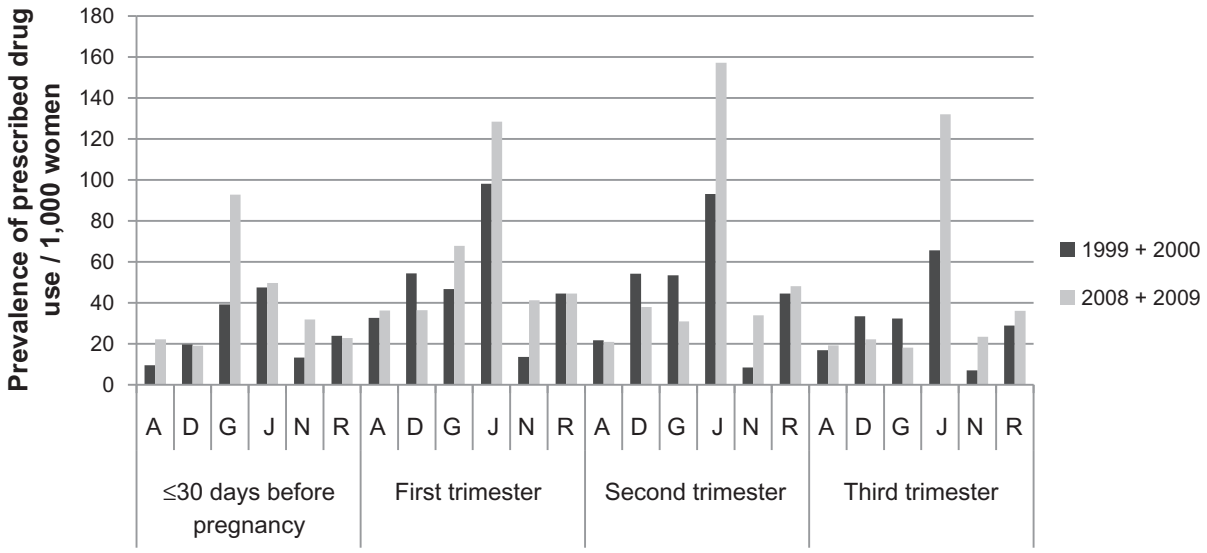

Figure 2 Prevalence (per 1000 women) of immediate pre-conception and trimester-specific drug use among primiparous women for the most commonly prescribed ATC groups[a] 1999–2000 and 2008–2009.

Notes: [a]ATC group A: drugs for alimentary tract and metabolism; ATC group D: dermatological drugs; ATC group G: gynecological drugs; ATC group J: anti-infective drugs for systemic use; ATC group N: neurological drugs; and ATC group R: drugs for the respiratory system.

observed modest increase in drug use among women giving first birth could thus be a reflection of this general population trend. The overall prevalence of drug use increased with age; however, increasing age of primiparous women did not explain the overall increase in prevalence of drug use over time. Anti-infective drugs were used with the highest prevalence as measured by one or more prescription dispensation. Prevalence of antidepressant use increased substantially over the observation period, but the absolute prevalence remained low.

Prevalence of drug use in pregnancy was on the same order of magnitude in other Nordic countries as in the present study. In Sweden (2007), the prevalence of drug use was 58% among pregnant women;[7] in 2004–2006, in Norway, prevalence of drug use during pregnancy was 57% at any time during trimester 1–3 among first singleton pregnancies,[3] while in Finland, the prevalence of use was 46% in 1999.[5] Comparability of findings is expected as Nordic countries have similar health care and record-keeping practices.[25]

Non-Nordic countries have reported higher prevalence of drug use during pregnancy.[1,2,4] According to records from the French Health Insurance Service, 99% of women in Southwest France receive prescribed drugs during pregnancy.[4] In the United States, 82% of pregnant women used prescribed drugs, based on data collected from the Health Maintenance Organizations (HMO) in 1996–2000.[1] Both in France and in the United States, the reported drug use includes certain OTC medications, such as iron, folic acid, and pregnancy vitamins. In particular, in the United States (in contrast to Denmark), pregnant women receive prescriptions for pregnancy vitamins in order to enable reimbursement, and therefore leading to a dispensation record. Thus, patterns of drug utilization during pregnancy can be expected to vary according to prescribing, reimbursement, and record-keeping practices, as well as socioeconomic differences.[1–5]

Drug utilization patterns varied by age and by type of drug. Our finding that women aged 35 years or older had a slightly higher prevalence of overall drug use than women in younger age groups is similar to findings in a recent Irish study including 61,252 women giving birth in Dublin from 2000 to 2007.[26] Young age, however, has been associated with a higher use of antibiotics, as observed in a German study of about 41,000 observations based on insurance claims. This observation was confirmed in our study. The German researchers attributed higher use of antibiotics by younger pregnant women to higher rates of infections in this age group.[27] Screening for bacteriuria as part of routine examination of pregnant women was introduced in Denmark in 1998,[22,23] probably partially explaining our observation of increased use of UTI-specific drugs among women giving first birth throughout the study period. In 2001, some drugs used in treatment of gynecological infections were re-coded from gynecological drugs to the anti-infective drugs for systemic use,[28] which may account for some of the decrease seen for gynecological drugs and some of the increase seen for anti-infective drug use observed in our study. An increased prevalence of prescribed antidepressant drug use seen in this study was also reported in the United States.[29,30] Exposure to antidepressants (selective serotonin reuptake inhibitors [SSRIs] in particular) in early pregnancy has been associated with an increased risk of adverse neonatal effects.[14,31,32] That, together with our findings that use of neurological drugs increased in early pregnancy during our study period, may call for further attention.

It is important to acknowledge different strengths and weaknesses of our study when interpreting our results. Our large and well defined study population contained data from a uniform health care system with complete coverage and universal access. We used data from a system of automatic reimbursement and routine electronic record-keeping. This enabled us to avoid recall bias, and estimate drug utilization systematically.[33]

We focused on trends in use of broad groups of prescription drugs according to major anatomical ATC-groups in order to give the general descriptive picture of drug utilization patterns among primiparous women. We did not aim to specifically address utilization of known or potential teratogens. Reports that almost 20% of Canadian women (study population = 18,575)[34] and 10% of US women (study population = 152,531)[1] used prescription drugs with potential or clear fetal risk during pregnancy call for further attention. However, the teratogenic potential of many drugs is unknown[35] and deserves special investigation.

The Aarhus University Prescription Database lacks information on dispensation of OTC drugs, in-hospital treatment, or sales of nonreimbursed prescribed drugs.[18] Therefore the overall prevalence of prescription drug use among women giving first birth is underestimated and caution about conclusions regarding the observed change of drug utilization patterns in specific ATC-groups,eg neurological drugs, need to be considered. Furthermore, because we used information on redeemed prescriptions, we had no data about the true drug intake, potentially leading to overestimation of the actual use of the purchased drugs.[36] Although we examined the effect on utilization of maternal

age and self-reported smoking, we had no data on other factors, such as social status and years of education, which could also explain some of the change in use. Further, we restricted the study population to primiparous women to maximally remove the effects of age from evaluating the trends of drug use.

Authors' contributions

AB, MN, EAN, and VE have all substantially contributed to study conception, design, and interpretation of data. AB and VE drafted the article. AB and HH analyzed the data, and HH participated in dataset creation. All authors revised the paper critically and approved the final version.

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Disclosure

The authors report no conflicts of interest in this work.

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Use of corticosteroids in early pregnancy is not associated with risk of oral clefts and other congenital malformations in offspring

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ABSTRACT

Corticosteroids are commonly used to treat inflammatory diseases. There is conflicting evidence regarding the association of corticosteroid use in pregnancy and congenital malformations in offspring.

We conducted a prevalence study of 83,043 primiparous women who gave birth to a live-born singleton in northern Denmark, in 1999-2009. Through medical registries, we identified prescriptions for corticosteroids, congenital malformations and covariates. Furthermore, we summarized previously published literature on this topic.

Overall, 1,449 women (1.7%) used inhaled or oral corticosteroids from 30 days before conception throughout the first trimester. Oral cleft in the offspring was recorded for one of the users (0.08%) and 145 of the non-users (0.2%), prevalence odds ratio (POR) 0.47 (95% confidence interval (CI), 0.07-3.34). The POR for congenital malformations overall was 1.02 (95% CI, 0.79-1.32). According to previously published studies, use of corticosteroids in early pregnancy was associated with congenital malformations overall with relative estimates ranging from 0.8 (95% CI, 0.4-1.7) to 2.1 (95% CI, 0.5-9.6). For oral clefts the odds ratios ranged from 0.6 (95% CI, 0.2-1.7) to 5.2 (95% CI, 1.5-17.1).

We found no evidence of an association between use of corticosteroids in early pregnancy and risk of congenital malformations in offspring.

Key words: Congenital malformation, corticosteroids, epidemiology, oral clefts, pregnancy.

Introduction

Because of their anti-inflammatory and immune-suppressive properties, corticosteroids are widely used to treat many conditions, including asthma, rheumatoid arthritis, eczema, and inflammatory bowel disease.[1,2]

Corticosteroids vary in their ability to cross the placenta.[3] Fetal endogenous levels of corticosteroids are much lower than maternal levels, which means that even a low contribution from the mother to the fetus may have substantial impact on the fetal environment.[4] Pregnancy increases maternal tissue perfusion, including that perfusion of the bronchial mucosa. This, which may enhance systemic absorption of inhaled corticosteroids, potentially causing high levels of corticosteroids within the maternal circulation.[5,6]

Animal studies have reported a teratogenic effect of corticosteroids, manifested as oral clefts.[7,8] A number of human studies have examined the association of first-trimester use of inhaled and oral corticosteroids and risk of oral clefts[1,9-13] or congenital malformations overall[11,14-17] (Table 1). Five case-control studies with 1-19 cases of oral clefts exposed to corticosteroids in early pregnancy,[1,9,10,12,13] reported odds ratios (OR) for oral clefts ranging from 0.6 (95% confidence interval (CI), 0.2-1.7)[12] to 5.2 (95% CI, 1.5-17.1).[13] Five studies examining the prevalence of congenital malformations overall in offspring following early-pregnancy use of corticosteroids[11,14-17] reported prevalence odds ratios (POR) ranging from 0.8 (95% CI, 0.4-1.7)[14] to 2.1 (95% CI, 0.5-9.6).[16] Thus, existing data are consistent with both presence and absence of an association.

We examined whether use of corticosteroids was associated with an increased risk of oral clefts and congenital malformations overall in offspring in a population-based study in northern Denmark. Furthermore, we summarized the previous published literature on this topic.

Materials and methods

Setting and study population

We used data from Danish medical registries, linked at the individual level by the unique 10-digit personal identifier, assigned to all Danish residents at birth and used in all public records.[18] To identify mothers and their newborns, we used the Danish Medical Birth Registry, which contains computerized records of all births in Denmark.[19] This registry holds data on characteristics of the mother (including age, citizenship, residence, marital status, parity, and self-reported smoking status) and the newborn (including vital status at birth, sex, birth weight, and gestational age). Gestational age is estimated mainly based on ultrasound[20] and during the relevant time period, gestational age was recorded in days.[21] We calculated the conception date as birth date minus gestational age in days plus 14 days.

We restricted our study population to women giving birth in northern Denmark (the Central and North Regions of Denmark, which together comprise about 33% of the entire Danish population or 1.8 million people). We included all women with a first-born singleton born alive at ≥ 22 weeks' gestation between 1 January 1999 and 31 December 2009. The restriction to first pregnancies was done because an adverse outcome in a previous pregnancy may influence a woman's drug use in a new pregnancy.[22] To ensure availability of full prescription record from a prescription database (described below) we started the study period in 1999 and also required that the women resided in one of the two regions from 30 days before conception through delivery.

Prescription data

To identify prescriptions for corticosteroids, we used the Aarhus University Prescription Database.[23] This database tracks prescriptions for reimbursed drugs redeemed at the regions' community pharmacies, which use electronic accounting systems to secure reimbursement

from the National Health Service. Complete records for the two regions are available since 1998. The tax-supported health care system in Denmark partially refunds costs of most prescribed drugs.[24] For all prescriptions, we noted date of redemption and type of medication using the Anatomical Therapeutic Chemical (ATC) classification.

We defined use of corticosteroids in early pregnancy as a record of at least one prescription for inhaled or oral corticosteroids redeemed from 30 days before estimated conception to the end of the first trimester (12 completed gestational weeks). We defined use of corticosteroids in late pregnancy as a record of at least one prescription for inhaled or oral corticosteroids redeemed from 13th gestational week until delivery and no use of corticosteroids in early pregnancy. We defined non-use as absence of inhaled or oral corticosteroid prescription redemptions from 30 days before estimated conception until delivery.

Data on congenital malformations

We retrieved data on congenital malformations from the Danish National Registry of Patients.[25] This registry was established in 1977 and records visits to all somatic hospitals in Denmark, including dates of admission and discharge, discharge diagnoses, and surgical procedures. Contacts to emergency rooms and outpatient clinics have been registered since 1995. Diagnoses are coded by medical doctors according to the *International Classification of Diseases*, eighth revision (ICD-8) until the end of 1993 and tenth revision (ICD-10) thereafter. We included congenital malformations registered during the first year of life to capture malformations that are not apparent or recorded at delivery.[26] We excluded registry diagnoses of congenital dislocation of the hip and undescended testes due to their expected lack of validity.[27] Further, we excluded infants with chromosomal disorders. Oral clefts were defined as diagnoses of cleft lip with or without cleft palate or isolated cleft palate.[28]

Data on covariates

From the Danish National Registry of Patients, we obtained information about maternal diagnoses of asthma, rheumatoid arthritis, and inflammatory bowel disease recorded from 1977 until delivery, as corticosteroids are used in medical treatment of these diseases and underlying diseases may themselves be risk factors for congenital malformations.[29,30] We also obtained information about maternal diagnoses of diabetes, because diabetes has been associated with an increased risk of congenital malformations[31] while use of corticosteroids may induce diabetes.[32] All relevant diagnostic codes and prescription codes are listed in the Appendix.

Literature search

To identify studies published in the last 25 years on the association between use of corticosteroids in early pregnancy and congenital malformations overall and oral clefts specifically, we search PubMed using the following MeSH terms "congenital abnormalities", "cleft palate", "glucocorticoids", "steroids", and "pregnancy outcome". If more than one study was conducted based on the same data sources and with overlapping study periods, we excluded all[33,34] but the most comprehensive study.

Statistical analyses

In the summary of the existing literature we computed relative risk estimates if absolute numbers were presented in the publication.

In the current study, we cross-tabulated use of corticosteroids with maternal characteristics. Use of corticosteroid was categorized as inhaled corticosteroids, oral corticosteroids, and concomitant use of inhaled and oral corticosteroids in early pregnancy. We computed prevalence of oral clefts and congenital malformations overall. We used logistic regression to estimate prevalence odds ratios (POR) with associated 95% confidence intervals (CI) for oral

clefts and congenital malformations overall among women who used corticosteroids in early pregnancy compared with non-users. The estimates for congenital malformations overall were further adjusted for maternal smoking during pregnancy, maternal age at delivery, and diabetes. We reported only unadjusted estimates for oral clefts because of sparse data. Because women who filled only a single prescription of corticosteroids in early pregnancy may not be actual users, we carried out an additional analysis where these women were excluded.

Analyses were performed using Stata software 10.0 (College Station, TX). The study was approved by the Danish Data Protection Agency (journal number: 2003-41-3103).

Results

Existing studies

We identified 10 studies on the association between use of corticosteroids in early pregnancy and congenital malformations overall or oral clefts specifically. These studies are summarized in Table 1. We found that use of corticosteroids in early pregnancy was associated with congenital malformations overall with relative estimates ranging from 0.8 (95% CI, 0.4-1.7) to 2.1 (95% CI, 0.5-9.6). Yet, the largest study found a relative risk of 1.1 (95% CI, 1.0-1.2). For oral clefts (including both cleft lip with or without cleft palate and isolated cleft palate), the OR ranged from 0.6 (95% CI, 0.2-1.7) to 5.2 (95% CI, 1.5-17.1). We found similar variations in the study that examined cleft lip with or without cleft palate and isolated cleft palate as two separate outcomes (Table 1).

Descriptive data

In the current study, we identified 83,043 primiparous women, of whom 1,449 (1.7%) used corticosteroids in early pregnancy; 1,223 women (1.5%) used inhaled corticosteroids, 226

women (0.3%) used oral corticosteroids, and 27 women (0.03%) had a concomitant use of inhaled and oral corticosteroids in early pregnancy. We excluded 644 women (0.8%) who used corticosteroids in late pregnancy.

Women who used corticosteroids in early pregnancy were slightly older and less likely to smoke during pregnancy compared with women who did not use corticosteroids. Among women who used corticosteroids in early pregnancy, 491 (33.9%) had a history of hospital diagnosis of asthma, rheumatoid arthritis or inflammatory bowel disease compared with 2.4% among the non-users (Table 2).

Oral clefts

Among the 1,223 women, who used inhaled corticosteroids during early pregnancy, one woman had an infant with an oral cleft (0.08%) compared with 145 (0.2%) among the 80,950 non-users. The unadjusted POR was 0.47 (95% CI, 0.07-3.34) (Table 3). This woman had filled more than one prescription for inhaled corticosteroids. When excluding women who redeemed only one corticosteroid prescription, the unadjusted POR was 0.64 (95% CI, 0.09-4.59).

Congenital malformations

In women who used corticosteroids during early pregnancy, the prevalence of congenital malformations was 4.3% similar to the non-users (unadjusted POR, 1.02; 95% CI, 0.79-1.32). Adjustment did not change this estimate substantially (data not shown). The prevalence of congenital malformations did not differ between users of inhaled or users of oral corticosteroids (Table 3). Of the 27 women who had a concomitant use of inhaled and oral corticosteroids in early pregnancy, two gave birth to infants with a malformation, corresponding to a prevalence of 7.4%.

When we excluded women who filled only a single prescription of corticosteroids we identified 30 women (3.5%) who gave birth to a malformed infant among users of inhaled corticosteroid in early pregnancy (unadjusted POR 0.80; 95%CI 0.56-1.16) and 7 women (6.7%) who gave birth to a malformed infant among users of oral corticosteroids in early pregnancy (unadjusted POR 1.62; 95%CI 0.75-3.50). Adjustment for smoking, maternal age, and diabetes did not change the estimates notably (data not shown).

Discussion

In this population based study, we found no association between use of corticosteroids in early pregnancy and risk of oral clefts or congenital malformations overall in the offspring. These findings do not corroborate previous studies that reported increased risk of oral cleft following corticosteroid exposure.[1,9,12,13] This difference may be explained by methodological differences between the studies. In previous studies, early pregnancy exposure information was based on retrospective data collected through interviews or questionnaires[1,9,12,13] with the risk of differential recall of drug use.[35] A previous study examining recall bias and misclassification in the Hungarian Congenital Abnormality Registry (a case-control surveillance system) found that differential recall may frequently cause spurious associations, with biased odds ratios up to a factor of 1.9.[36]

Our study is in agreement with earlier studies that showed no increased risk of congenital malformations in offspring following use of inhaled corticosteroids in early pregnancy.[11,14,17] On the other hand, use of oral corticosteroids have been reported in two previous studies to increase the risk of congenital malformations overall two-fold,[15,16] but in both studies the data were based on teratogen information systems; therefore, self-referral bias cannot be ruled out.[35]

Several issues should be considered in interpreting our results. We measured maternal drug use by using automated routine reimbursement record-keeping, which enabled us to avoid recall bias, and to assess drug use systematically.[37] Inhaled and oral corticosteroids are not sold over-the-counter in Denmark. We could not ascertain maternal drug use during hospitalizations nor could we rule out that some women used inhaled and oral corticosteroids without being recorded in the prescription registry because they could have used corticosteroids from storage at home. A high degree of agreement between self-reported drug intake and that recorded in the Aarhus University Prescription Database has been reported in a recent validation study found.[38] Furthermore, the co-payment requirements, associated with dispensation of prescription drugs, are expected to increase the likelihood of adherence to the drug. Finally, inhaled corticosteroids are used as a preventive long-term asthma medication and not as quick-relief asthma medication as e.g. β-agonist,[39] and higher compliance is expected for drugs taken for chronic conditions. It is possible that women who have filled only a single corticosteroid prescription may have a lower compliance than women with several corticosteroid prescriptions. Yet excluding the women who redeemed only one corticosteroid prescription did not affect the interpretation.

Because our well-defined study population contained data from a uniform health care system with complete coverage and universal free access, we did not have any selection problems for births recorded in the Medical Birth Registry. However, we could not capture congenital malformations leading to a miscarriage or to an induced abortion after prenatal diagnosis. If use of corticosteroid is related to an increased risk of congenital malformation-related miscarriage and/or induced abortion, the risk for congenital malformations among women who used corticosteroids would be underestimated when using the prevalence study design.[26]

The positive predictive value of a registry-recorded congenital malformation diagnosis has been estimated to be 88 %,[27] and since misclassification of the malformation status probably did not differ by maternal corticosteroid use our relative estimates were probably not affected by this misclassification. We were able to take age, self-reported smoking, and presence of diabetes into account in our analysis of congenital malformations overall. None of these factors affected our estimate noticeably. Binge-drinking (> 5 drinks per sitting) in early pregnancy has been associated with a two-fold increased risk of oral clefts in a Norwegian case-control study.[40] We lacked information on alcohol consumption in the Medical Birth Registry. Although it is possible that pregnant women with asthma has an even lower alcohol intake than pregnant women in general, preliminary data based on a sample of more than 4,800 pregnant women from the Danish National Birth Cohort, which is a nationwide study of 100,000 women and their offspring,[41] found that the prevalence of pregnant women who drank more than 3 drinks per week did not differ between women with and without asthma (Ellen Aagaard Nohr. Personal communication). We therefore do not consider binge-drinking to be a substantial confounder in our study.

Also, we cannot entirely rule out confounding by unknown factors, because of the null result, any confounding would need to be by factors that could have masked a risk of congenital malformation associated with the use of corticosteroids. Such factors would have to be associated with maternal use of corticosteroids and also be associated with a reduced risk of congenital malformations overall. No such factor is currently known, to the best of our knowledge. Among users of inhaled corticosteroids in our study, only about 30% had a record of asthma episode requiring a hospital contact. A validation of asthma diagnoses in the Danish National Registry of Patients showed that only 44% of independently confirmed asthma patients had a hospital contact with a diagnosis of asthma.[42] This reflects the practice of

treating of most asthma episodes in primary care. Although it may be difficult to separate the effect of corticosteroids from the effect of the underlying disease that indicated the treatment, confounding by indication is irrelevant because of the null result.

Low precision if the main limitation of our study: even in this large database, only one oral cleft event occurred in offspring of women who used corticosteroids in early pregnancy. We found a nearly null effect for oral clefts and malformations overall, still, our sample size only enabled us to detect a more than 3.5-fold increased risk for oral clefts and a 1.4-fold increased risk of malformations overall. Low overall prevalence of specific congenital malformations necessitates larger samples for providing robust evidence regarding safety of corticosteroids during pregnancy.[43]

In conclusion, we found no association between use of corticosteroids in early pregnancy and risk of oral clefts or congenital malformations overall in the offspring, but estimates were imprecise. The international literature seems to support our findings.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AB, MN, VE, EAN, and HTS have all substantially contributed to study conception, design, interpretation of data, and drafting of the article. AB and HH analyzed the data, HH participated in dataset creation. All authors revised the paper critically and approved the final version.

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Table 1. A summary of the previous observational studies of use of inhaled or oral corticosteroids in early pregnancy and risk of oral clefts and congenital malformations overall in offspring.

Study and data sources	Study design	Corticosteroid type examined	Relative risk estimates* (95% confidence interval);n of exposed offspring
Congenital malformations overall			
Källen[11] (Swedish Medical Birth Registry)	Prevalence study	Inhaled	1.1 (1.0-1.2); 627
Alexander[14] (Nova Scotia Atlee Perinatal Database, Halifax County, Canada, Grace Maternity Hospital)	Prevalence study	Inhaled	0.8 (0.4-1.7); 8
Gur[15]# (The Israeli Teratogen Information Service)	Prevalence study	Oral	2.0 (0.9-4.4); 10
Park-Wyllie[16] (Canadian Motherisk cohort)	Prevalence study	Oral	2.1 (0.5-9.6); 4
Schatz[17]# *(*Kaiser-Permanente Prospective	Prevalence study	Inhaled and oral	1.4 (0.9-2.5); 14

Study of Asthma During Pregnancy)					
Oral cleft			**Oral clefts**	**Cleft lip with or without cleft palate**	**Isolated cleft palate**
Källen[11] (Swedish Medical Birth Registry)	Prevalence study	Inhaled	1.4 (1.0; 1.9); 48		
Carmichael[1] (American National Birth Defects Prevention Study)	Case-control	Inhaled		1.5 (0.9-2.5); 19	0.7 (0.3-1.8); 5
Pradat[12] (Malformation Drug Exposure Surveillance Project - MADRE)	Case-control	Inhaled	0.6 (0.2-1.7); 4	0.7 (0.2-2.2); 3	0.6 (0.1-5.1); 1
Pradat[12] (Malformation Drug Exposure Surveillance Project - MADRE)	Case-control	Oral	1.3 (0.7-2.2); 15	1.8 (1.0-3.1); 13	0.3 (0.04-1.5);1
Carmichael[1] (American National Birth Defects Prevention Study)	Case-control	Oral		2.1 (0.9-4.7); 9	0.8 (0.2-3.6); 2
Carmichael[9]	Case-control	Oral		4.3 (1.1-17.2); 6	5.3 (1.1; 26.5); 3

(The California Birth Defects Monitoring Program)			
Rodriguez-Pinilla[13] (Spanish Collaborative Study of Congenital Malformations - ECEMC)	Case-control	Oral	5.2 (1.5-17.1); 5
Czeizel [10] (The Hungarian Congenital Abnormality Registry - HCCSCA)	Case-control	Oral	1.3 (0.8-2.0); 1

*the risk estimates are given as prevalence odds ratios for the prevalence studies and odds ratio for the case-control studies.

risk estimates calculated using the Episheet software (version 2011, by Kenneth J. Rothman)

Table 2. Characteristics of primiparous women with and without prescriptions for inhaled or oral corticosteroids in Northern Denmark, 1999-2009.

	Use of inhaled or oral corticosteroids in early pregnancy	**No use of inhaled or oral corticosteroids any time during pregnancy**
Number of women	1,449	80,950
Age, median (range)	29 (16; 47)	28 (15; 52)
Age group, number (%)		
<25 years	235 (16.2)	17,525 (21.7)
25-29 years	671 (46.3)	37,288 (46.1)
30-34 years	398 (27.5)	19,936 (24.6)
≥ 35 years	145 (10.0)	6,201 (7.7)
Smoking during pregnancy, Number (%)		
Yes	221 (15.3)	14,357 (17.7)
No	1,190 (82.1)	64,950 (80.2)
Missing	38 (2.6)	1,643 (2.0)
Hospital diagnosis, number (%)		
Asthma	437 (30.2)	1,228 (1.5)
Rheumatoid arthritis	19 (1.3)	121 (0.2)
Inflammatory bowel disease	35 (2.4)	568 (0.7)
Diabetes	7 (0.5)	405 (0.5)

Table 3. Prevalence and unadjusted prevalence odds ratios (POR) for congenital malformations according to corticosteroid use in early pregnancy in northern Denmark, 1999-2009.

	No use of inhaled or oral corticosteroids at any time during pregnancy	**Use of inhaled or oral corticosteroids in early pregnancy**	**Use of inhaled corticosteroids in early pregnancy**	**Use of oral corticosteroids in early pregnancy**
Number of women	80,950	1,449	1,223	226
Congenital malformations				
number	3,446	63	53	10
prevalence (%)	4.3	4.3	4.3	4.4
POR (95% CI)	*Reference*	1.02 (0.79-1.32)	1.02 (0.77-1.34)	1.04 (0.55-1.96)
Oral clefts				
number	145	1	1	0
prevalence (%)	0.2	0.07	0.08	-

POR (95% CI)	*Reference*	0.39 (0.05-2.75)	0.47 (0.07-3.34)	-

Abbreviations: POR, prevalence odds ratio; CI, confidence interval.

Appendix. Codes used in the present study from the international Classification of Diseases (ICD) and Anatomical Therapeutic Chemical (ATC) classification.

	ICD-8	ICD-10	ATC-codes
Congenital malformations		Q00-Q99	
Undescended testis		Q53	
Congenital dislocation of the hip		Q65.0-Q65.6	
Congenital chromosomal defects		Q90-Q99	
Oral clefts		Q35-Q37	
Asthma	493	J45-J46	
Rheumatoid arthritis	712.19, 712.39, 712.59	M05-M06	
Inflammatory bowel disease	563.00, 563.01, 563.10, 569.02	K51-K50	
Diabetes	250	E10-E14	
Inhaled corticosteroids			R03BA01, R03BA02, R03BA05, R03BA07, R03AK06, R03AK07
Oral corticosteroids			H02AB04, H02AB06, H02AB07, H02AB09

Study 3

Risk of miscarriage and use of corticosteroid hormones:

A population-based case-control study

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Condensation:

A slightly increased risk of first-trimester miscarriage was observed among women with a prescription for inhaled corticosteroids in 60 days before the miscarriage.

Short title:

Corticosteroid use and miscarriage

Abstract

Background: Data on the association between use of corticosteroids in pregnancy and risk of miscarriage are limited.

Study design: We conducted a registry-based case-control study in northern Denmark, in 1997-2009. Cases were women with a miscarriage before the 22nd gestational week and no previous delivery; controls were women with a first-time delivery and no previous miscarriage, matched to cases on conception year. Using conditional logistic regression, we estimated odds ratios (OR), adjusting for age, diabetes, epilepsy, and use of non-steroidal anti-inflammatory drugs (NSAIDs).

Results: Among 10,974 cases, 1.3%, and among 109,740 controls 1.0% redeemed a prescription for inhaled corticosteroids 60 days before the miscarriage/index date (adjusted OR = 1.20; 95% confidence interval (CI): 1.01-1.44). Among both cases and controls, 0.3% used oral corticosteroids (adjusted OR = 0.78; 95% CI: 0.53-1.15).

Conclusion: Use of inhaled corticosteroids in the preceding 60 days was associated with slightly increased risk of early miscarriage.

Keywords: case-control study, corticosteroid hormones, epidemiology, miscarriage.

Introduction

Corticosteroids have anti-inflammatory and immunosuppressive properties[1] and are used to treat asthma, rheumatoid arthritis, eczema, and inflammatory bowel disease.[2,3] These diseases may affect women of childbearing age.[4,5] In Denmark, an estimated 1.2% of women who give birth use inhaled or oral corticosteroids in the first trimester of pregnancy.[6]

Conflicting nature about safety or corticosteroids during pregnancy may stem from selection bias. For example, evidence on maternal use of inhaled and oral corticosteroids in early gestation and risk of congenital malformations in offspring[2,7-15] was inconclusive. Selection bias arising from early-gestation demise of malformed embryos could explain lack of an apparent association, if a true association were present.[16,17]

Most,[10,18,19] but not all,[12] previous studies have reported an increased risk of miscarriage among women with corticosteroid intake in early pregnancy, with risk ratio estimates ranging from 1.01 (95% confidence interval (CI): 0.48-2.11)[12] to 1.66 (95% CI: 1.12-2.48).[10] The largest prevalence study reported an odds ratio (OR) of 1.24 (95% CI: 1.17-1.32).[19] Information about gestational age at miscarriage was not available in any earlier studies, contributing to uncertainty about the gestational period of exposure. We conducted a case-control study to examine the relation between prenatal corticosteroid use and miscarriage, accounting for gestational age at miscarriage.

Material and methods

Study population and study period

This population-based case-control study was carried out in a well-defined geographic and administrative area of northern Denmark comprising about 1.8 million people or ~33% of the total Danish population. The study period extended from 1 January 1997 to 31 December

2009. Denmark's tax-funded health care system provides free health care, including partially refunded costs of most prescribed drugs, to all Danish inhabitants.[20] Since 1968, a unique ten-digit personal identifier (the CPR number) has been assigned to all Danish residents at birth by the Civil Registration System.[21] The CPR number, which encodes date of birth and sex, is used in all public records and permits unambiguous record linkage across databases.

Cases

Cases were women with a first-recorded miscarriage before 22nd gestational week and no previous delivery. Occurrences of miscarriage were identified from the Danish National Registry of Patients (DNPR), which tracks admissions to all Danish somatic hospitals. This registry, established in 1977, includes dates of admission and discharge, diagnoses, and surgical procedures, and from 1995 also outpatient visits.[22] Diagnoses are recorded by medical doctors at discharge, using the International Classification of Diseases (ICD), eighth revision (ICD-8) before 1994 and tenth revision (ICD-10) thereafter. Gestational age at time of miscarriage has been reported to the DNRP since 1997. Gestational age in Denmark is estimated mainly based on ultrasound examination.[23] We estimated the conception date as the birth date minus gestational age plus 14 days. The recorded hospital admission date for miscarriage was the index date.

Controls

Controls were women without a history of a miscarriage delivering their first live newborn. To identify controls, we used the Danish Medical Birth Registry,[24] which has tracked all births in Denmark since 1977. For each case, we sampled 10 controls from women whose estimated date of conception was in the same calendar year as that of the index case. The index date for each control was set as the date on which her fetus reached the same gestational age as that of her matched case at the time of miscarriage.

Use of corticosteroids

We used the Aarhus University Prescription Database to identify all prescriptions for inhaled and oral corticosteroids filled by cases and controls before their index date. This database tracks dispensations of reimbursed prescription drugs at the community pharmacies in the two regions of northern Denmark.[25] All pharmacies use electronic accounting systems to secure reimbursement from the National Health Service. Inhaled and oral corticosteroids, which are available by prescription only, are eligible for general reimbursement in Denmark and thus generate records in the database. For all relevant prescriptions filled by women in our study, we noted date of dispensation and type of drug, coded according to the Anatomical Therapeutic Chemical (ATC) classification system. In some areas of the two regions, data on dispensations were available starting in 1998. We restricted our sample to women whose prescriptions, based on their residence, would have been recorded in the database for a minimum of one year before the index date.

We defined the following categories of inhaled or oral corticosteroid users according to recency of the last prescription relative to the index date: (1) current users, with the most recent prescription filled within 60 days before the index date; (2) recent users, with the most recent prescription filled within 61 - 180 days before the index date; (3) former users, with the most recent prescription filled more than 180 days before the index date; and (4) never users, with no dispensation record of inhaled or oral corticosteroids in the prescription database before the index date (the reference group). Within the category of current users, we identified new users, whose first-recorded prescription of inhaled or oral corticosteroids was dispensed within 60 days before the index date.

Potential confounders

From the DNRP, we obtained information about maternal diagnoses of asthma, rheumatoid arthritis, and inflammatory bowel disease recorded from 1977 until delivery, as corticosteroids are used in medical treatment of these diseases. We identified history of diabetes or epilepsy before the index date by using hospital discharge diagnoses or prescriptions for antidiabetic or antiepileptic drugs. Both diseases have been associated with an increased risk of miscarriage.[26,27] For the same reason, we obtained data on women's prescriptions for non-steroidal anti-inflammatory drugs (NSAIDs) redeemed within 12 weeks before the index date.[28] Smoking has been associated with an increased risk of miscarriage,[29,30] but the DNRP contains no information on smoking status. Instead we used smoking status reported to the Medical Birth Registry. For cases, we collected smoking status from the first registration in the Medical Birth Registry following the miscarriage and used that information as a proxy measure of smoking status at the time of miscarriage. For controls, we used information on smoking recorded during the pregnancy.

All relevant diagnostic and drug codes are listed in the Appendix.

Statistical analysis

We summarized demographic and health characteristics of cases and controls. We used conditional logistic regression to estimate ORs with 95% CIs for the association between corticosteroid use and risk of miscarriage, separately for oral and inhaled drugs, adjusting for age, past medical history of diabetes and epilepsy, and use of NSAIDs. We then examined the association between steroid use and miscarriage according to gestational age, defining early miscarriage as that occurring in the first trimester (until 12 completed gestational weeks) and late miscarriage as that occurring in the second trimester (from gestational week 13 until gestational week 22).

Finally, we conducted a series of sensitivity analyses. First, we examined whether variation in exposure definition affected study results. Although few corticosteroid prescriptions in Denmark are expected to last more than 60 days (definition of current use in this study), we examined the impact of extending the definition of current and new use from 60 days to 90 days before the index date. Second, to examine the impact on the results of previous obstetric history, we recalculated odds ratios while excluding cases and controls with a history of induced abortion. Third, we examined the impact of unmeasured confounding by smoking (since information on smoking is only available for women giving birth) by stratifying corticosteroid users according to their smoking status.

We used SAS® software for all analyses (version 9.2; SAS Institute, Cary, NC, USA).
This study was approved by the Danish Data Protection Agency (journal number: 2003-41-3103).

Results

We identified 10,974 cases of miscarriage and 109,740 controls giving live birth. Cases were more likely than controls to be 30 years or older on the index date (34.1% vs. 26.9%). Overall, 1,381 (12.5%) of cases and 19,762 (17.9%) of controls were reported to be smokers. Information on smoking was missing for 3,352 (30.4%) cases and 2,546 (2.3%) controls. Cases and controls were similar with respect to prevalence of asthma, rheumatoid arthritis, inflammatory bowel diseases, diabetes, and epilepsy (Table 1). Current use of any (oral or inhaled) corticosteroids was recorded for 165 (1.5%) cases and 1,447 (1.3%) controls of which 19 (0.2%) cases and 245 (0.2%) controls were new users. We identified 118 (1.1%) cases and 1,286 (1.2%) controls as recent users; 976 (8.9%) cases and 9,213 (8.4%) controls

as former users. The adjusted OR for miscarriage was 1.11 (95% CI: 0.95-1.31) for current use of corticosteroids and 1.07 (95% CI: 0.99-1.14) for former use.

Among cases, 1.3% were current users of inhaled corticosteroids, 0.8% were recent users, 5.2% were former users, and 0.1% were new users. This distribution was almost identical among the controls (current use: 1.0%; recent use: 0.8%; former use: 5.0%; and new use: 0.1%). For inhaled corticosteroids, the adjusted OR of miscarriage was 1.20 (95% CI: 1.01-1.44) for current use and 1.05 (95% CI: 0.96-1.15) for former use.

Current, new, and recent use of oral corticosteroids did not differ among cases and controls. Among cases, 4.3% were former users compared with 4.0% of controls (Table 1). For current use of oral corticosteroids, the adjusted OR for miscarriage was 0.78 (95% CI: 0.53-1.15). For former use, the adjusted OR was 1.07 (95% CI: 0.97-1.18) (Table 2). Adding smoking to the adjusted analyses did not change the estimates notably (data not shown).

There were 9,735 (88.7%) early miscarriages and 1,239 (11.3%) late miscarriages. Among women with early miscarriage, 129 (1.3%) were current users of inhaled corticosteroids whereas this only accounted for 11 (0.9%) of women with late miscarriage. Table 3 shows the ORs for early and late miscarriage in relation to use of inhaled or oral corticosteroids. The adjusted OR for an early miscarriage associated with current use of inhaled corticosteroids was 1.22 (95% CI: 1.01-1.49) and that for a late miscarriage was 1.06 (95% CI: 0.56-1.99). Among women with early miscarriage, we identified 27 (0.3%) current users of oral corticosteroids while among women with late miscarriage there was only one (0.1%) current user of oral corticosteroids.

After extending the definition of current use to 90 days before the index date, prevalence of current use of inhaled corticosteroids was 1.5% among cases and 1.3% among controls (adjusted OR = 1.09; 95% CI: 0.92-1.28). Prevalence of newly-defined current use of oral corticosteroids was 0.3% among cases and 0.4% among controls (unadjusted OR = 0.74; 95% CI: 0.52-1.05). After excluding 1,585 cases (14.4%) and 13,197 controls (12.0%) with a record of induced abortion, the estimates did not change in ways that affected the interpretation (results available on request). Among women who reported to be smokers, the prevalence of inhaled corticosteroid use among cases and controls did not differ (1.0% respectively) (adjusted OR= 1.02; 95% CI: 0.50-2.08) whereas the prevalence of oral corticosteroid use among cases was 0.3% compared with 0.2% among controls (adjusted OR= 0.66; 95% CI: 0.13-3.38).

Discussion

In this large case-control study, redeeming a prescription for an inhaled corticosteroid before 60 days preceding a miscarriage was associated with a slightly increased risk of an early loss before 12 completed weeks of gestation. There was no evidence of an association between use of oral corticosteroids and risk of miscarriage.

Our study corroborates a large prevalence study based on The Health Improvement Network (THIN) in the United Kingdom of almost 300,000 pregnancies that was conducted to quantify risks of major adverse pregnancy outcomes and obstetric complications in women with and without asthma. They reported a higher risk of miscarriage (adjusted OR = 1.24; 95% CI: 1.17-1.34) among women who used inhaled corticosteroids compared with women who did not after controlling for age, smoking and body mass index.[19] Similarly, a cohort study based on data from an international asthma trial reported an unadjusted relative risk (RR) for

miscarriages of 1.25 (95% CI: 0.63-2.47) comparing users (n=196) and nonusers (n=117) of inhaled corticosteroids.[18] Like our study, a Canadian study based on the Motherisk Program found no increased risk of miscarriage (unadjusted RR = 1.01; 95% CI: 0.48-2.11) among users of oral corticosteroids (n=187) during pregnancy compared with non-users (n=188).[12] An Israeli study reported an unadjusted RR of 1.66 (95% CI: 1.12-2.48) for miscarriage among corticosteroid users (n=311) compared with nonusers (n=790).[10] Because it was based on data reported to the Teratogen Information Service, its results were susceptible to overestimation through self-referral bias.[31]

Our study extends the earlier studies by including information on gestational age at miscarriage. This allowed better estimation of timing of exposure in relation to embryogenesis. Because most fetal organs – and their malformations – develop during gestational weeks 5 to 12,[32] presence of an association between corticosteroid exposure and early but not late miscarriage is noteworthy and may represent fetal loss secondary to malformation incompatible with fetal survival. As hypothesized, selection bias due to fetal loss could constitute one explanation for lack of an apparent association between use of corticosteroids and congenital malformations[16,17] as observed in the literature.[2,7-15]

At the same time, inhaled corticosteroids are used to treat asthma, which may also be a risk factor for miscarriage.[15] Asthma severity may also play a role.[15,19] Possible biological mechanisms for increased risk of miscarriage in women with asthma include maternal hypoxia during asthma exacerbations and abnormal smooth muscle activity in the uterus, similar to airway smooth muscle contraction in asthma.[33,34] An observed association between an asthma medication and adverse pregnancy outcome could therefore be confounding by

indication.[35] Yet, we found no major difference in the prevalence of asthma diagnoses between cases and controls.

We had access to complete, independent registration of births, miscarriages, and prescriptions, which reduced the risk of selection and information biases. Availability of information on gestational age at miscarriage allowed us to select controls at the gestational period during which they were eligible to become cases and to ascertain corticosteroid use in the preceding comparable gestational period for both cases and controls. Data on gestational age also allowed differentiation between early and late miscarriage, which may have different etiologies.

We identified occurrence of miscarriage from hospital-based diagnoses. The positive predictive value of miscarriage diagnoses recorded in the DNRP has been estimated to be 97%.[36] Still, data may be incomplete because some women undergoing very early miscarriage are not hospitalized.[17] An estimated 25% of spontaneous abortions reported by women are not registered in the DNRP.[37] Furthermore, exact time of fetal death is unknown. As controls, we used women who gave birth to a live-born child, excluding women with induced abortions, ectopic pregnancies, or stillbirth. Thus we may have underestimated the level of exposure in the source population, if these outcomes are related to use of corticosteroids.[31] However, we observed a similar history of recorded induced abortions among cases and controls and have no reason to believe that extrauterine pregnancies are represented non-randomly among women.

Information about use of corticosteroids was based on prescriptions redeemed before the occurrence of miscarriage. Although inhaled and oral corticosteroids are not available over the counter, redeemed prescriptions do not fully reflect the timing of drug intake; nor could

we measure use of corticosteroids during hospitalizations. Such errors are unlikely to differ by miscarriage status; as a result, associations, if present, may be diluted.[38]

We were able to adjust for some confounding factors. Sensitivity analysis indicated that confounding by smoking is not likely to explain our findings. However, the information on cigarette smoking was incomplete.

In conclusion, we found a slightly increased risk of first-trimester miscarriage among women who used inhaled corticosteroids 60 days before the miscarriage.

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Table 1. Characteristics of women with a first-time miscarriage (cases) and women with a first live birth (controls). Northern Denmark, 1997-2009.

	Cases	**Controls**
	Number (%)	Number (%)
Total number	10,974	109,740
Corticosteroid use		
Corticosteroids, overall		
Current	165 (1.5)	1,447 (1.3)
Recent	118 (1.1)	1,286 (1.2)
Former	976 (8.9)	9,213(8.4)
Never	9,768 (89.0)	98,291 (89.6)
New	19 (0.2)	245 (0.2)
Inhaled corticosteroids		
Current	140 (1.3)	1,143 (1.0)
Recent	87 (0.8)	907 (0.8)
Former	575 (5.2)	5,496 (5.0)
Never	10,172 (92.7)	102,194 (93.1)
New	9 (0.1)	95 (0.1)
Oral corticosteroids		
Current	28 (0.3)	341 (0.3)
Recent	35 (0.3)	411 (0.4)
Former	474 (4.3)	4,387 (4.0)
Never	10,437(95.1)	104,601 (95.3)

New	10 (0.1)	171 (0.2)
Potential confounders		
Age at index date		
< 25 years	2,,931 (26.7)	28,523 (26.0)
25-29 years	4,302 (39.2)	51,694(47.1)
≥30 years	3,741 (34.1)	29,523 (26.9)
Smoking history[*]		
Yes	1,381 (12.5)	19,726 (17.9)
No	6,285 (57.0)	87,872 (79.8)
Missing	3,352 (30.4)	2,546 (2.3)
Past medical history		
Asthma	343 (3.1)	2,982 (2.7)
Rheumatoid arthritis	30 (0.3)	171 (0.2)
Inflammatory bowel disease	76 (0.7)	838 (0.8)
Diabetes	175 (1.6)	1,715 (1.6)
Epilepsy	233 (2.1)	2,034 (1.9)
Use of NSAIDs	441 (4.0)	3,469 (3.2)

[*] For cases, we collected smoking status from the first registration in the Medical Birth Registry following the miscarriage and used that information as a proxy measure of smoking status at the time of miscarriage. For controls, we used information on smoking recorded during the pregnancy.

Table 2. Use of inhaled and oral corticosteroids and miscarriage among women in northern Denmark, 1997-2009.

Corticosteroid use	**Case/control** ratio	**Unadjusted OR** (95% CI)	**Adjusted OR*** (95% CI)
Corticosteroids overall			
Current use	165/1,447	1.15 (0.97-1.35)	1.11 (0.95-1.31)
Recent	118/1,286	0.92 (0.76-1.12)	0.92 (0.76-1.11)
Former use	976/9,213	1.07 (1.00-1.14)	1.07 (0.99-1.14)
Never use	9,768/98,291	*reference*	*reference*
New use	19/245	0.78 (0.49-1.25)	0.75 (0.47-1.19)
Inhaled corticosteroids			
Current use	140/1,143	1.23 (1.03-1.47)	1.20 (1.01-1.44)
Recent	87/907	0.95 (0.76-1.19)	0.94 (0.75-1.17)
Former use	575/5,496	1.05 (0.96-1.15)	1.05 (0.96-1.15)
Never use	10,172/102,194	*reference*	*reference*
New use	9/95	0.92 (0.46-1.82)	0.86 (0.43-1.72)
Oral corticosteroids			
Current use	28/341	0.82 (0.56-1.21)	0.78 (0.53-1.15)
Recent	35/411	0.85 (0.60-1.21)	0.85 (0.60-1.20)
Former use	474/4,387	1.08 (0.98-1.19)	1.07 (0.97-1.18)
Never use	10,437/104,601	*reference*	*reference*
New use	10/171	0.60 (0.32-1.14)	0.57 (0.30-1.07)

*Adjusted for age at the index date, history of diabetes and epilepsy, and use of NSAIDS 12

weeks before the index date.

OR: odds ratio; CI: confidence interval.

Table3. Use of oral and inhaled corticosteroid prescriptions stratified by early miscarriage (gestational weeks 1-12) or late miscarriage (gestational weeks 13-21). Northern Denmark, 1997-2009.

Corticosteroid use	Early miscarriage			Late miscarriage		
	Case/control ratio	**Unadjusted OR** (95% CI)	**Adjusted OR** (95% CI)	**Case/control** ratio	**Unadjusted OR** (95% CI)	**Adjusted OR** (95% CI)
Inhaled corticosteroids						
Current	129/1,047	1.24 (1.03-1.49)	1.22 (1.01-1.49)	11/96	1.09 (0.58-2.04)	1.06 (0.56-1.99)
Recent	74/786	0.93 (0.73-1.18)	0.92 (0.72-1.17)	13/121	1.07 (0.60-1.91)	1.06 (0.59-1.89)
Former	515/4,867	1.06 (0.97-1.17)	1.07 (0.97-1.17)	60/629	0.95 (0.72-1.25)	0.97 (0.74-1.27)
Never	9017/90,650	*reference*	*reference*	1,155/11,544	*reference*	*reference*
New	9/88	0.99 (0.50-1.97)	0.93 (0.47-1.86)	0/7	-	-
Oral corticosteroids						
Current	27/318	0.86 (0.58-1.27)	0.81 (0.55-1.20)	1/23	0.41 (0.06-3.06)	-
Recent	32/367	0.87 (0.61-1.25)	0.87 (0.60-1.25)	3/44	0.70 (0.22-2.26)	0.68 (0.21-2.20)
Former	431/3,884	1.11 (1.01-1.23)	1.10 (0.99-1.22)	43/503	0.85 (0.62-1.17)	0.83 (0.61-1.14)
Never	9,245/92,781	*reference*	*reference*	1,192/11,820	*reference*	*reference*

New	10/164	0.63 (0.33-1.19)	0.59 (0.31-1.12)	0/7	-	-

*Adjusted for age at the index date, history of diabetes and epilepsy, and use of NSAIDS in the 12 weeks before the index date.

OR: odds ratio; CI: confidence interval.

Appendix. Codes from the International Classification of Diseases (ICD) and Anatomical Therapeutic Chemical (ATC) classification used to identify diagnoses from the Danish National Registry of Patients and to identify use of prescribed drugs from the Aarhus University Prescription Database.

	ICD-8 codes	ICD-10 codes	ATC codes
Miscarriage	643	O02-O03	
Induced abortion	640, 641, 642	O04	
Asthma	493	J45-J46	
Rheumatoid arthritis	712.19, 712.39, 712.59	M05-M06	
Inflammatory bowel disease	563.00, 563.01, 563.10, 569.02	K51-K50	
Diabetes	250	E10-E14	
Epilepsy	345	G40	
Inhaled corticosteroids			R03BA01, R03BA02, R03BA05, R03BA07, R03AK06, R03AK07
Oral corticosteroids			H02AB04, H02AB06, H02AB07, H02AB09
Non-steroidal anti-inflammatory drugs (NSAIDs)			M01A

Antidiabetics	A10
Antiepileptics	N03A

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