Risk of congenital malformations and miscarriages following maternal use of oral fluconazole during the first trimester of pregnancy: a systematic review and meta-analysis

Mathilde Latour, Catherine Vauzelle, Elisabeth Elefant, Florence Tubach, Stephanie Padberg, Brigitte Martin, Kristen Joseph-Delaffon, Agnès Dechartres, Benoit Marin

Title Page

Title: Risk of congenital malformations and miscarriages following maternal use of oral fluconazole during the first trimester of pregnancy: a systematic review and meta-analysis

Authors: Mathilde Latour[1], Catherine Vauzelle[1], Elisabeth Elefant[1], Florence Tubach[2], Stephanie Padberg[3], Brigitte Martin[4], Kristen Joseph-Delaffon[5], Agnès Dechartres[2], Benoit Marin[6]

Affiliations:

1. AP-HP.Sorbonne Université, Hôpital Trousseau, Département de Santé Publique, Centre de Référence sur les Agents Tératogènes (CRAT), F75012, Paris, France.
2. Sorbonne Université, INSERM, Institut Pierre Louis d'Epidémiologie et de Santé Publique, AP-HP, Hôpital Pitié Salpêtrière, Département de Santé Publique, Centre de Pharmacoépidémiologie (Cephepi), Unité de Recherche Clinique PSL-CFX, CIC-1901, AP-HP, 75013 Paris, France.
3. Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Institute of Clinical Pharmacology and Toxicology, Embryotox Center of Clinical Teratology and Drug Safety in Pregnancy, Augustenburger Platz 1, 13353 Berlin, Germany.
4. Centre IMAGe (Info-Médicaments en Allaitement et Grossesse), CHU Sainte-Justine, H3T 1C5, Montréal, Québec, Canada.
5. Department of Neonatology, Bretonneau Hospital, François Rabelais University, F-37000 Tours, France, Sorbonne Université, INSERM, Institut Pierre Louis d'Epidémiologie et de Santé Publique, AP-HP, Hôpital Trousseau, Département de Santé Publique, Centre de Référence sur les Agents Tératogènes (CRAT), F75012, Paris, France
6. Sorbonne Université, INSERM, Institut Pierre Louis d'Epidémiologie et de Santé Publique, AP-HP, Hôpital Trousseau, Département de Santé Publique, Centre de Référence sur les Agents Tératogènes (CRAT), F75012, Paris, France.

Competing interests: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding sources/sponsors: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

PROSPERO Registration: The protocol was registered on the 23rd September 2021 (registration number: CRD42021274003).

Paper presentation information: This paper and the data have not previously been published. Nevertheless, preliminary results have been presented in the 34th Annual ENTIS (European Network of Teratology Information Services) Conference in Dublin, Ireland, from 31st August 2023 to 2nd September 2023.

Data availability: Data extracted from original articles can be provided upon request.

Corresponding author: Benoît Marin

e-mail: benoit.marin@aphp.fr

Centre de Référence sur les Agents Tératogènes (CRAT)

APHP.Sorbonne Université. Hôpital Armand Trousseau.

26 avenue du Dr Arnold Netter

75012 Paris

Word count:

Abstract: 254

Manuscript: 5,584/7,451

Abstract (254 words)

Background

The risks related to fluconazole use during the first trimester of pregnancy (T1) remain controversial. The aims of this systematic review and meta-analysis were to assess the association between oral fluconazole during T1 and major congenital malformations (MCM) overall and by subtype, minor malformations and miscarriages.

Methods

We searched MEDLINE, EMBASE, Cochrane, ICTRP and ClinicalTrials.gov from inception to 02/12/24. Randomized controlled trials and observational studies were included. ROBINS-I was used for risk of bias assessment. Both fixed- and random-effects models meta-analyses were performed. GRADE was used to assess the certainty of the evidence.

Results

Among 1,403 references, nine observational studies were included (3,764,897 pregnancies, including 116,425 exposed to fluconazole). The association between any fluconazole use during T1 and overall MCM was significant when combining crude estimates (ORc 1.18, 95%CI (1.08-1.29), I^2 23%, seven studies), but not when combining adjusted estimates (ORa 1.02, 95%CI (0.98-1.07), I^2 0%, six studies). Results were consistent for cumulative dose of fluconazole. In sensitivity analyses considering only studies with a valid definition of MCM, the association between fluconazole >150 mg and overall MCM remained significant when combining adjusted estimates. For the subtypes of MCM (cardiac, genito-urinary, musculoskeletal) we found no significant association. A significant association was found between fluconazole use and miscarriages (ORa 1.60, 95% CI (1.06-2.42).

Conclusion

Fluconazole use during T1 does not significantly increase the risk of MCM overall or by subtype when considering adjusted estimates. However, potential risks, particularly at cumulative doses greater than 150 mg which show a potential association with MCM, deserve much attention.

Keywords: oral fluconazole, pregnancy, first trimester, congenital malformation, teratogenicity, miscarriage

1. Introduction

Fluconazole, a first-generation triazole antifungal drug, is widely used for treating vaginal candidiasis, which is particularly common during pregnancy, and for preventing and treating invasive fungal infections, especially in immunocompromised patients.

Fluconazole can be administered intravenously or orally, with a very good bioavailability by the oral route (>90%) [1]. It is particularly effective against Candida albicans. Dosage and treatment duration vary according to the indication. Two main patterns of prescription regimens can be distinguished according to indication: a single low dose scheme (150 mg once, which may be repeated once if needed) mainly for vaginal candidiasis, and a high-cumulated dose scheme for disseminated fungal infections (50 to 800 mg per day for a given period, mostly several weeks).

In animal studies, high doses of fluconazole increased the frequency of anomalies [2,3], a pattern of malformations similar to those reported in humans [4–7]. Indeed, a few cases of congenital malformations have been reported in newborns of pregnant women treated for severe visceral fungal infections with high dose fluconazole during a prolonged time: especially craniofacial malformations (e.g. craniostenosis, hypoplasia of the facial bones), skeletal malformations (e.g. radiohumeral synostosis, curvature of the long bones), cardiac malformations and cleft palates [4–7]. A recent observational study also suggested a moderate increased risk of musculoskeletal malformations [8]. Fluconazole works by inhibiting the fungal CYP51 enzyme, crucial for ergosterol synthesis in the cell membrane and essential for the construction of fungal cell walls [9]. Inhibition of these enzymes has been discussed as an explanation for a possible teratogenic mechanism [6].

Several meta-analyses have found no significant increased risk of overall major congenital malformations associated with fluconazole use during pregnancy, but some have suggested an increased risk of fetal heart defects [10–12] as well as an increased risk of early miscarriages [11]. Nevertheless, two main reasons conducted us to perform a new systematic review and meta-analysis (SR/MA): serious methodological concerns [13] regarding the most recent meta-analysis [14] on this topic (limited search of the literature, no information on eligibility and data extraction process, no assessment of the risk of bias, heterogeneity in the definition of outcomes, discrepancies between text and table, errors in data extraction…) which also reported an increased risk of cardiac malformation ,

and a recent publication from a large population-based study [8] which was not included in previous SR/MA [11].

The main objective of this study was to assess the association between maternal use of oral fluconazole during the first trimester of pregnancy (T1) and the risk of major congenital malformations (MCM), overall and by type of malformation, among all pregnancies (including live births, stillborn and medical terminations of pregnancy). Our secondary aims were to assess the association between maternal use of oral fluconazole during the first trimester and: (i) minor congenital malformations among all pregnancies (including live births, stillborn and medical terminations of pregnancies); (ii) miscarriages: early miscarriages (loss of pregnancy before 14 gestational weeks [GW = weeks after the Last Menstrual Periods]) or late miscarriages (loss of pregnancy between 14 and 22 GW).

2. Methods

This study adhered to methodologies outlined in the Cochrane Handbook [15] and its report conformed to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) and the Meta-analyses Of Observational Studies in Epidemiology (MOOSE) guidelines [16,17]. The protocol was registered in PROSPERO (CRD42021274003) [18]. The modifications made to the protocol are reported and justified in Online Resource Appendix 1.

Data sources and search strategy

We searched MEDLINE via PubMed, EMBASE, the Cochrane Database of Systematic Reviews, Cochrane Central Register of Controlled Trials (CENTRAL), WHO International Clinical Trials Registry Platform (ICTRP), and ClinicalTrials.gov from inception through February 12th, 2024.

A dedicated search algorithm using keywords and free-text words was developed for each database (Online Resource Appendix 2).

We reviewed the past five years of abstracts from key international conferences such as: Organization of Teratology Information Specialists (OTIS), European Teratology Society (ETS), European Network of Teratology Information Services (ENTIS), Teratology Society (TS), American College of Obstetricians and Gynaecologists (ACOG). We asked the pharmaceutical companies for pregnancy register and relevant non-published studies, if any. Finally, we screened the reference lists of all systematic reviews and selected studies and asked two independent Teratology Information Service (TIS) experts to assess list of included articles and to complete with further references, if any.

Eligibility criteria

We planned to include all reports of randomized controlled trials (RCT) and prospective or retrospective comparative observational studies (case control and cohort studies, including multi-arm studies) [19] evaluating the association between oral fluconazole intake, whatever the indication, during the first trimester of pregnancy and the risk of adverse fetal outcomes. Hence single-arm studies were not included. Studies regarding paternal pre-conceptional exposure to fluconazole were not included and studies involving maternal pre-conceptional exposure were included only if the exposure began after the last menstrual period date. Systematic reviews and meta-analyses were not included but their references examined. Conference abstracts corresponding to published studies, expert opinions,

editorials, letters, case-reports and studies reporting non-human research were not included. There was no restriction on publication date and language.

Our primary endpoint was major congenital malformations (MCM) among the pregnancy outcomes (including live births, stillborn and medical terminations of pregnancy), overall and by type of malformation, following the definition proposed by the European Surveillance for Congenital Anomalies (EUROCAT) [20]. According to this definition, major congenital anomalies "*are structural changes that have significant medical, surgical, social or cosmetic consequences for the affected individual, and typically require medical intervention*".

Our secondary endpoints were (i) minor congenital malformations in the pregnancy outcomes (including live births, stillborn and medical terminations of pregnancy), following the definition proposed by the EUROCAT [20] which states that minor malformations "*are those which do not in themselves have serious medical, functional or cosmetic consequences for the child*", and (ii) miscarriages defined as early miscarriages (loss of pregnancy before 14 GW) or late miscarriages (loss of pregnancy between 14 and 22 GW).

Selection process

Records were managed with Zotero v5.0 and duplicates deleted. One review author identified eligible studies by screening titles and abstracts, then two review authors independently read the full texts [21]. Two other reviewers were involved to reach consensus in case of disagreements.

Data collection process and risk of bias assessment

Two review authors independently extracted data from the included studies by using a standardized data extraction form and assessed the risk of bias using the updated version of the Non-Randomized Studies of Interventions (ROBINS-I) for non-randomized studies [22]. The risk of bias was assessed focusing first on primary endpoint and then on secondary endpoints. Two other reviewers were involved to reach consensus in case of disagreements. In case of missing or unclear data in the manuscript, we attempted to contact the study authors.

For each study the following data were collected: study design, population characteristics, fluconazole exposure, control group and results [18]. Concerning confounding factors, we identified whether the authors had adjusted for a list of potential confounders identified by us *a priori* (Online Resource Appendix 3) for the risk of major congenital malformations and the risk of miscarriages respectively and which other factors had been taken into account in the analyses. For each arm, we collected the number of participants, the number of events for each outcome in each group. Crude and adjusted estimates of measures of association as well as 95% confidence intervals were collected. When some subtypes of malformations within a specific group of malformations were presented separately in publications (for example genito-urinary [23] and musculoskeletal MCM [8,24]), in order to have homogeneous outcomes within the included studies, we recalculated, based on the data given in the original articles, the frequencies of each event into congruent categories according to the organs affected (Online Resource Appendix 4).

Data synthesis

Main and secondary analyses

For each endpoint, the measure of association estimated in the meta-analysis was the odds ratio (OR). We *a priori* decided to perform both fixed- and random-effects models and report both results in forest plots [18].

We conducted meta-analyses based on crude associations on one hand and on adjusted associations on the other hand. Pooled estimates of crude association were based on the frequencies reported in the original papers allowing direct calculation of crude OR (ORc). In these cases, we verified that the calculated ORc in our report were congruent with ORc reported in the original papers. Pooled estimates of adjusted associations were based on adjusted OR (ORa), adjusted relative risks (RRa) and adjusted hazard ratio (HRa) reported in the original papers. As it can be assumed that OR approximates RR when the disease prevalence is low (i.e. <10%, which was the case in our study which focused on MCM), RR and OR were combined in the meta-analysis [25]. As regards Hazard Ratio, this assumption is more debatable [26] although some authors consider that the same approach can be used [27]. Given this uncertainty we decided to perform first an analyse including OR, RR and HR, and then a sensitivity analyse excluding HR to assess the robustness of our results.

Meta-analyses were first performed considering exposure to “any dose” of fluconazole and then considering two types of cumulative dose of fluconazole (≤ 150 mg and > 150 mg) to differentiate between the two types of exposure, related to the indications. When the authors reported other cumulative exposure categories (e.g. 3 categories: 150 mg or in between 150 and 450 mg or > 450 mg), we used the frequencies reported in the original papers to recalculate relevant ORc. In such cases, to obtain the relevant ORa for the two types of cumulative dose of fluconazole, we first asked authors to provide us the missing data and when no information was provided, we performed a calculation of a weighted average of the adjusted effect size following the approach proposed by Borenstein et al. [28] recommended in the Cochrane Handbook [29].

Heterogeneity assessment and exploration

We evaluated statistical heterogeneity across studies by visually inspecting forest plots and by the Cochrane Q test, Tau^2 and I^2 statistics. An I^2 value > 50% or p-value of heterogeneity (p_{het}) < 0.1 was considered as substantial heterogeneity [15]. We planned *a priori* subgroup analyses according to relevant study level covariates, namely single dose versus repeated low-dose treatment, timing of exposure and reference group (no treatment, topical azole) and also a random-effects meta-regression to evaluate the effect of the cumulated dose on the risk of MCM, nevertheless, these analyses were not performed due to the lack of data [15].

Sensitivity analyses

Sensitivity analyses were performed to assess the robustness of the results considering (i) the validity of MCM definition (i.e. excluding publications where the definition of MCM was considered imprecise by the review authors) (ii) the type of reference group (i.e. when a study considered various reference groups: "not exposed to oral fluconazole" and "exposed to topical azole") (iii) the estimate reported (i.e. excluding studies reporting HR) and (iv) potential duplicate material between studies. A sensitivity analysis considering the risk of bias was planned but was not possible.

Analyses were done using metabin and metagen using the following packages: dplyr v1.1.2, fivemat, meta v6.2-1, tidyverse v2.0.0, readxl v1.4.3, R v4.2.2 [30].

Certainty of evidence

GRADE approach [31] and GRADEpro software [32] were used to assess the certainty of evidence for all the endpoints.

3. Results

Search results

Our search identified 1,403 studies for title and abstract screening. Of these, 15 were eligible for full text review (Figure 1) and nine observational studies ultimately met the inclusion criteria [8,23,24,33–38]. One of these nine studies was in abstract form for which we contacted the authors but while the article is not yet published, it was not possible for this group to share more data with us [36].

Study characteristics

Studies were conducted in North America [8,23,33] or in Europe [24,34,35,37,38]. One study was conducted in several countries (USA, Canada and UK) [36] (Table 1). There were seven cohort studies [8,24,34–38] and two case-control studies [23,33], mostly performed in the general population [8,23,24,33,34,36–38] (n=8), based on medico-administrative data with access to prescription or delivery drug information (n=7) [8,23,24,34,36–38] or on registry data (n=1) [33]. There was one cohort study based on clinical data from several TIS [35]. In the majority of the studies, the data were prospectively collected (n=8) and for one study the data were retrospectively collected [33]. The period of recruitment was reported for all but one of the studies and ranged from the late 1990s to 2016.

Number of pregnancies

In total, the nine studies included in the meta-analysis made it possible to evaluate 3,764,897 pregnancies, including 116,425 pregnancies exposed to fluconazole during the first trimester of pregnancy.

Population

Among eight studies that evaluated the risk of congenital malformation (Table 1 and Online Resource eTable 1a), four of these were based on live birth only [8,23,38], two studies considered live birth and medical termination of pregnancy [24,35], one study considered live births, stillbirth and induced abortion [33]. For two studies, this information was not available [34,36].

Among four studies that evaluated the risk of miscarriages (Table 1 and Online Resource eTable 1b), one study based the analysis on all pregnancies excluding induced abortions. For the other three studies, no information was available.

Exposure definition

Four studies clearly defined exposure as any dose of fluconazole whatever the cumulative or daily dosage was (referred as “any dose”) [33–35,38] while for another one it was speculated from the data given in the abstract that the exposure was also defined as any dose [36]. One study considered the cumulative dose of fluconazole as categories [23] and three studies considered both any dose and different levels of cumulative dose of fluconazole [8,24,37]. The studies assessing any dose of fluconazole either failed to specify a minimum dosage considered for this category [33,34,36,38] or provided imprecise information about the minimal dose included [35]. Besides, for cumulative dose, there were different ways to categorize exposure: (i) ≤ 150 mg or > 150 mg [23]; (ii) 150 mg or 300 mg or ≥ 350 mg [24]; (iv) in between 150 mg and 300 mg or ≥ 350 mg [37]; (v) 150 mg or in between 150 mg and 450 mg or > 450 mg [8].

In most studies, the reference group of included articles consisted in subjects not exposed to oral fluconazole (n=7) [23,24,33,35–38]. In two instances there were several reference groups (not exposed, exposure to topical azole and exposure to another oral azole) [8,34].

Definition of the endpoints

Five studies focused exclusively on MCM [23,24,33–35] whose definition were considered valid by the review authors (Online Resource eTable 2a). Indeed, these studies either provided an accurate definition of MCM excluding minor malformations [23,24,33], especially relying on ICD codes or a list of malformations (including the EUROCAT definition [23] and the definition from the National Birth Defect Prevention Study [33]), or provided an acceptable narrative definition of MCM such as “congenital anomalies that warranted medical or surgical treatment” [35] and “malformations present at birth that resulted in surgery or other treatment for functional or cosmetic reasons” [34] without other precision (“approximate definition”). Two other studies either included minor malformations [38] or left doubts about the current exclusion/inclusion of minor malformations [8]. Lastly, one study stated the evaluation of MCM without giving a definition in the abstract material available [36]. These last three studies were qualified as studies with an imprecise definition of MCM.

A total of 35 specific types of malformations were studied, 20 of which were evaluated in a single study (Online Resource eTable 2b). The remaining 15 were evaluated in several studies and are presented in Online Resource eTable 2c.

For some specific types of malformations (n=5), the number of patients provided in the article allowed us the recalculation of estimates (Online Resource Appendix 4).

The risk of minor congenital malformations associated with fluconazole use in the first trimester of pregnancy was not assessed as a separated outcome in the included studies, so no meta-analysis was possible for this outcome.

Only one study evaluated specifically miscarriages before 12 GW [35]. The other studies did not distinguish between early and late miscarriages [23] [37] or did not specify the definition [36].

Risk of bias in studies

Overall, seven studies had a serious risk of bias [8,23,24,33,34,37,38], one a moderate risk of bias [35] and one had non-informative data [36] (Table 2). Online Resource eTable 3 provides more information about control for confounding of the respective studies.

Meta-analyses

As per protocol, both fixed- and random-effects meta-analyses were performed. Results based on the random-effects model are detailed in the text, and both fixed- and random-effects meta-analyses are shown in the figures (Figures 2 to 4 and eFigures 1 to 9 in the Online Resource).

<u>Association between fluconazole exposure during T1 and MCM</u>

The overall pooled crude ORc was 1.18, 95%CI (1.08-1.29), p_{het}=0.25, I^2 23% (Figure 2a) for the risk of MCM with any dose of fluconazole in the first trimester of pregnancy. The pooled adjusted association was non-significant (ORa 1.02, 95%CI (0.98-1.07), p_{het}=0.49, I^2 0%, Figure 2b). Sensitivity analyses did not modify the results (Online Resource eFigure 1).

There was a significant crude association between the two types of fluconazole cumulative dose and the risk of MCM, respectively ORc 1.12, 95%CI (1.04-1.19), p_{het}=0.48, I^2 0% for a dose ≤ 150 mg and ORc 1.22, 95%CI (1.06-1.40), p_{het}=0.17, I^2 44% for a dose > 150 mg (Figure 3a) considering no fluconazole exposure as the reference group. Nevertheless, the pooled adjusted association were non-significant (≤ 150 mg: ORa 1.03, 95%CI (0.97-1.10), p_{het}=0.84, I^2 0%, and > 150 mg: ORa 1.08, 95CI (0.90-1.29), p_{het}=0.06, I^2 65%, Figure 3b).

In the sensitivity analysis according to the validity of the definition of MCM, when considering the crude estimates, no significant increased risk of MCM was found for a dose of fluconazole ≤ 150 mg (ORc 1.09, 95%CI (0.92-1.28), p_{het}=0.26, I^2 22%) but the point estimate was higher and significant for a dose of fluconazole > 150 mg (ORc 1.32, 95%CI (1.07-1.63), p_{het}=0.22, I^2 33%) (Online Resource eFigure 2a). After considering adjusted estimates, the results did not differ from the main analysis for a dose of fluconazole ≤ 150 mg (ORa 1.03 95%CI (0.89-1.19), p_{het}=0.55, I^2 0%) but a significant association was persistent for a dose of fluconazole > 150 mg (ORa 1.19, 95%CI (1.01-1.40), p_{het}=0.47, I^2 0%), based on the two studies that could be included in this analysis (Online Resource eFigure 2b).

Association between fluconazole exposure during T1 and miscarriages

The overall pooled ORc was 1.62, 95%CI (0.70-3.75), p_{het}<0.01, I^2 98% (Figure 4a) for the risk of miscarriages associated to any dose of fluconazole during the first trimester of pregnancy and the overall pooled ORa was 1.60, 95%CI (1.06-2.42), p_{het}<0.01, I^2 97% (Figure 4b). There was no significant association in sensitivity analysis (Online Resource eFigure 3). It was not possible to conduct an analysis according to the cumulative dose of fluconazole due to lack of data.

Association between fluconazole exposure during T1 and subtypes of MCM

- Cardiac MCM

The overall pooled ORc for the risk of cardiac malformation associated to any dose of fluconazole exposure was 1.24, 95%CI (1.14-1.36), p_{het}=0.78, I^2 0% (Online Resource eFigure 4a) while the pooled ORa was 1.06, 95%CI (0.97-1.16), p_{het}=0.41, I^2 0% (Online Resource eFigure 4b). Results of sensitivity analyses according to the reference group (topical azole instead of no fluconazole [8,34] did not differ from the above-mentioned results (Online Resource eFigure 5).

As regards cumulative dose of fluconazole exposure, there was a significant crude association between a dose of fluconazole ≤ 150 mg and cardiac MCM (ORc 1.17, 95%CI (1.04-1.33), p_{het}= 0.65, I^2 0%) but the association was not significant for a dose > 150 mg while the point estimate was higher (ORc 1.35, 95%CI (0.98-1.87), p_{het}=0.07, I^2 63%, Online Resource eFigure 6a). When considering adjusted estimates, both the pooled adjusted associations were not significant (Online Resource eFigure 6b). It was not possible to conduct sensitivity analyses due to lack of data.

- Genito-urinary MCM

The overall pooled ORc was 1.19, 95%CI (1.07-1.33), p_{het}=0.49, I^2 0%) between any dose of fluconazole exposure during the first trimester and genito-urinary MCM (Online Resource eFigure 7). Results of sensitivity analyses according to the definition of MCM did not differ from the above-mentioned results. Sensitivity analyses according to the reference group was not significant (Online Resource eFigure 8). Due to lack of data, it was not possible to perform analyses with adjusted estimates and according to cumulative dose of fluconazole.

- Musculoskeletal MCM

The overall pooled ORc was 1.18, 95%CI (0.98-1.43), p_{het}=0.12, I^2 54% between exposure to any dose of fluconazole during the first trimester and the risk of musculoskeletal MCM (Online Resource eFigure 9a). There was no significant association between a dose of fluconazole ≤ 150 mg and the risk of musculoskeletal MCM (ORc 1.01, 95%CI (0.69-1.46), p_{het}= 0.02, I^2 75%) but it was significant for a dose > 150 mg (ORc 1.33, 95%CI (1.13-1.56), p_{het}=0.89, I^2 0%, Online Resource eFigure 9b). Results of sensitivity analyses according to the definition of MCM did not differ from the above-mentioned results (Online Resource eFigure 10). Due to lack of data, it was not possible to perform analyses with adjusted estimates for both any dose and cumulative dose.

Small study effect

We did not assess small study effect because no meta-analysis included at least 10 studies.

Grade evaluation

Overall, the certainty of evidence for the respective meta-analyses ranged from very low to low (Online Resource eTable 4).

4. Discussion

This SR/MA aimed to assess the association between maternal use of oral fluconazole during T1 and the risk of major congenital malformations, minor congenital malformations and miscarriages. It included 9 studies based on more than 100,000 pregnancies exposed to oral fluconazole during T1. While we found a significant crude association between fluconazole exposure and MCM overall, this association disappeared when combining adjusted estimates. Indeed, all associations failed to reach statistical significance in the meta-analysis based on adjusted estimates except for the sensitivity analysis of overall MCM considering the studies with a valid definition of MCM for cumulative dose of fluconazole > 150 mg, based on only two studies [23,24]. Similarly, we found no significant adjusted association between fluconazole exposure and the risk of cardiac MCM whatever the dose. For other subtypes of MCM, the associations were significant for genito-urinary MCM according to any dose of fluconazole and for musculoskeletal MCM considering cumulative dose of fluconazole > 150 mg but based on crude estimates only. Unfortunately, it was not possible to perform meta-analysis based on adjusted estimates. Overall, the certainty of evidence according to GRADE ranged from very low to low.

A significant association between any dose of fluconazole exposure during T1 and the risk of miscarriages was also found, based on adjusted estimates, but it was not possible to perform a meta-analysis for cumulative doses of fluconazole. It was also not possible to perform a meta-analysis to assess the risk of miscarriages by distinguishing between early and late miscarriages because only one study specified the term of the miscarriages [35]. The certainty of evidence for this endpoint was very low.

The major strengths of this SR/MA are the important number of exposed pregnancies included and its robust methodology. It followed the Cochrane Handbook, included a systematic and comprehensive search of the literature using multiple data sources and evaluated the risk of bias using ROBINS-I. A detailed collection of the potential confounders and adjustment variables and a robust examination of the diagnostic codes used by the authors of the original papers was performed to be sure of the congruence of the different endpoints. Besides, contacts with the authors of the selected studies and teratology experts, when possible, allowed us to obtain additional relevant information. Also, we performed a numerous number of sensitivity analyses and found a significant association for the cumulative dose of fluconazole > 150 mg considering only studies with a valid definition of MCM. This

result may be due to chance, to the fact that some effect sizes were recalculated (and may therefore be less reliable) or to the fact that this analysis does not discriminate between 150 mg repeated one time (300 mg) and 1500 mg in cumulative dose during the first trimester of pregnancy. For this last point, indeed, according to the authors of the included studies, the main indication of treatment was vulvovaginal candidiasis. However, a proportion of the patients received doses of fluconazole higher than 150 mg, although it was not possible to determine whether patients received two doses of 150 mg as part of a repeated dose for the treatment of vaginal mycosis, or higher doses over a prolonged period of treatment as part of an opportunistic infection. Yet, in these two different types of indications the systemic exposure is probably different and might be associated with different level of risk which, unfortunately, we were unable to evaluate.

The main limitations of our study are that we have not been able to obtain all the information we needed for some endpoints. Based on the data provided by the authors, we were able, for some subtypes of malformation, to make recalculations, nevertheless as we did not have access to individual data, this approach may have led to a risk of duplicate counting of events (for example, for the subtype of "musculoskeletal" malformations, we may have taken into account twice a same child considered in the original papers as having two different musculoskeletal malformations) and our results may therefore be less reliable. We tried to reduce the risk of duplication by contacting the authors to ask for the frequencies of reconstructed groups but only one replied. We wanted to carry out sensitivity and subgroup analyses but this was not possible due to a lack of information in the selected articles (Online Resource Appendix 1). We were not able to collect all the data we planned to, especially data concerning the fluconazole treatment and the population characteristics. In some cases, the number of studies taken into account in the sensitivity analyses was very low. Pre-planned sensitivity analysis according to the risk of bias could not be performed because all studies evaluating the risk of malformations were considered at serious risk of bias, except one that was "no informative" and only one study evaluating the risk of miscarriages was at moderate risk of bias. We also recognize the inherent limitation of the primary endpoint "any MCM" because it is a composite, heterogeneous outcome [39]. Nevertheless, our study was not limited to this single endpoint but considered as well specific subtypes of MCM previously discussed in literature, and miscarriage. Finally, we were not able to assess small study effect because no meta-analysis included at least ten studies.

We faced also limitations of the included material which in short consisted in the great variability in the definition used for the exposure (any dose, cumulative dose), for the endpoints (malformations, miscarriages) and for the population (live births, medical termination of pregnancies) as well as in terms of control for confounding.

First, regarding the exposure, the minimal dose of fluconazole taken into account was sometimes not mentioned by the authors and the categories of cumulative dose of fluconazole in each study were not superposable. Similarly, detailed information on the total duration of treatment of fluconazole was not available in the studies. Although it could be assumed that most patients receiving cumulative dose ≤ 150 mg were being treated for vulvovaginal candidiasis (information sometimes reported in the selected articles), it was not always possible to obtain details for fluconazole exposure > 150 mg (distribution of treatment indications or dosages). This group may therefore include either patients receiving a 150 mg dose repeated once or twice, or patients treated with daily doses of fluconazole as a long-term treatment for prophylaxis/treatment of invasive fungal infection, without any possibility to distinguish these two very different expositions. Besides, the source for exposure assessment was variable among the studies: the majority of them used a national prescription database with no possibility to ensure that the drug was actually taken, and one study based on clinical chart used structured interviews with the risk of recall bias.

Second, regarding the definition of the malformations, some authors provided validated diagnostic codes or algorithms while others reported the diagnostic of malformations according to their functional or aesthetic impact. This is inherent to the fact that the procedures for identifying these outcomes have changed over time, in particular with the evolution of the definitions of major malformations and the development or improvement in prenatal diagnosis techniques (ultrasound, genetic analyses…). Thus, the inclusion or exclusion of minor congenital malformation was not systematically mentioned in the articles and some minor malformations were differently managed: for example, Molgaard-Nielsen et al. [24] excluded ICD-10 code Q65 (congenital deformities of hip) whereas Bérard et al. [23] included Q65 (Online Resource Appendix 5). Minor anomalies are inconsistently reported in the literature, as they are sometimes undiagnosed due to their limited prognostic, functional or cosmetic impact. Besides, the objective assessment of the severity of the malformations could differ between health professionals or international classifications and this could explain disparities in the distinction between major and minor congenital malformations.

Third, the definition of miscarriages varied also greatly and made it impossible to assess early and late miscarriages separately. Besides, since fluconazole is mainly a single dose or a short-term treatment, the time-dependency and the competing risks (spontaneous abortion, elective termination, ongoing pregnancy) should be taken into account when analysing miscarriages.

Fourth, in the majority of the studies and especially key studies that contribute heavily to the overall analysis, such as the study of Zhu et al., Bérard et al. or Molgaard-Nielsen et al., some important confounding factors (e.g., BMI, alcohol consumption, familial history of malformations) were not controlled in all cases. This raises concerns in terms of residual confounding, which is most likely to be important, and this explains why we considered the risk of bias as “serious” for most studies including those who displayed adjusted estimates.

We acknowledge that there are previous SR/MA published on this topic [10–12,14]. Nevertheless, due to methodological limitations of some articles and recent publications of an observational study, we consider our SR/MA as timely and relevant. Alsaad et al. [10] suggested an increased risk of cardiac malformation and no increased risk of overall malformation nevertheless the search strategy was limited and up to 2014. Zhang et al. [11] found an increased risk of cardiac malformation for any dose of fluconazole, an increased risk of overall malformation for > 150 mg fluconazole in a subgroup analysis and an increased risk of miscarriages. This SR/MA did not include the recent study of Zhu et al.[8] which represents the more important weight in our SR/MA and in particular for the analysis of MCM where its weight is approaching 80%. Nevertheless, our results may be due to residual confounding bias present in the study of Zhu et al., as some covariates were not taken into account in this study such as the personal and familial history of malformation, or were poorly measured such as body mass index, alcohol consumption, illicit drug exposure. Besides as compared to Zhang, for the evaluation of miscarriages our analysis based on adjusted estimates included one more reference [36], providing an increase in the precision of the estimate compared with the results reported by Zhang et al., while the interpretation was unchanged. Finally, two SR/MA [12,14] suggested an increased risk of cardiac malformations but the first one [12] used a different analysis strategy and compared frequencies of malformations with frequencies reported according to EUROCAT while the other [14] presented heterogenous definitions of endpoints and errors in data extraction [13]. Moreover all these SR/MA did not take into account the risk of potential duplication of material from the studies based on the Danish

Medical Birth Registry including both the studies of Molgaard-Nielsen et al. [24] and the study of Norgaard et al. [40] that are superposable (Figure 1). Even more important, except one [12], these studies did not mention if the results were based on crude or adjusted estimates.

Previous comparative studies that assessed separately genital MCM and urinary MCM did not find a significant association between these malformations and oral fluconazole exposure during the first trimester of pregnancy. Our meta-analysis is the first to suggest an increased risk of genito-urinary MCM. However, as mentioned above, there is a risk of duplicate endpoints in our study and our results, based on crude estimates, need to be confirmed.

In summary, our study which followed the standards of SR/MA methodology (Cochrane Handbook) found no significant increased risk of MCM overall or of cardiac MCM whatever the dose of fluconazole exposure during the first trimester of pregnancy, contrary to previous SR/MA. For other subtypes of MCM mentioned in previous observational studies, the lack of adequate data did not allow us to conduct meta-analyses based on adjusted estimates. Besides, in line with previous studies, the significant association found in the sensitivity analysis for the cumulative dose of fluconazole > 150 mg when we considered studies with a valid definition of MCM deserves attention. The same applies for the significant association found for the risk of miscarriages. Further research is needed to elaborate guidelines and therapeutic strategies, especially since the certainty of evidence in our study ranged from very low to low. Given the wide heterogeneity between studies, this study highlights the importance to use standardised definitions for pregnancy outcomes and when possible consensual and homogeneous exposition definitions to facilitate the comparison across studies and the realization of SR/MA.

Acknowledgment: We would like to thank the authors who were contacted and could help us by providing supplementary data (Teresa Mazzone, Henrik Toft Sørensen, Anick Bérard) as well as David Hajage for statistical expertise.

References

1. Brammer KW, Farrow PR, Faulkner JK. Pharmacokinetics and Tissue Penetration of Fluconazole in Humans. Clinical Infectious Diseases. 1990;12:S318–26.

2. Chaube S, Murphy ML. The teratogenic effects of 5-fluorocytosine in the rat. Cancer Res. 1969 Mar;29(3):554-7. PMID: 5813093.

3. Menegola E, Broccia ML, Di Renzo F, Giavini E. Pathogenic pathways in fluconazole-induced branchial arch malformations. Birth Defects Research Part A: Clinical and Molecular Teratology. 2003;67:116–24.

4. Lopez-Rangel E, Van Allen MI. Prenatal exposure to fluconazole: an identifiable dysmorphic phenotype. Birth Defects Res A Clin Mol Teratol. 2005;73:919–23.

5. Lee BE, Feinberg M, Abraham JJ, Murthy AR. Congenital malformations in an infant born to a woman treated with fluconazole. Pediatr Infect Dis J. 1992;11:1062–4.

6. Aleck KA, Bartley DL. Multiple malformation syndrome following fluconazole use in pregnancy: report of an additional patient. Am J Med Genet. 1997;72:253–6.

7. Pursley TJ, Blomquist IK, Abraham J, Andersen HF, Bartley JA. Fluconazole-induced congenital anomalies in three infants. CLIN INFECT DIS. 1996;22:336–40.

8. Zhu Y, Bateman BT, Gray KJ, Hernandez-Diaz S, Mogun H, Straub L, et al. Oral fluconazole use in the first trimester and risk of congenital malformations: population based cohort study. BMJ. 2020;369:m1494.

9. Zhang J, Li L, Lv Q, Yan L, Wang Y, Jiang Y. The Fungal CYP51s: Their Functions, Structures, Related Drug Resistance, and Inhibitors. Front Microbiol. 2019;10:691.

10. Alsaad AMS, Kaplan YC, Koren G. Exposure to fluconazole and risk of congenital malformations in the offspring: A systematic review and meta-analysis. Reprod Toxicol. 2015;52:78–82.

11. Zhang Z, Zhang X, Zhou Y-Y, Jiang C-M, Jiang H-Y. The safety of oral fluconazole during the first trimester of pregnancy: a systematic review and meta-analysis. BJOG. 2019;126:1546–52.

12. Liu D, Zhang C, Wu L, Zhang L, Zhang L. Fetal outcomes after maternal exposure to oral antifungal agents during pregnancy: A systematic review and meta-analysis. Int J Gynaecol Obstet. 2020;148:6–13.

13. Marin B, Vauzelle C, Coulm B, Beghin D, Latour M, Tubach F, et al. Comment on “Maternal use of fluconazole and congenital malformations in the progeny: A meta-analysis of the literature.” Reprod Toxicol. 2021;104:168.

14. Budani MC, Fensore S, Di Marzio M, Tiboni GM. Maternal use of fluconazole and congenital malformations in the progeny: A meta-analysis of the literature. Reprod Toxicol. 2021;100:42–51.

15. Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors). *Cochrane Handbook for Systematic Reviews of Interventions* version 6.3 (updated February 2022). Cochrane, 2022. Available from www.training.cochrane.org/handbook.

16. Moher D, Liberati A, Tetzlaff J, Altman D, Group The PRISMA. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: the PRISMA Statement. [Internet]. PLoS Med; 2009. Available from: https://doi.org/10.1371/journal. pmed.1000097.

17. Stroup DF. Meta-analysis of Observational Studies in EpidemiologyA Proposal for Reporting. JAMA. 2000;283:2008.

18. Mathilde Latour, Catherine Vauzelle, Elisabeth Elefant, Agnès Dechartres, Benoit Marin, Florence Tubach. Risk of congenital malformation following maternal use of oral fluconazole (low dose/high dose) during the first trimester of pregnancy: a systematic review and meta-analysis. PROSPERO 2021 CRD42021274003 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42021274003.

19. Gordis L., Epidemiology. 4th.ed.Amsterdam: Elsevier;2009.

20. EUROCAT (2022). EUROCAT Guide 1.5: Instruction for the registration of congenital anomalies. EUROCAT Central Registry, University of Ulster.

21. Lefebvre C, Glanville J, Briscoe S, Featherstone R, Littlewood A, Metzendorf M-I, Noel-Storr A, Paynter R, Rader T, Thomas J, Wieland LS. Chapter 4: Searching for and selecting studies. In: Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors). Cochrane Handbook for Systematic Reviews of Interventions version 6.4 (updated October 2023). Cochrane, 2023. Available from www.training.cochrane.org/handbook.

22. Sterne JA, Hernán MA, Reeves BC, Savović J, Berkman ND, Viswanathan M, et al. The Risk Of Bias In Non-randomized Studies – of Interventions (ROBINS-I) assessment tool.

23. Bérard A, Sheehy O, Zhao J-P, Gorgui J, Bernatsky S, de Moura CS, et al. Associations between low- and high-dose oral fluconazole and pregnancy outcomes: 3 nested case-control studies. CMAJ. 2019;191:E179–87.

24. Mølgaard-Nielsen D, Pasternak B, Hviid A. Use of oral fluconazole during pregnancy and the risk of birth defects. N Engl J Med. 2013;369:830–9.

25. Sedgwick P, Relative risks versus odds ratios. BMJ 2014;348:g1407.

26. George A, Stead TS, Ganti L. What's the Risk: Differentiating Risk Ratios, Odds Ratios, and Hazard Ratios? Cureus [Internet]. 2020 [cited 2024 May 27]; Available from: https://www.cureus.com/articles/39455-whats-the-risk-differentiating-risk-ratios-odds-ratios-and-hazard-ratios

27. VanderWeele TJ. Optimal approximate conversions of odds ratios and hazard ratios to risk ratios. Biometrics. 2020;76:746–52.

28. Borenstein M, Hedges L, Higgins J, Rothstein H. Introduction to Meta-analysis. Chichester (UK): John Wiley&Sons. 2008.

29. Higgins JPT, Eldridge S, Li T (editors). Chapter 23: Including variants on randomized trials. Section 23.3.4:How to include multiple groups from one study. In: Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors). Cochrane Handbook for Systematic Reviews of Interventions version 6.3 (updated February 2022). Cochrane, 2022. Available from www.training.cochrane.org/handbook.

30. Core Team. R: A language and environment for statistical computing. [Internet]. Vienna, Austria: R Foundation for Statistical Computing; 2023. Available from: https://www.R-project.org/.

31. Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Alonso-Coello P, et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. BMJ. 2008;336:924–6.

32. McMaster University and Evidence Prime. GRADEpro GDT: GRADEpro Guideline Development Tool. [Internet]. 2022. Available from: gradepro.org.

33. Howley MM, Carter TC, Browne ML, Romitti PA, Cunniff CM, Druschel CM, et al. Fluconazole use and birth defects in the National Birth Defects Prevention Study. Am J Obstet Gynecol. 2016;214:657.e1-9.

34. Jick SS. Pregnancy outcomes after maternal exposure to fluconazole. Pharmacotherapy. 1999;19:221–2.

35. Mastroiacovo P, Mazzone T, Botto LD, Serafini MA, Finardi A, Caramelli L, et al. Prospective assessment of pregnancy outcomes after first-trimester exposure to fluconazole. Am J Obstet Gynecol. 1996;175:1645–50.

36. Dormuth C, Winquist B, Fisher A, Wu F, Reynier P, Dahl M, et al. Use of oral fluconazole during pregnancy and risks of spontaneous abortion, stillbirth and major congenital malformation: An international, multi-centre cohort study. Pharmacoepidemiol Drug Saf. 2019;28:14.

37. Mølgaard-Nielsen D, Svanström H, Melbye M, Hviid A, Pasternak B. Association Between Use of Oral Fluconazole During Pregnancy and Risk of Spontaneous Abortion and Stillbirth. JAMA. 2016;315:58–67.

38. Sorensen HT, Nielsen GL, Olesen C, Larsen H, Steffensen FH, Schønheyder HC, et al. Risk of malformations and other outcomes in children exposed to fluconazole in utero. Br J Clin Pharmacol. 1999;48:234–8.

39. The European Network of Centres for Pharmacoepidemiology and Pharmacovigilance (ENCePP) Guide on Methodological Standards in Pharmacoepidemiology. Annex 2. Guidance on methods for the evaluation of medicines in pregnancy and breastfeeding. Chapter 4. Measuring maternal, pregnancy, and neonatal outcomes. Section 4.2. Safety outcomes. https://www.encepp.eu/standards_and_guidances/methodologicalGuideAnnex2.shtml#nine [accessed on 02.06.2024].

40. Nørgaard M, Pedersen L, Gislum M, Erichsen R, Søgaard KK, Schønheyder HC, et al. Maternal use of fluconazole and risk of congenital malformations: a Danish population-based cohort study. J Antimicrob Chemother. 2008;62:172–6.

Figure legends

Fig. 1 Flow chart eligibility criteria

Fig. 2 Pooled odds ratios (OR) for major congenital malformation associated with exposure to any dose of fluconazole (2a crude OR; 2b adjusted OR)

Fig. 3 Pooled odds ratios (OR) for major congenital malformation associated with exposure to cumulative dose of fluconazole (≤ 150 mg; >150 mg) (3a crude OR; 3b adjusted OR) in the first trimester of pregnancy

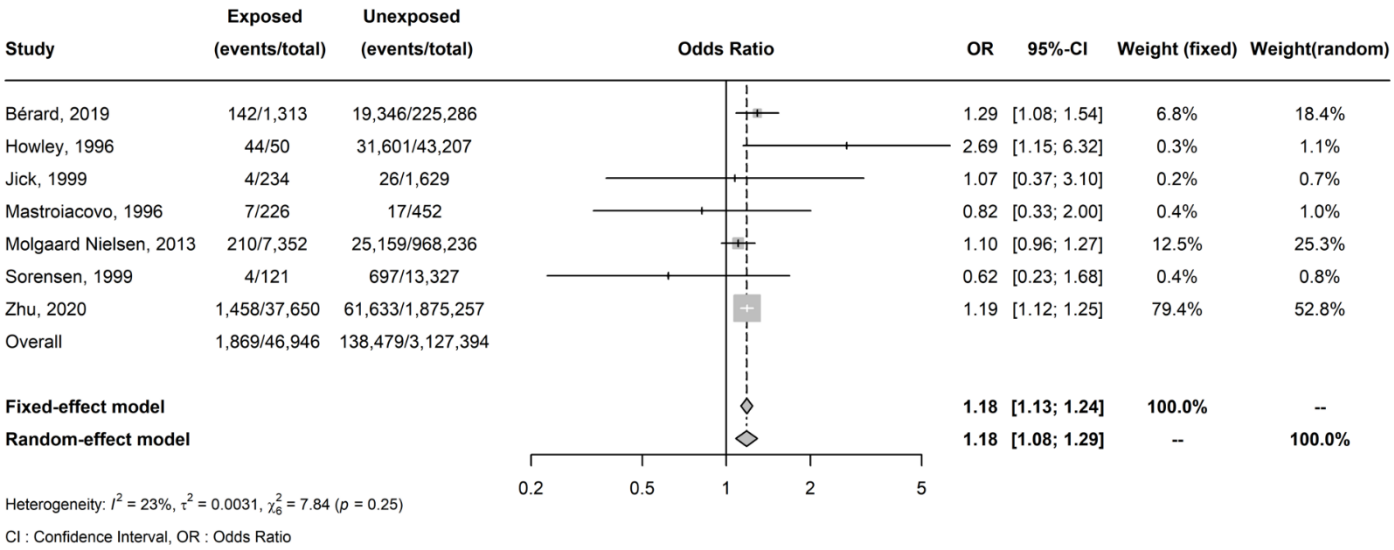
Fig. 4 Pooled odds ratios (OR) for miscarriage associated with exposure to any dose of fluconazole in the first trimester of pregnancy (4a crude OR; 4b adjusted OR)

Table legends

Table 1 Characteristics of included studies

Table 2 Evaluation of the risk of bias of included studies for MCM and miscarriages according to Sterne JA, Hernán MA, Reeves BC, Savović J, Berkman ND, Viswanathan M, et al. The Risk Of Bias In Non-randomized Studies – of Interventions (ROBINS-I) assessment tool

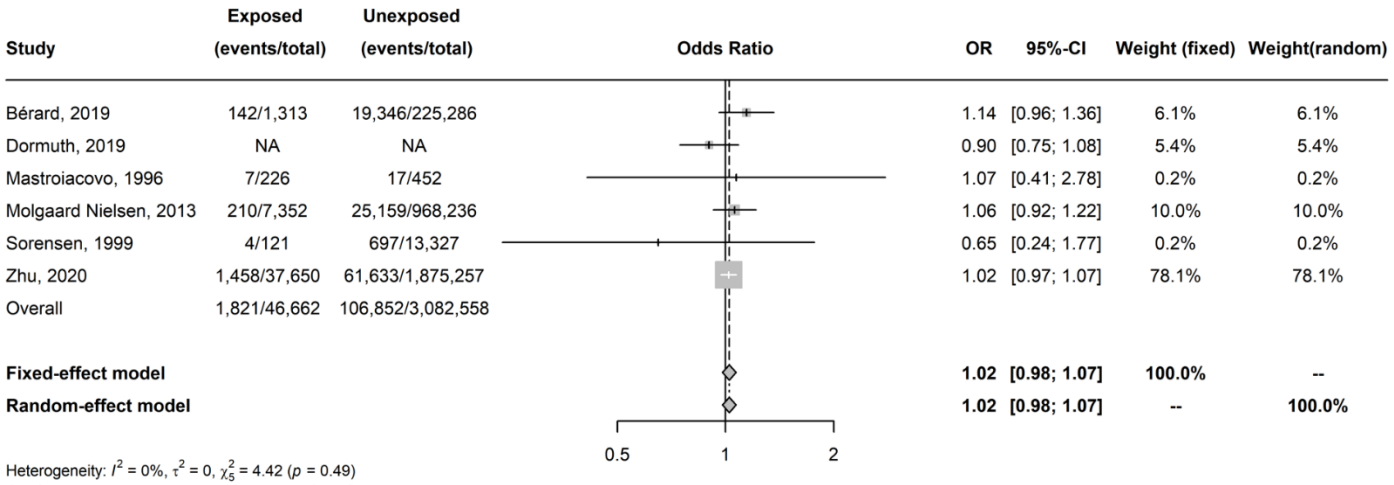
2a: MCM / Any dose (crude OR)



Heterogeneity: I^2 = 23%, τ^2 = 0.0031, χ^2_6 = 7.84 (p = 0.25)

CI : Confidence Interval, OR : Odds Ratio

2b: MCM / Any dose (adjusted OR)



Heterogeneity: I^2 = 0%, τ^2 = 0, χ^2_5 = 4.42 (p = 0.49)

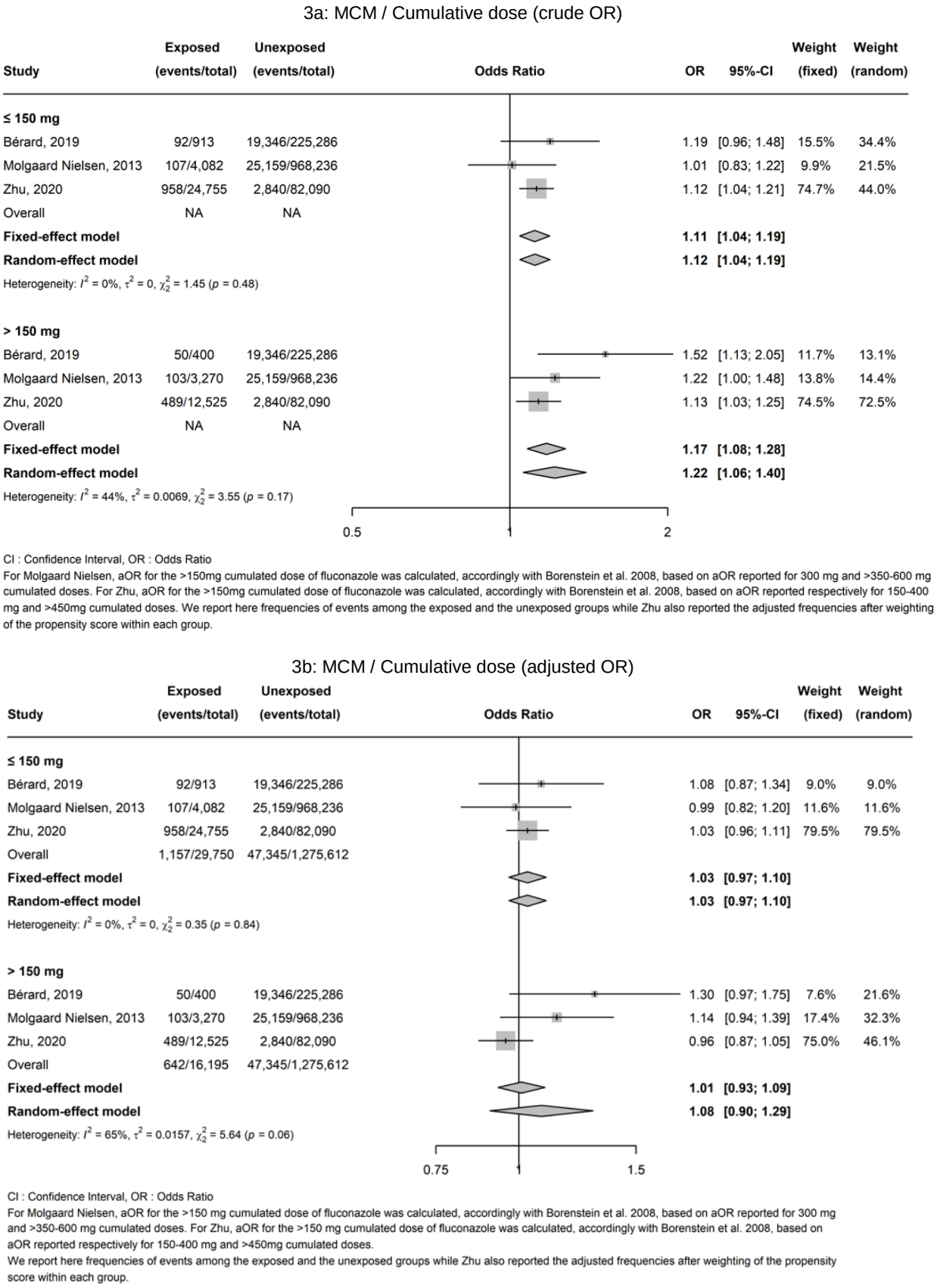
CI : Confidence Interval, NA : not available, OR : Odds Ratio

For Bérard, aOR for any dose of fluconazole was calculated, accordingly with Borenstein et al. 2008, based on aOR reported for ≤150 mg and >150mg cumulated doses.

For Dormuth, the number of events in each group was not reported in the available material but the total number of pregnancies exposed to oral fluconazole was 63,346 and the total number of pregnancies in the control group was 107,212.

We report here frequencies of events among the exposed and the unexposed groups while Zhu also reported the adjusted frequencies after weighting of the propensity score within each group.

Fig. 2 Pooled odds ratios (OR) for major congenital malformation associated with exposure to any dose of fluconazole (2a crude OR; 2b adjusted OR)



CI : Confidence Interval, OR : Odds Ratio

For Molgaard Nielsen, aOR for the >150mg cumulated dose of fluconazole was calculated, accordingly with Borenstein et al. 2008, based on aOR reported for 300 mg and >350-600 mg cumulated doses. For Zhu, aOR for the >150mg cumulated dose of fluconazole was calculated, accordingly with Borenstein et al. 2008, based on aOR reported respectively for 150-400 mg and >450mg cumulated doses. We report here frequencies of events among the exposed and the unexposed groups while Zhu also reported the adjusted frequencies after weighting of the propensity score within each group.

CI : Confidence Interval, OR : Odds Ratio

For Molgaard Nielsen, aOR for the >150 mg cumulated dose of fluconazole was calculated, accordingly with Borenstein et al. 2008, based on aOR reported for 300 mg and >350-600 mg cumulated doses. For Zhu, aOR for the >150 mg cumulated dose of fluconazole was calculated, accordingly with Borenstein et al. 2008, based on aOR reported respectively for 150-400 mg and >450mg cumulated doses.

We report here frequencies of events among the exposed and the unexposed groups while Zhu also reported the adjusted frequencies after weighting of the propensity score within each group.

Fig. 3 Pooled odds ratios (OR) for major congenital malformation associated with exposure to cumulative dose of fluconazole (≤ 150 mg; >150 mg) (3a crude OR; 3b adjusted OR) in the first trimester of pregnancy

Click here to access/download;Figure;Figure 4a 4b.docx

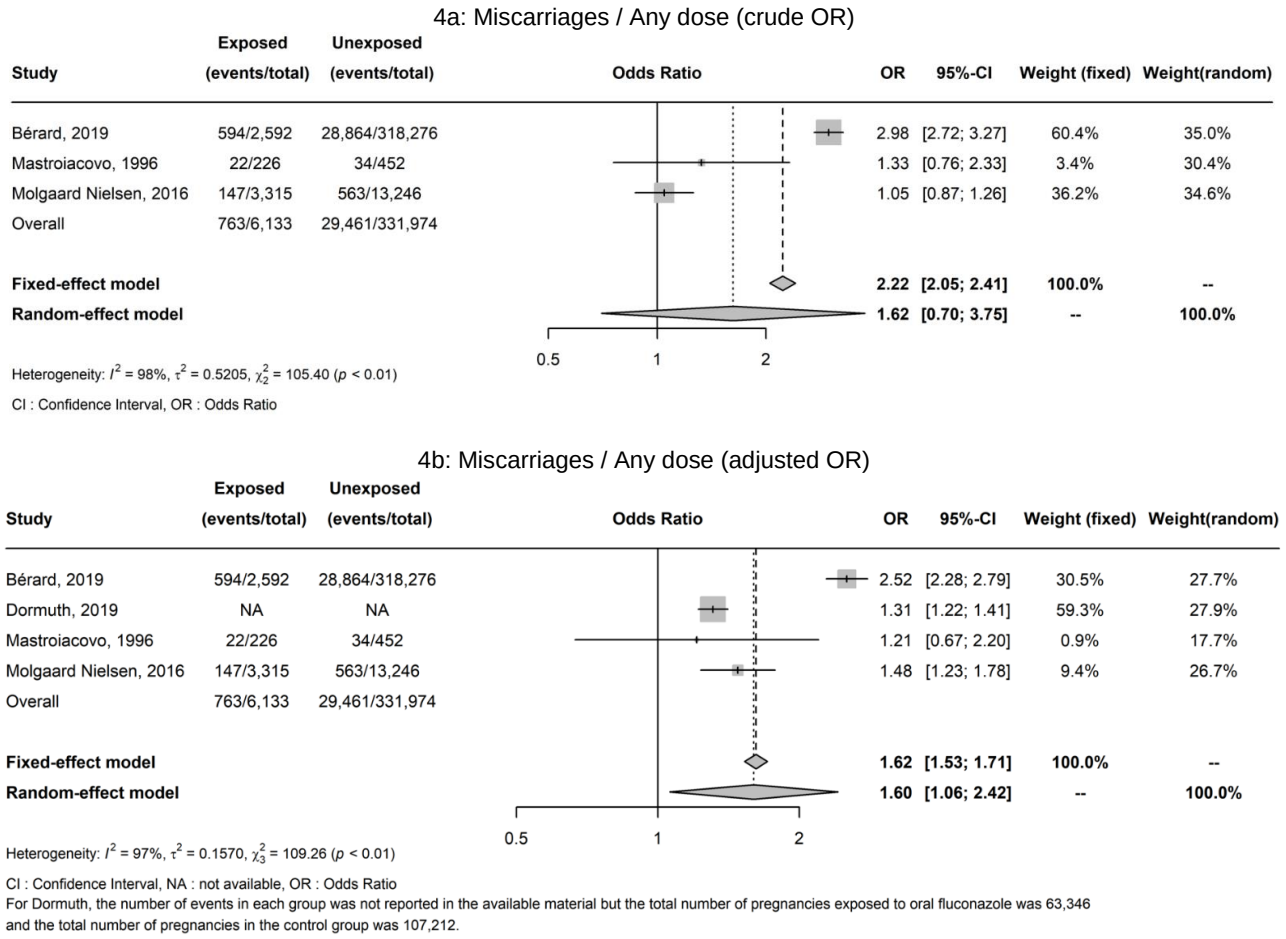


Fig. 4 Pooled odds ratios (OR) for miscarriage associated with exposure to any dose of fluconazole in the first trimester of pregnancy (4a crude OR; 4b adjusted OR)

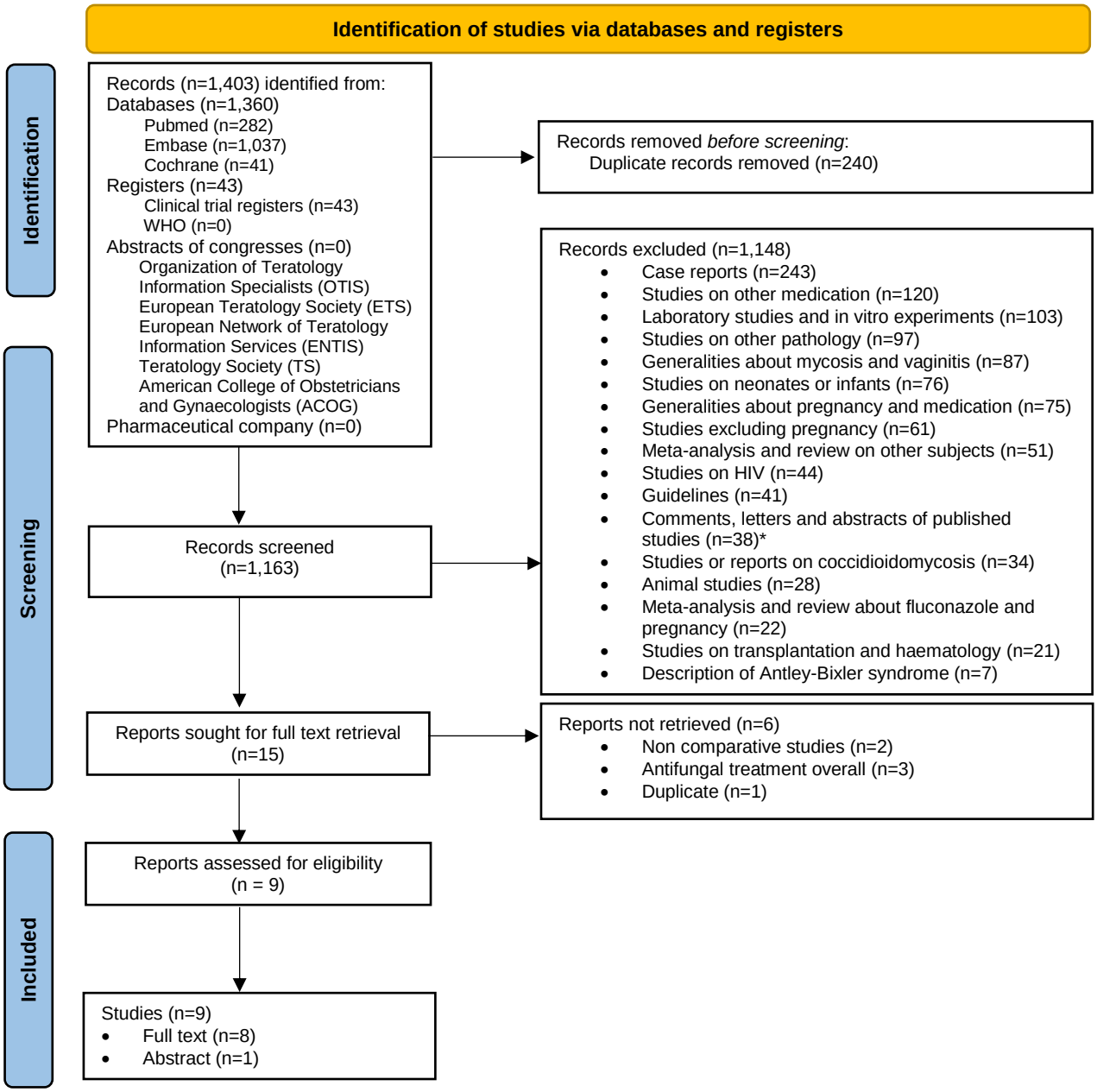


Fig. 1 Flow chart eligibility criteria[1,2]

1. During the eligibility process, three Danish studies were especially examined for a concern of duplication of data [29, 32, 33]. In details, Norgaard et al. [33] was excluded because it was geographically and temporally included in the work of Molgaard-Nielsen et al. [29] (1991–2005 for North Jutland County, 1996– 2005 for Aarhus County and 1998– 2005 for Ringkjøbing and Viborg Counties, Denmark). Besides, Sorensen [32] (01/01/1991-31/12/1996, North Jutland in Denmark) was included because there was only a partial duplication of data as compared to Molgaard-Nielsen et al. paper (only the year 1996 from North Jutland was common to both studies).

2. Two TIS experts who examined the list of the included references did not identify missing studies.

*Latour et al.

Table 1 Characteristics of included studies

First author (year)	Settings (geographical area, period of recruitment)	Study design	Data collection	Population based yes/no	Type of data	Sample size	Type of exposition	Definition of exposition	Outcomes (MCM, malformation or miscarriages)	Definition of outcomes
Bérard (2019) [23]	Quebec 1998-2015	case-control	PRO	yes	MA	Total: 226,599 Cases: 19,488 Controls: 207,111 Total: 320,868 Cases: 29,458 Controls: 291,410	cum	(i) ≤ 150 mg (ii) > 150 mg	MCM Miscarriages	Validated congenital malformation diagnostic codes in ICD-9 and ICD-10 6-20 GW
Sorensen (1999) [38]	North Jutland in Denmark 01/01/1991-31/12/1996	cohort	PRO	yes	MA	Total: 13,348 Exposed: 121 Non exposed: 13,327	any	NA	Malformation	Diagnostic codes in ICD8 and ICD10
Howley (2016) [33]	USA 1997-2011	case-control	RETRO	yes	R	Total: 43,257 Cases: 31,645 Controls: 11,612	any	NA	MCM	List of major defects (those that have surgical, medical, or serious cosmetic

										importance) reviewed by clinician NB: list of minor defects that seemed to be excluded in this study
Jick (1999) [34]	United Kingdom unknown	cohort	PRO	yes	MA	Total: 2,443 Exposed: 234 Non exposed: 1,629 Topical azole: 492 Itraconazole: 88	any	NA	MCM	Malformations present at birth that resulted in surgery or other treatment for functional or cosmetic reasons
Mastroiacovo (1996) [35]	Italy 01/1992 - 06/1994	cohort	PRO	no	C	Total: 678 Exposed: 226 Non exposed: 452	any	NA	MCM Miscarriages	Congenital anomalies that warranted medical or surgical treatment Before 12 GW°
Molgaard Nielsen (2013) [24]	Denmark 01/01/1996-31/03/2011	cohort	PRO	yes	MA	Total: 975,588 Exposed: 7,352 Non exposed: 968,236	any and cum	(i) 150 mg (ii) 300 mg (iii) ≥ 350 mg	MCM	EUROCAT / codes according to ICD-10

Molgaard Nielsen (2016) [37]	Denmark 01/01/1997-31/12/2013	cohort	PRO	yes	MA	Total: 16,561 Exposed: 3,315 Non exposed: 13,246	any and cum	(i) 150-300 mg (ii) ≥ 350 mg	Miscarriages	7-22 GW
Zhu (2020) [8]	USA 2000-2014	cohort	PRO	yes	MA	Total: 1,994,997 Exposed: 37,650 Non exposed: 1,875,257 Topical azole: 82,090	any and cum	(i) 150 mg (ii) 150-450 mg (iii) >450 mg	Malformation	Validated highly specific algorithms based on codes from ICD-9
Dormuth (2019) [36]	5 Canadian provinces, USA, United Kingdom 04/2002-03/2016	cohort	PRO	yes	MA	Total: 170,558 Exposed: 63,346 Non exposed: 107,212	any	NA	Malformation Miscarriages	NA

Legend: any = any dose; any and cum = any dose and cumulative dose; C = clinical data (medical chart); cum = cumulative dose; GW = gestational week; MA = medico-administrative data; MCM = major congenital malformation; NA= not available; PRO = prospective collection of data; R = register; RETRO = study based on previously collected data; USA = United State of America; °information provided by the authors

Table 2 Evaluation of the risk of bias of included studies for MCM and miscarriages according to Sterne JA, Hernán MA, Reeves BC, Savović J, Berkman ND, Viswanathan M, et al. The Risk Of Bias In Non-randomized Studies – of Interventions (ROBINS-I) assessment tool

Type of risk of bias	Bérard (2019)	Sorensen (1999)	Howley (2016)	Jick (1999)	Mastroiacovo (1996)	Mastroiacovo (1996)	Molgaard-Nielsen (2013)	Molgaard-Nielsen (2016)	Zhu (2020)	Dormuth (2019)
Outcomes considered	**MCM and miscarriages**	**MCM**	**MCM**	**MCM**	**MCM**	**Miscarriages**	**MCM**	**Miscarriages**	**MCM**	**MCM and miscarriages**
Confounding	serious	serious	serious	serious	serious	serious	serious	serious	serious	NI
Selection	low	low	low	-	low	low	low	low	low	NI
Classification of intervention	low	low	serious	low	low	low	low	low	low	NI
Deviation from intended intervention	-	-	-	-	-	-	-	-	-	-
Missing data	low	NI	moderate	NI	moderate	moderate	low	low	low	NI
Measurement of outcome	low	low	serious	low	moderate	low	low	low	low	NI
Selection of the reported result	-	-	-	-	-	-	-	-	-	-
CONCLUSION	**serious**	**serious**	**serious**	**serious**	**serious**	**serious**	**serious**	**serious**	**serious**	**NI**

Legend: MCM=major congenital malformation.
Of the four studies evaluating the risk of MCM and the risk of miscarriages associated with oral fluconazole exposure during the first trimester of pregnancy, only the study of Mastroiacovo et al. presented a different assessment of the risk of bias for the two outcomes (moderate for miscarriages and serious for malformations). For the other three studies (Bérard et al., Molgaard-Nielsen et al., Dormuth et al.) the assessment of the risk of bias was identical for the two outcomes.

Table 1 Characteristics of included studies

First author (year)	Settings (geographical area, period of recruitment)	Study design	Data collection	Population based yes/no	Type of data	Sample size	Type of exposition	Definition of exposition	Outcomes (MCM, malformation or miscarriages)	Definition of outcomes
Bérard (2019) [239]	Quebec 1998-2015	case-control	PRO	yes	MA	Total: 226,599 Cases: 19,488 Controls: 207,111 Total: 320,868 Cases: 29,458 Controls: 291,410	cum	(i) ≤ 150 mg (ii) > 150 mg	MCM Miscarriages	Validated congenital malformation diagnostic codes in ICD-9 and ICD-10 6-20 GW
Sorensen (1999) [3638]	North Jutland in Denmark 01/01/1991-31/12/1996	cohort	PRO	yes	MA	Total: 13,348 Exposed: 121 Non exposed: 13,327	any	NA	Malformation	Diagnostic codes in ICD8 and ICD10
Howley (2016) [330]	USA 1997-2011	case-control	RETRO	yes	R	Total: 43,257 Cases: 31,645 Controls: 11,612	any	NA	MCM	List of major defects (those that have surgical, medical, or serious cosmetic

										importance) reviewed by clinician NB: list of minor defects that seemed to be excluded in this study
Jick (1999) [~~31~~34]	United Kingdom unknown	cohort	PRO	yes	MA	Total: 2,443 Exposed: 234 Non exposed: 1,629 Topical azole: 492 Itraconazole: 88	any	NA	MCM	Malformations present at birth that resulted in surgery or other treatment for functional or cosmetic reasons
Mastroiacovo (1996) [~~32~~35]	Italy 01/1992 - 06/1994	cohort	PRO	no	C	Total: 678 Exposed: 226 Non exposed: 452	any	NA	MCM Miscarriages	Congenital anomalies that warranted medical or surgical treatment Before 12 GW°
Molgaard Nielsen (2013) [~~33~~24]	Denmark 01/01/1996-31/03/2011	cohort	PRO	yes	MA	Total: 975,588 Exposed: 7,352 Non exposed: 968,236	any and cum	(i) 150 mg (ii) 300 mg (iii) ≥ 350 mg	MCM	EUROCAT / codes according to ICD-10

Molgaard Nielsen (2016) [3537]	Denmark 01/01/1997-31/12/2013	cohort	PRO	yes	MA	Total: 16,561 Exposed: 3,315 Non exposed: 13,246	any and cum	(i) 150-300 mg (ii) ≥ 350 mg	Miscarriages	7-22 GW
Zhu (2020) [8]	USA 2000-2014	cohort	PRO	yes	MA	Total: 1,994,997 Exposed: 37,650 Non exposed: 1,875,257 Topical azole: 82,090	any and cum	(i) 150 mg (ii) 150-450 mg (iii) >450 mg	Malformation	Validated highly specific algorithms based on codes from ICD-9
Dormuth (2019) [3436]	5 Canadian provinces, USA, United Kingdom 04/2002-03/2016	cohort	PRO	yes	MA	Total: 170,558 Exposed: 63,346 Non exposed: 107,212	any	NA	Malformation Miscarriages	NA

Legend: any = any dose; any and cum = any dose and cumulative dose; C = clinical data (medical chart); cum = cumulative dose; GW = gestational week; MA = medico-administrative data; MCM = major congenital malformation; NA= not available; PRO = prospective collection of data; R = register; RETRO = study based on previously collected data; USA = United State of America; °information provided by the authors

Table 2 Evaluation of the risk of bias of included studies for MCM and miscarriages according to Sterne JA, Hernán MA, Reeves BC, Savović J, Berkman ND, Viswanathan M, et al. The Risk Of Bias In Non-randomized Studies – of Interventions (ROBINS-I) assessment tool

Type of risk of bias	Bérard (2019)	Sorensen (1999)	Howley (2016)	Jick (1999)	Mastroiacovo (1996)	Mastroiacovo (1996)	Molgaard-Nielsen (2013)	Molgaard-Nielsen (2016)	Zhu (2020)	Dormuth (2019)
Outcomes considered	**MCM and miscarriages**	**MCM**	**MCM**	**MCM**	**MCM**	**Miscarriages**	**MCM**	**Miscarriages**	**MCM**	**MCM and miscarriages**
Confounding	serious	serious	serious	serious	serious	serious~~moderate~~	serious	serious	serious	NI
Selection	low	low	low	-	low	low	low	low	low	NI
Classification of intervention	low	low	serious	low	low	low	low	low	low	NI
Deviation from intended intervention	-	-	-	-	-	-	-	-	-	-
Missing data	low	NI	moderate	NI	moderate	moderate	low	low	low	NI
Measurement of outcome	low	low	serious	low	moderate	low	low	low	low	NI
Selection of the reported result	-	-	-	-	-	-	-	-	-	-
CONCLUSION	**serious**	**serious**	**serious**	**serious**	**serious**	**serious~~moderate~~**	**serious**	**serious**	**serious**	**NI**

Legend: MCM=major congenital malformation.

Of the four studies evaluating the risk of MCM and the risk of miscarriages associated with oral fluconazole exposure during the first trimester of pregnancy, only the study of Mastroiacovo et al. presented a different assessment of the risk of bias for the two outcomes (moderate for miscarriages and serious for malformations). For the other three studies (Bérard et al., Molgaard-Nielsen et al., Dormuth et al.) the assessment of the risk of bias was identical for the two outcomes.

Supplementary material – Online Resource

eTables

Figures

eTables

eTable 1a Characteristics of the included studies evaluating the risk of congenital malformation and the use of fluconazole in early pregnancy

First author (year) and settings (geographical area, period of recruitment)	Study design	Data collection	Population based yes/no	Type of data	Precision about data source	Sample size	MCM definition	Definition of malformation	MCM analyses based on…	Exclusion	Type of exposition	Definition of exposition	Distribution of exposition or indication	Reference group	Results reported in the study
Bérard (2019) Quebec 1998-2015 [23]	case-control	PRO	yes	MA	Quebec Prescription Drug Insurance Quebec Pregnancy Cohort RAMQ (medical database) MED-ECHO (hospitalization archive database) Quebec Statistics Database	Total: 226,599 Cases: 19,488 Controls: 207,111	accurate	Validated congenital malformation diagnostic codes in ICD-9 and ICD-10	Singleton live births during the first 6 months of life	mcm alone, chromosomal abnormalities, exposure to known teratogens during the first trimester (0–14 GW)	cum	(i) ≤ 150 mg (ii) > 150 mg	n=913 exposed to ≤ 150 mg and n=400 to > 150 mg	no fluconazole	≤ 150 mg : cOR 1.19 (0.96–1.48) aOR 1.08 (0.87–1.34) > 150 mg : cOR 1.51 (1.12–2.03) aOR 1.30 (0.97–1.75)
Sorensen (1999) North Jutland in Denmark 01/01/1991-31/12/1996 [38]	cohort	PRO	yes	MA	The North Jutland Pharmaco-Epidemiological Prescription Database Danish Medical Birth Registry Regional Hospital Discharge Registry	Total: 13,348 Exposed: 121 Non exposed: 13,327	imprecise	Diagnostic codes in ICD8 and ICD10	Live births	NA	any	NA	NA	no reimbursed treatment	cRR 0.62 (0.23-1.68) aRR 0.65 (0.24-1.77)
Howley (2016) USA 1997-2011 [33]	case-control	RETRO	yes	R	Register of the National Birth Defect Prevention Study (birth defect surveillance program in 10 States)	Total: 43,257 Cases: 31,645 Controls: 11,612	accurate	List of major defects (those that have surgical, medical, or serious cosmetic	Live births	malformations attributed to a known chromosomal or single-gene abnormality	any	NA	72% for vulvovaginal candidiasis and 98% for a short period of time (less than 1 week)	no fluconazole	/

							importance) reviewed by clinicians NB: list of minor defects that seemed to be excluded in this study							
Jick (1999) United Kingdom Unknown [34]	cohort	PRO	yes	MA	The United Kingdom based General Practice Research (medical and administrative database recorder by general practitioners)	Total: 2,443 Exposed: 234 Non exposed: 1,629 Topical azole: 492 Itraconazole: 88	approximate	Malformations present at birth that resulted in surgery or other treatment for functional or cosmetic reasons	NA	NA	any	NA	92% received the single 150-mg dose preparation	(i) no fluconazole (ii) topical (iii) itraconazole	cRR 1.1 (0.4-3.3)
Mastroiacovo (1996) Italy 01/1992 - 06/1994 [35]	cohort	PRO	no	C	3 Italian Teratology Information Services	Total: 678 Exposed: 226 Non exposed: 452	approximate	Congenital anomalies that warranted medical or surgical treatment	Live births and medical termination of pregnancy for malformation	NA	any	NA	150 mg: 46.5% single dose and 35.8% multiple dose 50 mg: n=3 single dose and n=23 multiple dose 100 mg: n=5 single dose and n=9 multiple dose The total dose exposure was in the range of 100 to	no fluconazole and no teratogenic treatment	aOR 1.07 (0.41-2.77)

													2100 mg, with a median of 200 mg (interquartile range 150 to 300 mg)	
Molgaard Nielsen (2013) Denmark 01/01/1996-31/03/2011 [24]	cohort	PRO	yes	MA	The National Prescription Registry The National Patient Register The Danish Civil Registration System Statistics Denmark The Medical Birth Registry	Total: 975,588 Exposed: 7,352 Non exposed: 968,236	accurate	EUROCAT / codes according to ICD-10	Live births during the first year of life in the primary analysis, live births and medical termination of pregnancy in the secondary analysis	minor malformations, chromosomal aberrations, genetic syndromes, birth-defect syndromes with known causes, congenital viral infections associated with malformation	any and cum	(i) 150 mg (ii) 300 mg (iii) ≥ 350 mg	150 mg: 56% 300 mg: 31% 350–6000 mg: 14% (mean dose: 722±689 mg) 90.7% vaginal candidiasis	no azole	Any dose: cOR 1.10 (0.96-1.27) aOR 1.06 (0.92-1.21) 150 mg: cOR 1.01 (0.83-1.22) aOR 0.99 (0.82-1.20) 300 mg: cOR 1.22 (0.96-1.55) aOR 1.15 (0.91-1.46) ≥ 350 mg: cOR 1.22 (0.86-1.73) aOR 1.12 (0.79-1.59)
Zhu (2020) USA 2000-2014 [8]	cohort	PRO	yes	MA	Medicaid analytic eXtract (clinical chart and socio-economic data, drug delivery data)	Total: 1,994,997 Exposed: 37,650 Non exposed: 1,875,257 Topical: 82,090	imprecise	Validated highly specific algorithms based on codes from ICD-9-CM	Live births during the first 3 months of life	chromosomal abnormality or exposure to a known teratogenic drug during the first trimester; fungal infection between 90 days before the last menstrual	any and cum	(i) 150 mg (ii) >150-450 mg (iii) >450 mg	65.8% cumulative dose of 150 mg, 27.7% more than 150 mg up to 450 mg, and 5.6% more than 450 mg (highest cumulative dose included: 6000 mg)	(i) no azole (ii) topical	cRR 1.18 (1.12 1.24) aRR 1.02 (0.97 1.07)

										period (baseline) and the end of the first trimester, diagnoses of oropharyngeal or oesophageal candidiasis, cryptococcal meningitis, or systemic candidiasis during the baseline and first trimester; and diagnoses of HIV infection, malignancy, or transplant during the baseline and first trimester		Indications: vaginal candidiasis 19,4% ; UTI 26,3% ; other superficial/mucosal candidiasis 0,2% ; other candidiasis 1,7% ; other fungal infection 1% ; other non-fungal infection 28,2%.			
Dormuth (2019) Canada, USA, United Kingdom 04/2002-03/2016 [36]	cohort	PRO	yes	MA	Administrative data from 5 Canadian provinces US MarketScan UK Clinical Practice Research Datalink	Total: 170,558 Exposed: 63,346 Non exposed: 107,212	NA	NA	NA	NA	any	NA	20% received a cumulative dose >300 mg during pregnancy	topical azole	aOR 0,90 (0,75-1,09)

Legend: any = any dose; any and cum = any and cumulative dose; C = clinical data (medical chart); cum = cumulative dose; MA = medico-administrative; MCM = major congenital malformations; NA = not available; PRO = prospective collection of data; R = register; RETRO = study based on previously collected data; USA = United State of America; aOR = adjusted Odds Ratio, cOR = crude Odds Ratio, aRR = adjusted Relative Risk, cRR = crude Relative Risk

eTable 1b Characteristics of the included studies evaluating the risk of miscarriages and the use of fluconazole in early pregnancy

First author (year) and settings (geographical area, period of recruitment)	Study design	Data collection	Population based yes/no	Type of data	Precision about data source	Sample size	Term of miscarriages	Analyses based on…	Exclusion	Type of exposition	Definition of exposition	Distribution of exposition or indication	Reference group	Results reported in the study
Bérard (2019) Quebec 1998-2015 [23]	case-control	PRO	yes	MA	Quebec Prescription Drug Insurance Quebec Pregnancy Cohort RAMQ (medical database) MED-ECHO (hospitalization archive database) Quebec Statistics Database	Total: 320,868 Cases: 29,458 Controls: 291,410	6-20 GW	All pregnancies	induced abortions; exposure to known teratogens during the first trimester (0–14 GW)	cum	(i) ≤ 150 mg (ii) > 150 mg	0.53% exposed to ≤ 150 mg and 0.28% exposed to > 150 mg	no fluconazole	≤ 150 mg : cOR 2.51 (2.21–2.85) aOR 2.23 (1.96–2.54) > 150 mg : cOR 3.91 (3.26–4.45) aOR 3.20 (2.73–3.75)
Mastroiacovo (1996) Italy 01/1992 - 06/1994 [35]	cohort	PRO	no	C	3 Italian Teratology Information Services	Total: 678 Exposed: 226 Non exposed: 452	Before 12 GW*	NA	NA	any	NA	150 mg: 46,5% single dose and 35,8% multiple dose 50 mg: n=3 single dose and n= 23 multiple dose 100 mg: n=5 single dose and n=9 multiple dose The total dose exposure was in the	no fluconazole and no teratogenic treatment	aOR 1.21 (0.67-2.21)

												range of 100 to 2100 mg, with a median of 200 mg (interquartile range 150 to 300 mg)		
Molgaard Nielsen (2016) Denmark 01/01/1997-31/12/2013 [37]	cohort	PRO	yes	MA	The Medical Birth Register The National Patient Register The National Prescription Register The Central Person Register Statistics Denmark	Total: 16,561 Exposed: 3,315 Non exposed: 13,246	7-22 GW	NA	Pregnancies with a missing or implausible gestational age and pregnancies with multiple records on overlapping dates	any and cum	(i) 150-300 mg (ii) ≥ 350 mg	85,8% cumulative dose 150-300 mg	no azole	Any dose: cHR 1.48 (1.23-1.77) 150-300 mg: cHR 1.47 (1.22-1.77) ≥ 350 mg: cHR 1.55 (0.94-2.58)
Dormuth (2019) Canada, USA, United Kingdom 04/2002-03/2016 [36]	cohort	PRO	yes	MA	Administrative data from 5 Canadian provinces US MarketScan UK Clinical Practice Research Datalink	Total: 170,558 Exposed: 63,346 Non exposed: 107,212	NA	NA	NA	any	NA	20% received a cumulative dose >300 mg	topical azole	aHR 1.31 (1.22-1.41)

Legend: any = any dose; any and cum = any and cumulative dose; C = clinical (medical chart); cum = cumulative dose; GW = gestational week; MA = medico-administrative data; NA = not available; PRO = prospective collection of data; USA = United State of America; *information provided by the author; cOR = crude Odds Ratio, aOR = adjusted Odds Ratio, cHR = crude Hazard Ratio, aHR = adjusted Hazard Ratio

eTable 2a Any congenital malformation by validity of MCM definition

Occurrence of malformations	Definition of MCM	Bérard (2019)	Sorensen (1999)	Howley (2016)	Jick (1999)	Mastroiacovo (1996)	Molgaard Nielsen (2013)	Zhu (2020)	Dormuth (2019)
3	accurate	x	o	x	o	o	x	o	o
2	approximate	o	o	o	x	x	o	o	o
3	imprecise	o	x	o	o	o	o	x	x

Legend: o red = no, x green = yes; MCM: major congenital malformation

eTable 2b Congenital malformation with only one occurrence in the included studies

Occurrence of malformations	Type of malformation	Bérard (2019)	Sorensen (1999)	Howley (2016)	Jick (1999)	Mastroiacovo (1996)	Molgaard Nielsen (2013)	Zhu (2020)	Dormuth (2019)
1	Spina bifida	o	o	o	x	o	o	o	o
1	Middle ear defect	o	o	o	o	o	x	o	o
1	Conotroncal	o	o	o	o	o	o	x	o
1	Pulmonary valve stenosis	o	o	x	o	o	o	o	o
1	Atrial septal defect	o	o	x	o	o	o	o	o
1	Pulmonary artery hypoplasia	o	o	o	o	o	x	o	o
1	Ventricular septal defects	o	o	o	o	o	x	o	o
1	Hypoplastic left heart	o	o	o	o	o	x	o	o
1	Diaphragmatic hernia	o	o	o	o	o	x	o	o
1	Hypospadias	o	o	x	o	o	o	o	o
1	Cleft lip or palate	o	o	o	x	o	o	o	o
1	Polydactyly	o	o	o	o	o	x	o	o
1	Syndactyly	o	o	o	o	o	x	o	o
1	Other limb disorders (not fingers)	o	o	o	x	o	o	o	o
1	Limb defect total	o	o	o	o	o	x	o	o
1	Limb reduction defect	o	o	o	o	o	x	o	o
1	Limb defect without limb deformities	o	o	o	o	o	o	x	o
1	Other cranial defect (not clefts)	o	o	o	o	o	x	o	o
1	Craniosynostosis	o	o	o	o	o	x	o	o
1	Musculoskeletal alone	o	o	o	o	o	o	x	o

Legend: o red = no, x green = yes

eTable 2c Congenital malformation with more than one occurrence in the included studies

Occurrence of malformations	Type of malformation	Bérard (2019)	Sorensen (1999)	Howley (2016)	Jick (1999)	Mastroiacovo (1996)	Molgaard Nielsen (2013)	Zhu (2020)	Dormuth (2019)
2	Nervous	x	o	o	o	o	o	x	o
2	Eyes	x	o	o	o	o	o	x	o
4	Cardiac	x	o	o	x	o	x	x	o
3	Tetralogy of Fallot	o	o	x	o	o	x	x	o
2	TGV (including dTGA)	o	o	x	o	o	o	x	o
2	Digestive	x	o	o	o	o	o	x	o
2	Genital	x	o	o	o	o	o	x	o
2	Urinary	x	o	o	o	o	o	x	o
3	Genito-urinary	x	o	o	x	o	o	x	o
2	Cleft lip	o	o	x	o	o	o	x	o
3	Cleft palate	o	o	x	o	o	x	x	o
2	Cleft lip with palate	o	o	x	o	o	o	x	o
3	Cleft lip and/or palate	o	o	x	o	o	x	x	o
2	Poly or syndactyly	o	o	o	x	o	x	o	o
3	Musculoskeletal overall	x	o	o	o	o	x	x	o

Legend: o red = no, x green = yes

eTable 3a Control for confounding in the studies evaluating the risk of congenital malformation

First author (year)	Control for confounding	Maternal age	Weight	Diabetes	Personal or familial history of malformation	Smoking	Alcohol consumption	Exposure to other medication	Exposure to other drugs	Comorbidities	Socio-economic status	Paternal status (age, exposure to medication or drug, comorbidities)	Other
Bérard (2019)	adjusting for potential confounders	yes	yes but*	yes but#	no	yes but~	yes but~	yes	no	yes	yes	no	parity, medical visit
Sorensen (1999)	adjusting for potential confounders	yes	no	no	no	yes	no	no	no	no	no	no	no
Howley (2016)	adjusting for potential confounders	yes	no	no	no	yes but^	no	no	no	no	no	no	ethnicity
Jick (1999)	not adjusting for potential confounders; some groups were matched	yes	no	no	no	no	no	no	no	no	no	no	no
Mastroiacovo (1996)	adjusting for potential confounders	yes	no	no	yes	yes	yes	no	no	no	yes	no	parity, history of pregnancy loss, gestational age at inclusion
Molgaard Nielsen (2013)	adjusting for potential confounders	yes	no	yes	yes	yes	no	yes	no	yes	yes	no	Parity, HIV before or during the first trimester, other STI/infections during the first trimester
Zhu (2020)	stratification of the propensity score	yes	yes but§	yes	no	yes	yes but§	yes	yes but§	yes	no	no	ethnicity, region, year of delivery, parity, vaginal candidiasis, urinary tract infection, other candidiasis, medical visit
Dormuth (2019)	adjusting for potential confounders without precision	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Legend: MCM = major congenital malformations; NA = not available; USA = United State of America; *only obesity, #only 6 months before conception, ~only dependence, ^before conception and during the first trimester, §not well defined or poorly measured according to the authors

eTable 3b Control for confounding in the studies evaluating the risk of miscarriage

First author (year)	Control for confounding	Maternal age	Weight	Diabetes	Personal or familial history of spontaneous abortion	Smoking	Alcohol consumption	Exposure to other medication	Exposure to other drugs	Comorbidities	Socio-economic status	Paternal status (age, exposure to medication or drug, comorbidities)	Other
Bérard (2019)	adjusting for potential confounders	yes	yes but*	yes but#	no	yes but~	yes but~	yes	no	yes	yes	no	parity, medical visit
Mastroiacovo (1996)	adjusting for potential confounders	yes	no	no	yes	yes	yes	no	no	yes but§	yes	no	parity,gestational age at inclusion
Molgaard Nielsen (2016)	matched groups with a propensity score	yes	no	yes	yes	no	no	yes but%	no	yes but°	yes	no	Parity, gestational age
Dormuth (2019)	adjusting for potential confounders without precision	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Legend: NA = not available; *only obesity; #only 6 months before conception; ~only dependence; °HIV, other immunosuppression, hospitalizations; § is part of the interview but does not appear in statistical analyses or results; %only anti-infective and anti-hypertensive medication

eTable 4 GRADE assessment for associations between oral fluconazole during the first trimester of pregnancy and the risk of (i) overall MCM (ii) miscarriages (iii) subtypes of MCM

Outcomes № of participants (studies)	Type of estimates	Relative effect (95% CI)	**Anticipated absolute effects (95% CI)**			Certainty	What happens
			Not exposed	Fluconazole exposed	**Difference**		
Overall MCM (any fluconazole exposure) № of participants: 3,174,340 (7 observational studies)	crude	**OR 1.18** (1.08 to 1.29)	4.4%	**5.2%** (4.8 to 5.6)	**0.8% more** (0,3 more to 1,2 more)	⊕◯◯◯ Very low	The evidence suggests that any fluconazole in utero exposure results in a slight increase in overall MCM.
Overall MCM (any fluconazole exposure) № of participants: 3,129,220 (6 observational studies)	adjusted	**OR 1.02** (0.98 to 1.07)	3.5%	**3.5%** (3.4 to 3.7)	**0.1% more** (0,1 fewer to 0,2 more)	⊕⊕◯◯ Low	Any fluconazole in utero exposure probably does not increase overall MCM.
Overall MCM (cumulative fluconazole exposure)							

≤ 150 mg № of participants: 1,305,362 (3 observational studies)	crude	**OR 1.12** (1.04 to 1.19)	3.7%	**4.1%** (3.9 to 4.4)	**0.4% more** (0,1 more to 0,7 more)	⊕◯◯◯ Very low	The evidence suggests that fluconazole in utero exposure with cumulative dose ≤ 150 mg results in a slight increase in overall MCM.
>150 mg № of participants: 1,291,807 (3 observational studies)	crude	**OR 1.22** (1.06 to 1.40)	3.7%	**4.5%** (3.9 to 5.1)	**0.8% more** (0,2 more to 1,4 more)	⊕◯◯◯ Very low	The evidence suggests that fluconazole in utero exposure with cumulative dose > 150 mg results in a slight increase in overall MCM.
Overall MCM (cumulative fluconazole exposure)							
≤ 150 mg № of participants: 1,305,362 (3 observational studies)	adjusted	**OR 1.03** (0.97 to 1.10)	3.7%	**3.8%** (3.6 to 4.1)	**0.1% more** (0,1 fewer to 0,4 more)	⊕⊕◯◯ Low	≤ 150 mg fluconazole in utero exposure probably does not increase overall MCM.

>150 mg № of participants: 1,291,807 (3 observational studies)	adjusted	**OR 1.08** (0.90 to 1.29)	3.7%	**4.0%** (3.4 to 4.7)	**0.3% more** (0,4 fewer to 1 more)	⊕◯◯◯ Very low	-
Miscarriage (any fluconazole exposure) № of participants: 338,107 (3 observational studies)	crude	**OR 1.62** (0.70 to 3.75)	8.9%	**13.6%** (6.4 to 26.8)	**4.8% more** (2,5 fewer to 17,9 more)	⊕◯◯◯ Very low	-
Miscarriage (any fluconazole exposure) № of participants: 338,107 (4 observational studies)	adjusted	**OR 1.60** (1.06 to 2.42)	8.9%	**13.5%** (9.4 to 19.1)	**4.6% more** (0,5 more to 10,2 more)	⊕◯◯◯ Very low	The evidence is very uncertain about the effect of any fluconazole in utero exposure on miscarriage.
Cardiac MCM (any fluconazole exposure) № of participants: 3,116,957 (4 observational studies)	crude	**OR 1.24** (1.14 to 1.36)	0.9%	**1.1%** (1 to 1.2)	**0.2% more** (0,1 more to 0,3 more)	⊕◯◯◯ Very low	The evidence suggests that any fluconazole in utero exposure results in a slight increase in cardiac MCM.

Cardiac MCM (any fluconazole exposure) № of participants: 3,115,094 (3 observational studies)	adjusted	**OR 1.06** (0.97 to 1.16)	0.9%	**0.9%** (0.9 to 1)	**0.1% more** (0 fewer to 0,1 more)	⊕⊕◯◯ Low	Any fluconazole in utero exposure probably does not increase cardiac MCM.
Cardiac MCM (cumulative fluconazole exposure)							
≤ 150 mg № of participants: 1,305,362 (3 observational studies)	crude	**OR 1.17** (1.04 to 1.33)	0.9%	**1.1%** (1 to 1.2)	**0.2% more** (0 fewer to 0,3 more)	⊕◯◯◯ Very low	The evidence suggests that fluconazole in utero exposure with cumulative dose ≤ 150 mg results in a slight increase in cardiac MCM.
>150mg № of participants: 1,291,807 (3 observational studies)	crude	**OR 1.35** (0.98 to 1.87)	0.9%	**1.3%** (0.9 to 1.7)	**0.3% more** (0 fewer to 0,8 more)	⊕◯◯◯ Very low	-
Cardiac MCM (cumulative fluconazole exposure)							

≤ 150 mg № of participants: 1,305,362 (3 observational studies)	adjusted	**OR 1.07** (0.95 to 1.22)	0.9%	**1.0%** (0.9 to 1.1)	**0.1% more** (0 fewer to 0,2 more)	⊕⊕◯◯ Low	≤ 150 mg fluconazole in utero exposure probably does not increase cardiac MCM.
> 150mg № of participants: 1,291,807 (3 observational studies)	adjusted	**OR 1.12** (0.80 to 1.56)	0.9%	**1.0%** (0.7 to 1.4)	**0.1% more** (0,2 fewer to 0,5 more)	⊕◯◯◯ Very low	-
Genito-urinary MCM (any fluconazole exposure) № of participants: 2,141,369 (3 observational studies)	crude	**OR 1.19** (1.07 to 1.33)	0.7%	**0.9%** (0.8 to 1)	**0.1% more** (0,1 more to 0,2 more)	⊕◯◯◯ Very low	The evidence is very uncertain about the effect of fluconazole in utero exposure on genito-urinary MCM.
Musculoskeletal MCM (any fluconazole exposure) № of participants: 3,115,094 (3 observational studies)	crude	**OR 1.18** (0.98 to 1.43)	0.9%	**1.1%** (0.9 to 1.3)	**0.2% more** (0 fewer to 0,4 more)	⊕◯◯◯ Very low	-
Musculoskeletal MCM (cumulative fluconazole exposure)							

≤ 150 mg № of participants: 1,305,362 (3 observational studies)	crude	**OR 1.01** (0.69 to 1.46)	1.1%	**1.1%** (0.7 to 1.6)	**0.0% fewer** (0,3 fewer to 0,5 more)	⨁◯◯◯ Very low	-
> 150 mg № of participants: 1,291,806 (3 observational studies)	crude	**OR 1.33** (1.13 to 1.56)	1.1%	**1.4%** (1.2 to 1.7)	**0.4% more** (0,1 more to 0,6 more)	⨁◯◯◯ Very low	The evidence suggests that fluconazole in utero exposure with cumulative dose > 150 mg results in a slight increase in musculoskeletal MCM.

GRADE Working Group grades of evidence

High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.

Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.

Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.

Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

The risk in the intervention group (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI). **CI:** confidence interval; **OR:** odds ratio. **MCM**: major congenital malformation

eFigures

eFig. 1 Sensitivity analyses for major congenital malformation associated with exposure to any dose of fluconazole according to: the validity of the definition of MCM (1a crude OR; 1b adjusted OR), the reference group (1c crude OR; 1d adjusted OR) and excluding duplicate data (Sorensen et al.) (1e crude OR; 1f adjusted OR)

eFig. 2 Sensitivity analyses for major congenital malformation associated with exposure to cumulative dose of fluconazole (≤ 150 mg; >150 mg) according to the validity of the definition of MCM (2a crude OR; 2b adjusted OR)

eFig. 3 Sensitivity analyses for miscarriages associated with exposure to any dose of fluconazole excluding the publications with Hazard Ratio (3a crude OR; 3b adjusted OR)

eFig. 4 Pooled odds ratios (OR) for major cardiac malformation and exposure to any dose of fluconazole (4a crude OR; 4b adjusted OR)

eFig. 5 Sensitivity analyses for major cardiac congenital malformation associated with exposure to any dose of fluconazole according to the reference group (5a crude OR; 5b adjusted OR)

eFig. 6 Pooled odds ratios (OR) for major cardiac malformation and exposure to cumulative dose of fluconazole (≤ 150 mg; >150 mg) (6a crude OR; 6b adjusted OR)

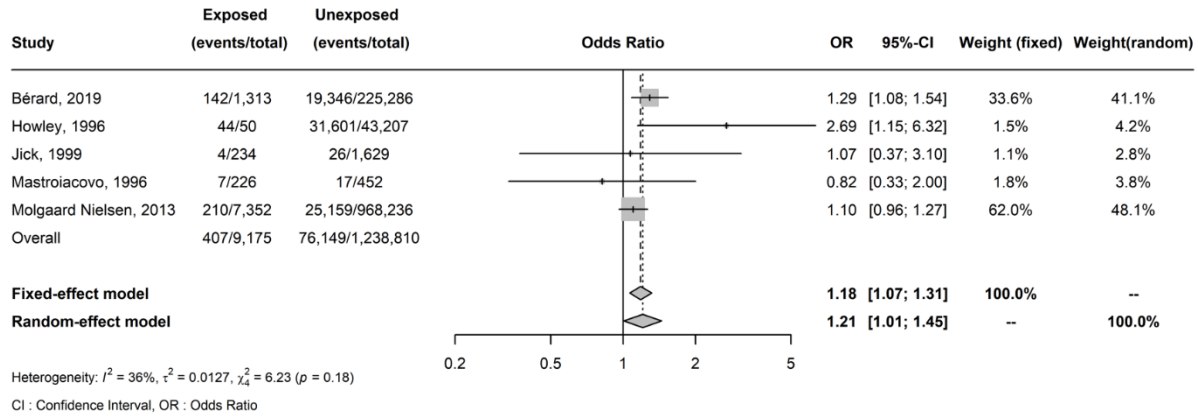
eFig. 7 Pooled odds ratios (OR) for major genito-urinary malformation associated with exposure to any dose of fluconazole (crude OR)

eFig. 8 Sensitivity analyses for major genito-urinary congenital malformation associated with exposure to any dose of fluconazole according to: the definition of MCM (8a crude OR) and to the reference group (8b crude OR)

eFig. 9 Pooled odds ratios (OR) for major musculoskeletal malformation associated with exposure to any dose of fluconazole (9a crude OR) and to cumulative dose of fluconazole (≤ 150 mg; >150 mg) (9b crude OR)

eFig. 10 Sensitivity analyses for major musculoskeletal congenital malformation associated with exposure to fluconazole according to: the definition of MCM (10a any dose, crude OR; 10b cumulative dose, crude OR) and to the reference group (10c any dose, crude OR)

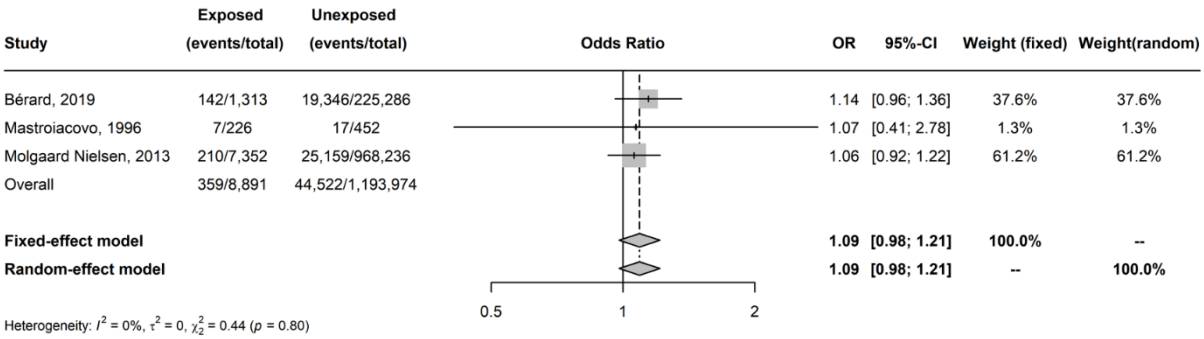
1a: Sensitivity analyse for MCM Any dose / Definition of MCM (crude estimates)



Heterogeneity: I^2 = 36%, τ^2 = 0.0127, χ^2_4 = 6.23 (p = 0.18)

CI : Confidence Interval, OR : Odds Ratio

1b: Sensitivity analyse for MCM Any dose / Definition of MCM (adjusted estimates)

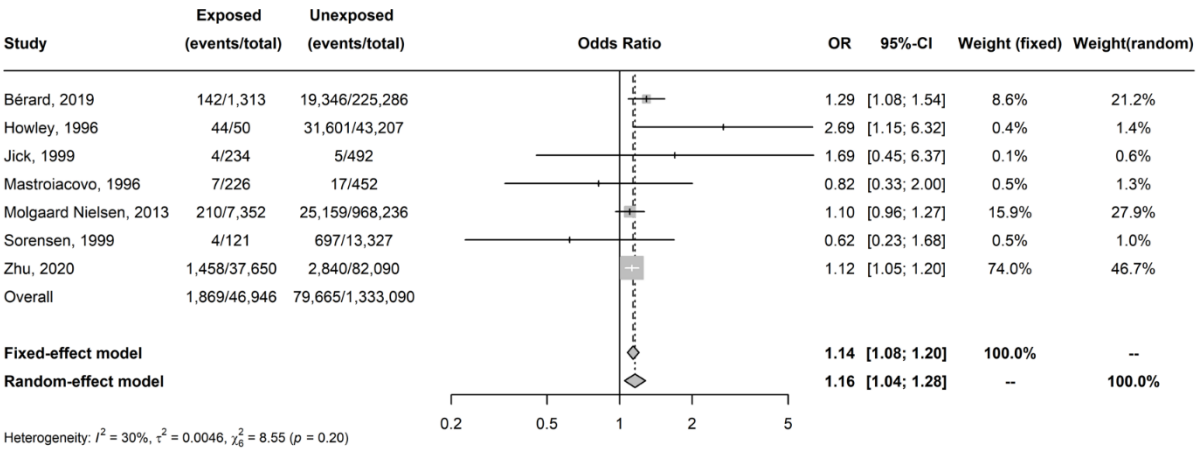


Heterogeneity: I^2 = 0%, τ^2 = 0, χ^2_2 = 0.44 (p = 0.80)

CI : Confidence Interval, NA : not available, OR : Odds Ratio

For Bérard, aOR for any dose of fluconazole was calculated, accordingly with Borenstein et al. 2008, based on aOR reported for ≤150 mg and >150mg cumulated doses.

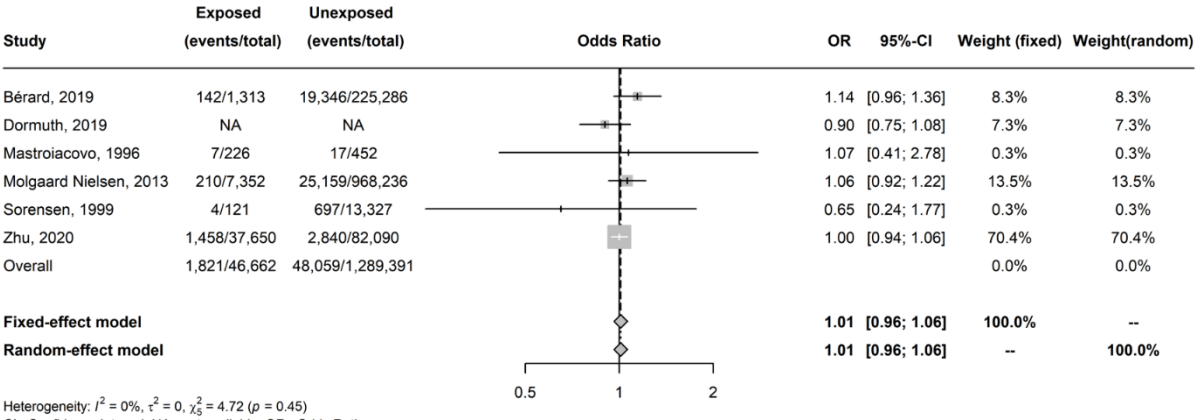
1c: Sensitivity analyse for MCM Any dose / Reference group (crude estimates)



Heterogeneity: I^2 = 30%, τ^2 = 0.0046, χ^2_6 = 8.55 (p = 0.20)

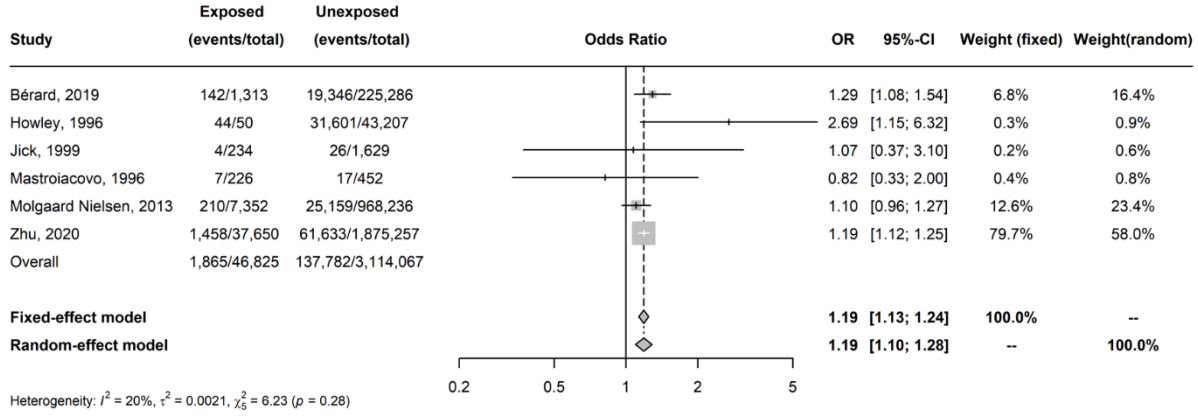
CI : Confidence Interval, OR : Odds Ratio

1d: Sensitivity analyse for MCM Any dose / Reference group (adjusted estimates)



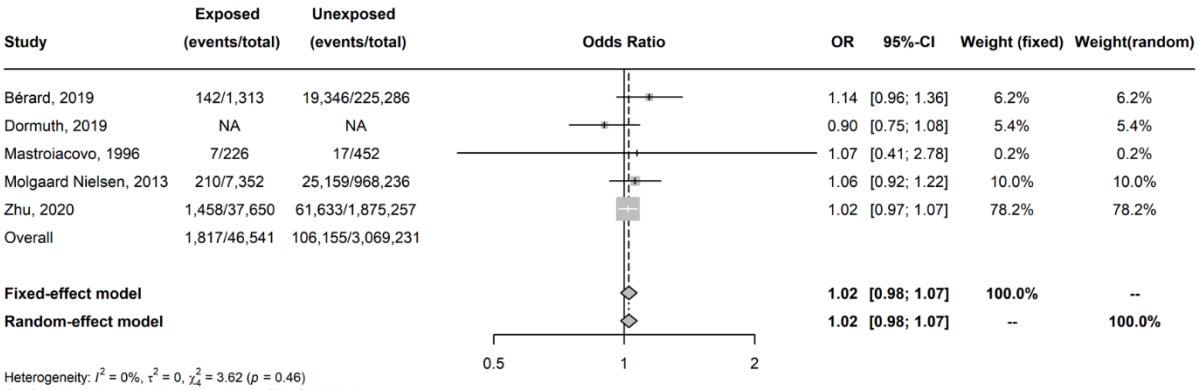
Heterogeneity: I^2 = 0%, τ^2 = 0, χ^2_5 = 4.72 (p = 0.45)
CI : Confidence Interval, NA : not available, OR : Odds Ratio
For Bérard, aOR for any dose of fluconazole was calculated, accordingly with Borenstein et al. 2008, based on aOR reported for ≤150 mg and >150mg cumulated doses.
For Dormuth, the number of events in each group was not reported in the available material but the total number of pregnancies exposed to oral fluconazole was 63,346 and the total number of pregnancies in the control group was 107,212.
We report here frequencies of events among the exposed and the unexposed groups while Zhu also reported the adjusted frequencies after weighting of the propensity score within each group.

1e: Sensitivity analyse for MCM Any dose / Excluding duplicate data (crude estimates)



Heterogeneity: I^2 = 20%, τ^2 = 0.0021, χ^2_5 = 6.23 (p = 0.28)
CI : Confidence Interval, OR : Odds Ratio

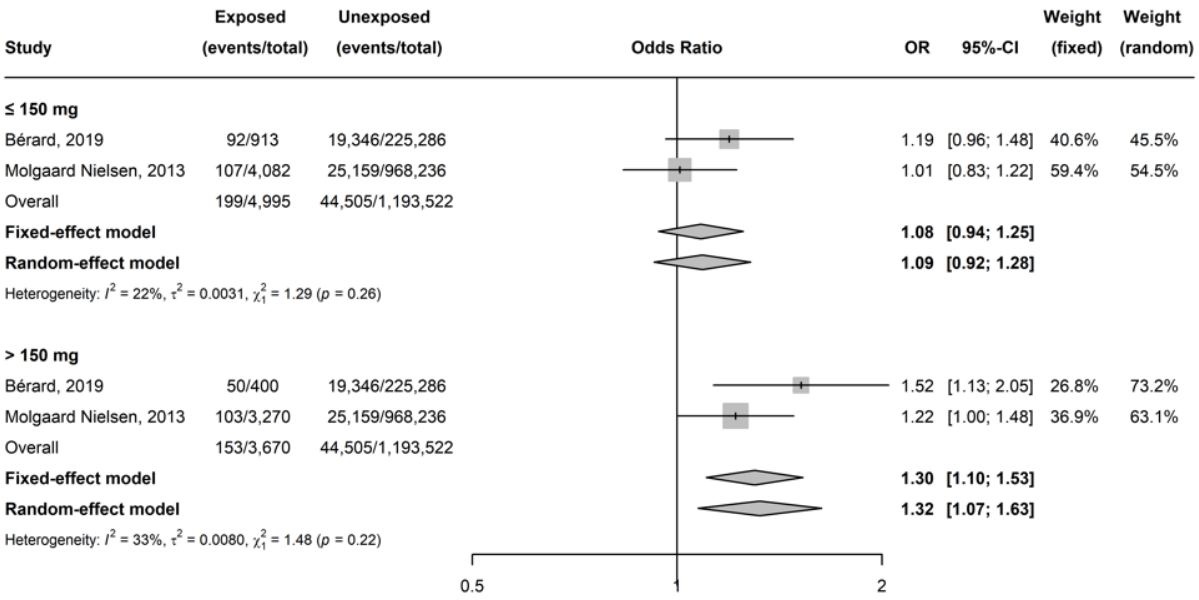
1f: Sensitivity analyse for MCM Any dose / Excluding duplicate data (adjusted estimates)



Heterogeneity: I^2 = 0%, τ^2 = 0, χ^2_4 = 3.62 (p = 0.46)
CI : Confidence Interval, NA : not available, OR : Odds Ratio
For Bérard, aOR for any dose of fluconazole was calculated, accordingly with Borenstein et al. 2008, based on aOR reported for ≤150 mg and >150mg cumulated doses.
For Dormuth, the number of events in each group was not reported in the available material but the total number of pregnancies exposed to oral fluconazole was 63,346 and the total number of pregnancies in the control group was 107,212.
We report here frequencies of events among the exposed and the unexposed groups while Zhu also reported the adjusted frequencies after weighting of the propensity score within each group.

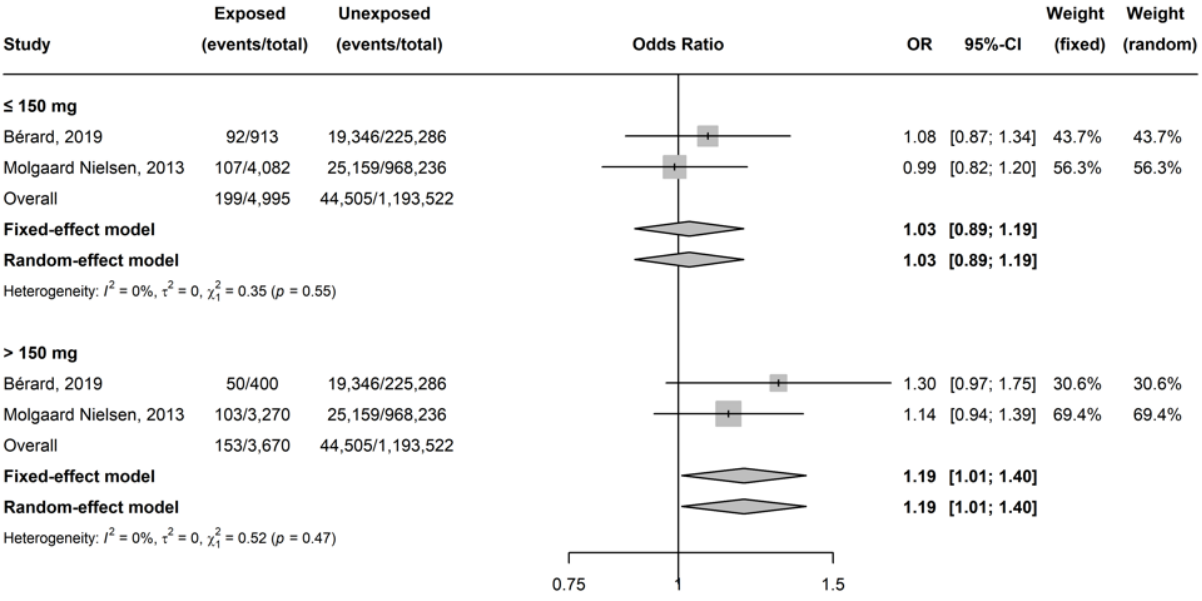
eFig. 1 Sensitivity analyses for major congenital malformation associated with exposure to any dose of fluconazole according to: the validity of the definition of MCM (1a crude OR; 1b adjusted OR), the reference group (1c crude OR; 1d adjusted OR) and excluding duplicate data (Sorensen et al.) (1e crude OR; 1f adjusted OR)

2a: Sensitivity analyse for MCM Cumulative dose / Definition of MCM (crude estimates)



CI : Confidence Interval, OR : Odds Ratio
For Molgaard Nielsen, aOR for the >150mg cumulated dose of fluconazole was calculated, accordingly with Borenstein et al. 2008, based on aOR reported for 300 mg and >350-600mg cumulated doses. For Zhu, aOR for the >150mg cumulated dose of fluconazole was calculated, accordingly with Borenstein et al. 2008, based on aOR reported respectively for 150-400 mg and >450mg cumulated doses.

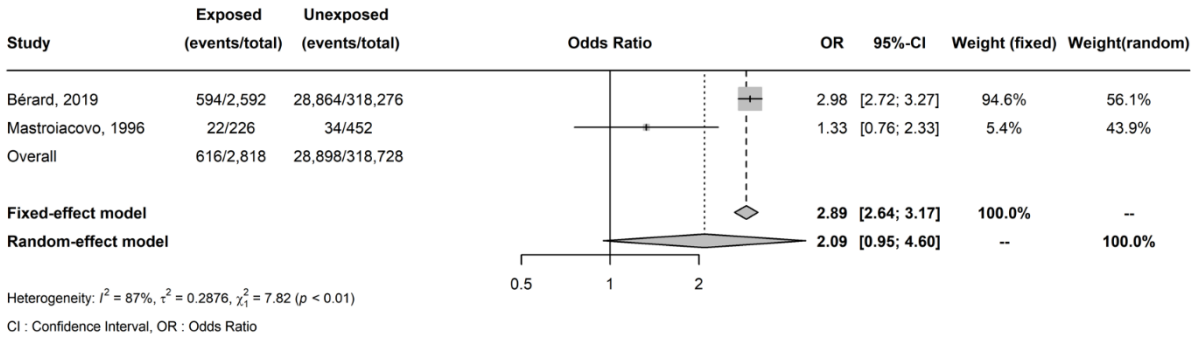
2b: Sensitivity analyse for MCM Cumulative dose / Definition of MCM (adjusted estimates)



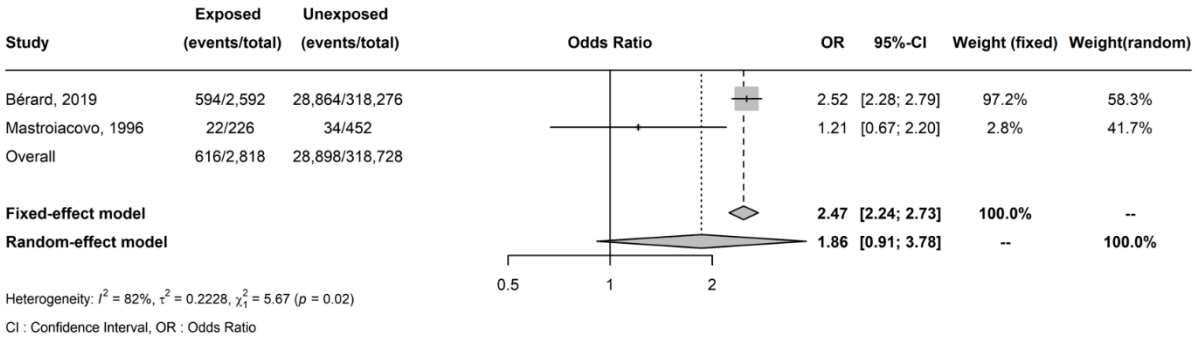
CI : Confidence Interval, OR : Odds Ratio
For Molgaard Nielsen, aOR for the >150mg cumulated dose of fluconazole was calculated, accordingly with Borenstein et al. 2008, based on aOR reported for 300 mg and >350-600mg cumulated doses. For Zhu, aOR for the >150mg cumulated dose of fluconazole was calculated, accordingly with Borenstein et al. 2008, based on aOR reported respectively for 150-400 mg and >450mg cumulated doses.

eFig. 2 Sensitivity analyses for major congenital malformation associated with exposure to cumulative dose of fluconazole (≤ 150 mg; >150 mg) according to the validity of the definition of MCM (2a crude OR; 2b adjusted OR)

3a: Sensitivity analyse for miscarriages any dose / Excluding publication with Hazard Ratio (crude estimates)

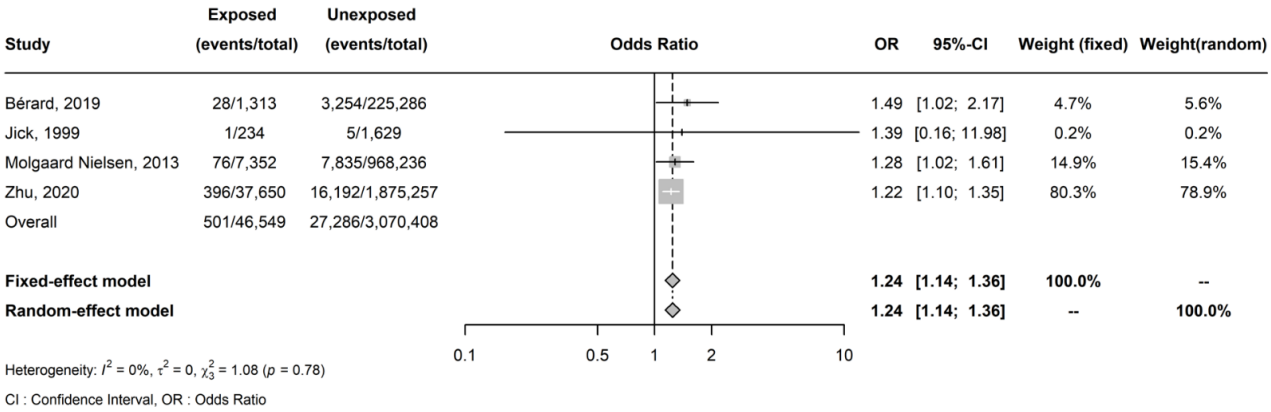


3b: Sensitivity analyse for miscarriages any dose / Excluding publication with Hazard Ratio (adjusted estimates)



eFig. 3 Sensitivity analyses for miscarriages associated with exposure to any dose of fluconazole excluding the publications with Hazard Ratio (3a crude OR; 3b adjusted OR)

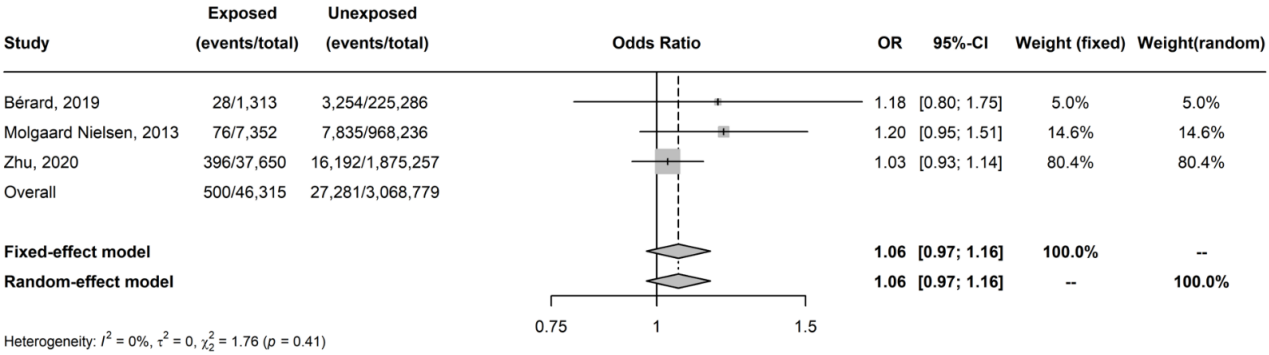
4a: Cardiac MCM / Any dose (crude OR)



Heterogeneity: I^2 = 0%, τ^2 = 0, χ^2_3 = 1.08 (p = 0.78)

CI : Confidence Interval, OR : Odds Ratio

4b: Cardiac MCM / Any dose (adjusted OR)



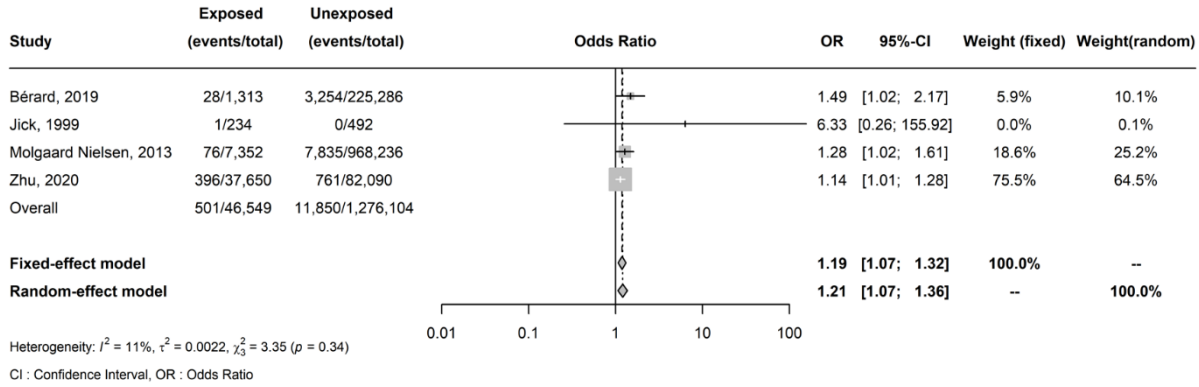
Heterogeneity: I^2 = 0%, τ^2 = 0, χ^2_2 = 1.76 (p = 0.41)

CI : Confidence Interval, OR : Odds Ratio

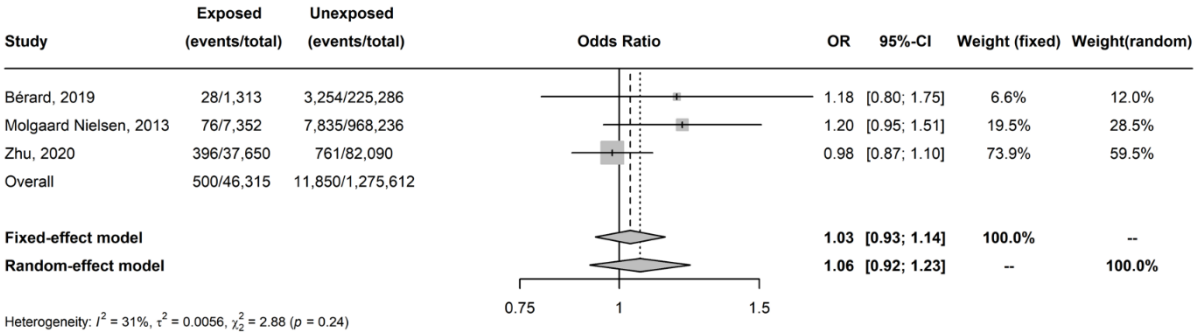
We report here frequencies of events among the exposed and the unexposed groups while Zhu also reported the adjusted frequencies after weighting of the propensity score within each group.

eFig. 4 Pooled odds ratios (OR) for major cardiac malformation and exposure to any dose of fluconazole (4a crude OR; 4b adjusted OR)

5a: Sensitivity analyse for cardiac MCM Any dose / Reference group (crudes estimates)

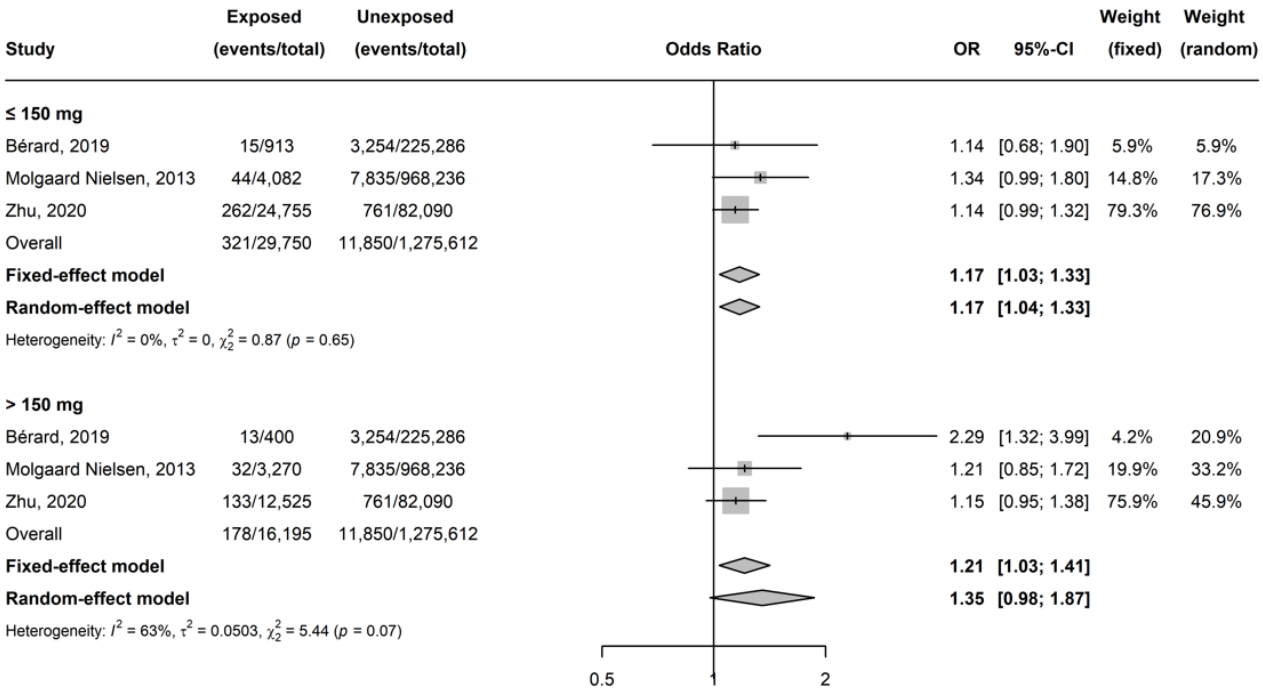


5b: Sensitivity analyse for cardiac MCM Any dose / Reference group (adjusted estimates)



eFig. 5 Sensitivity analyses for major cardiac congenital malformation associated with exposure to any dose of fluconazole according to the reference group (5a crude OR; 5b adjusted OR)

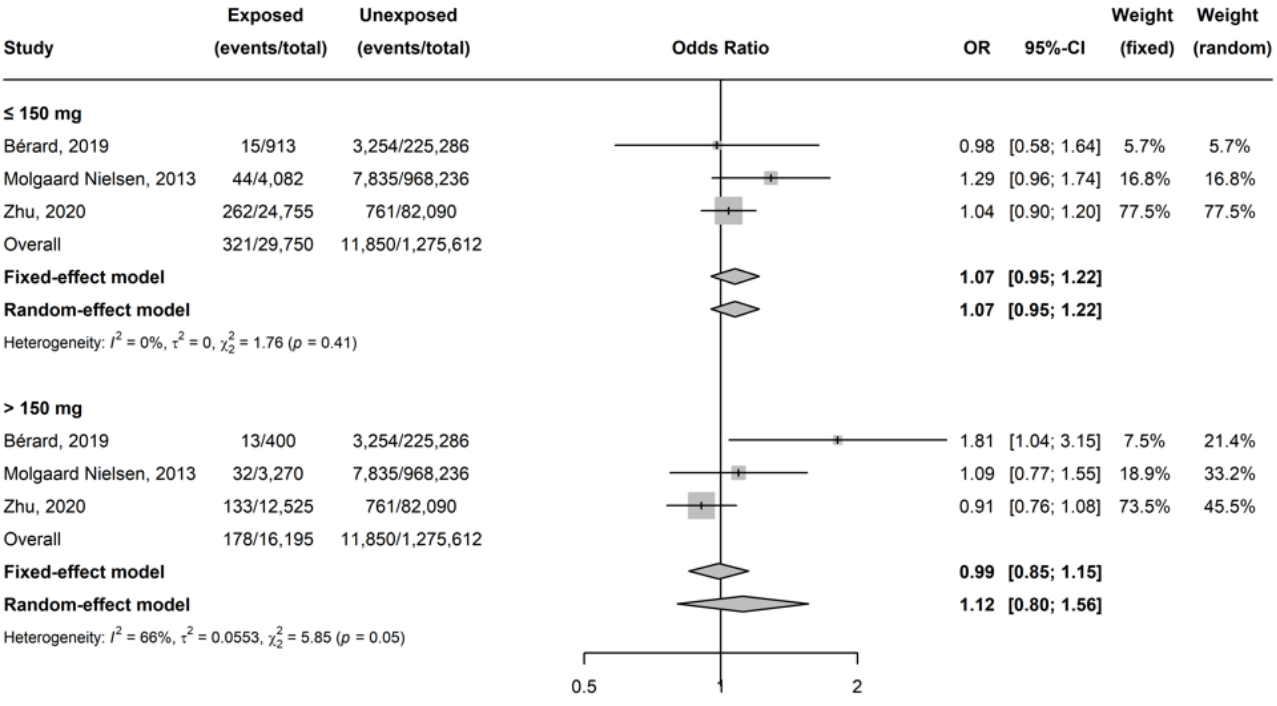
6a: Cardiac MCM / Cumulative dose (crude OR)



CI : Confidence Interval, OR : Odds Ratio

For Molgaard Nielsen, aOR for the >150mg cumulated dose of fluconazole was calculated, accordingly with Borenstein et al. 2008, based on aOR reported for 300 mg and >350-600mg cumulated doses. For Zhu, aOR for the >150mg cumulated dose of fluconazole was calculated, accordingly with Borenstein et al. 2008, based on aOR reported respectively for 150-400 mg and >450mg cumulated doses.

6b: Cardiac MCM / Cumulative dose (adjusted OR)

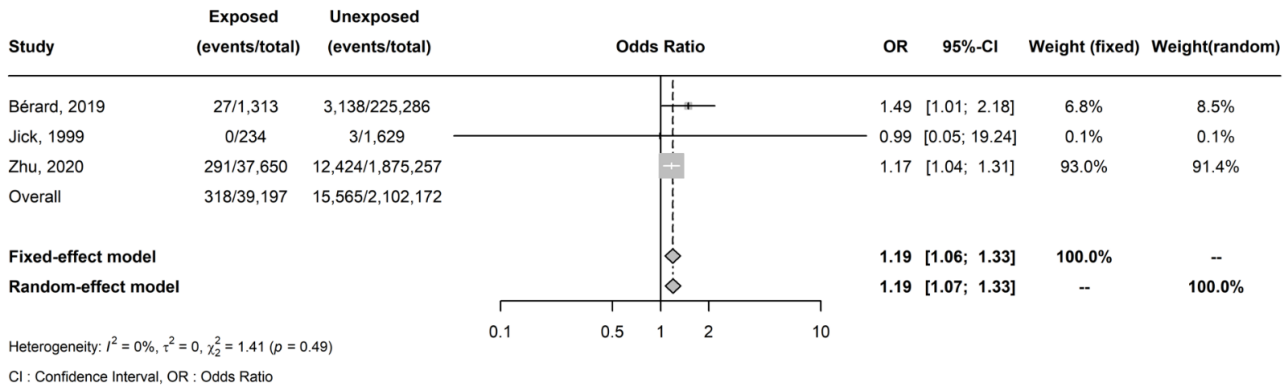


CI : Confidence Interval, OR : Odds Ratio

For Molgaard Nielsen, aOR for the >150mg cumulated dose of fluconazole was calculated, accordingly with Borenstein et al. 2008, based on aOR reported for 300 mg and >350-600mg cumulated doses. For Zhu, aOR for the >150mg cumulated dose of fluconazole was calculated, accordingly with Borenstein et al. 2008, based on aOR reported respectively for 150-400 mg and >450mg cumulated doses.

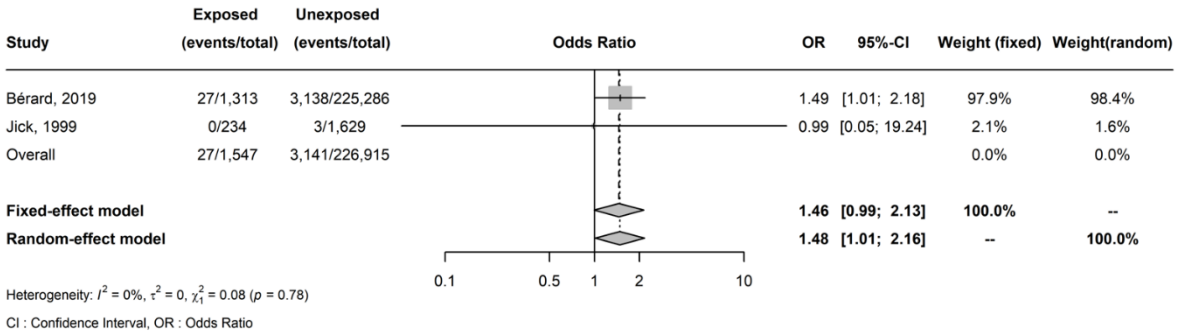
We report here frequencies of events among the exposed and the unexposed groups while Zhu also reported the adjusted frequencies after weighting of the propensity score within each group.

eFig. 6 Pooled odds ratios (OR) for major cardiac malformation and exposure to cumulative dose of fluconazole (≤ 150 mg; >150 mg) (6a crude OR; 6b adjusted OR)

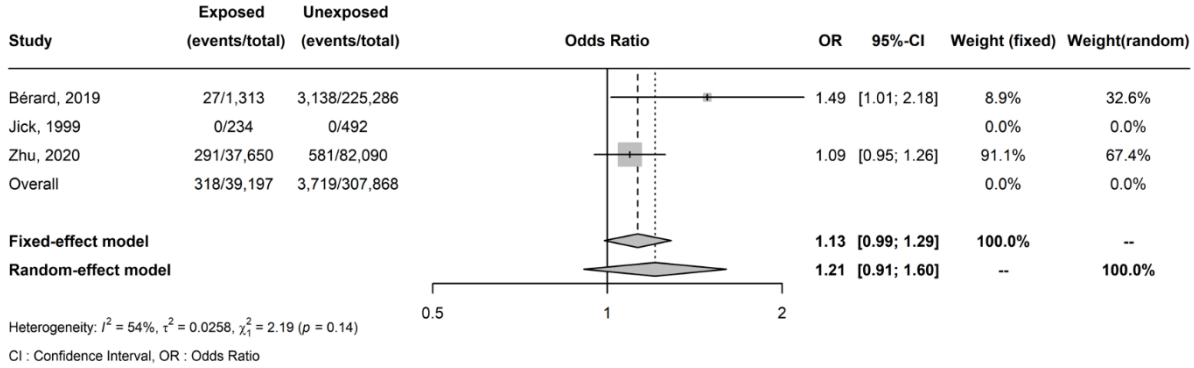


eFig. 7 Pooled odds ratios (OR) for major genito-urinary malformation associated with exposure to any dose of fluconazole (crude OR)

8a: Sensitivity analyse for genito-urinary MCM Any dose / Definition of MCM (crude estimates)



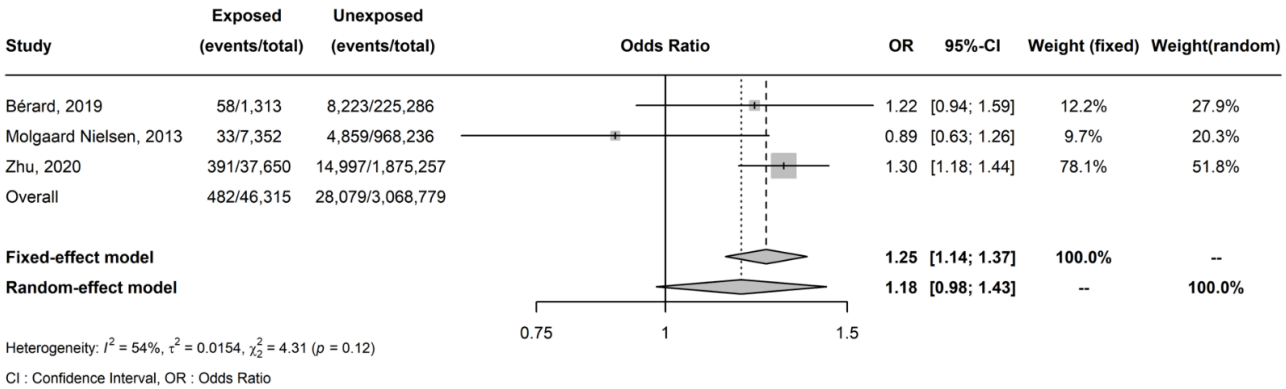
8b: Sensitivity analyse for genito-urinary MCM Any dose / Reference group (crude estimates)



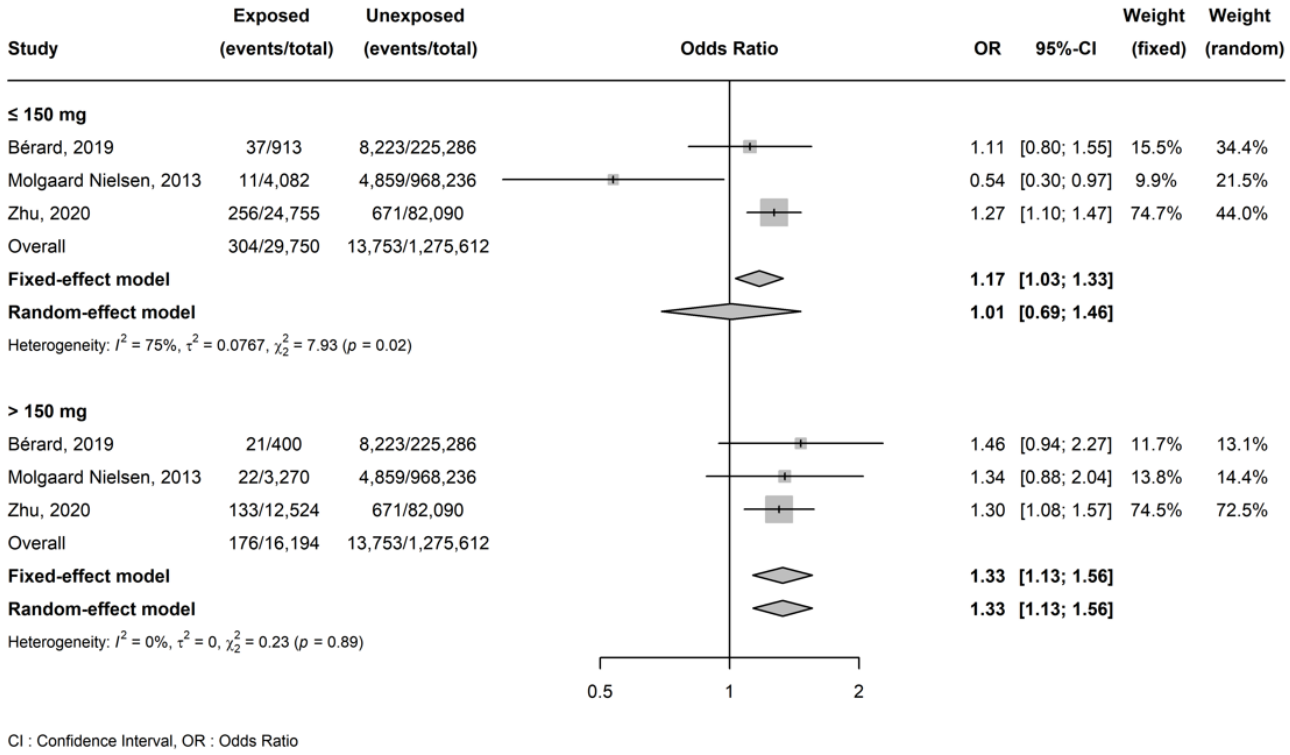
Because no event occurred in both groups (exposed and unexposed), Jick's study could not be included in the analysis

eFig. 8 Sensitivity analyses for major genito-urinary congenital malformation associated with exposure to any dose of fluconazole according to: the definition of MCM (8a crude OR) and to the reference group (8b crude OR)

9a: Musculoskeletal MCM / Any dose (crude OR)

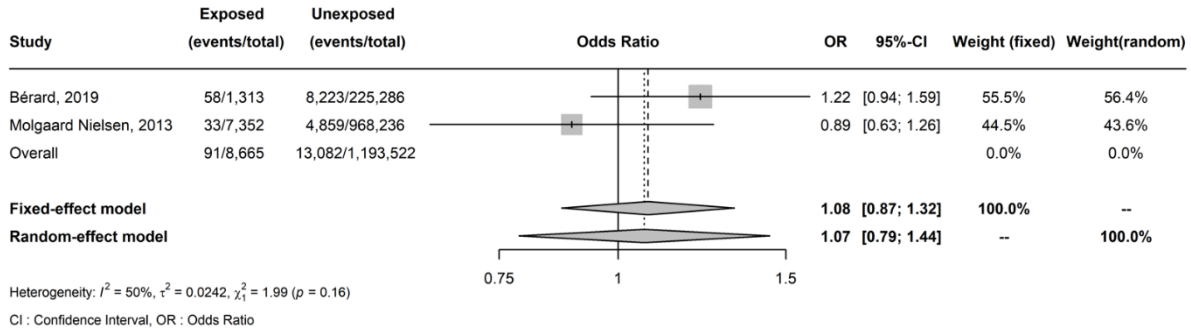


9b: Musculoskeletal MCM / Cumulative dose (crude OR)

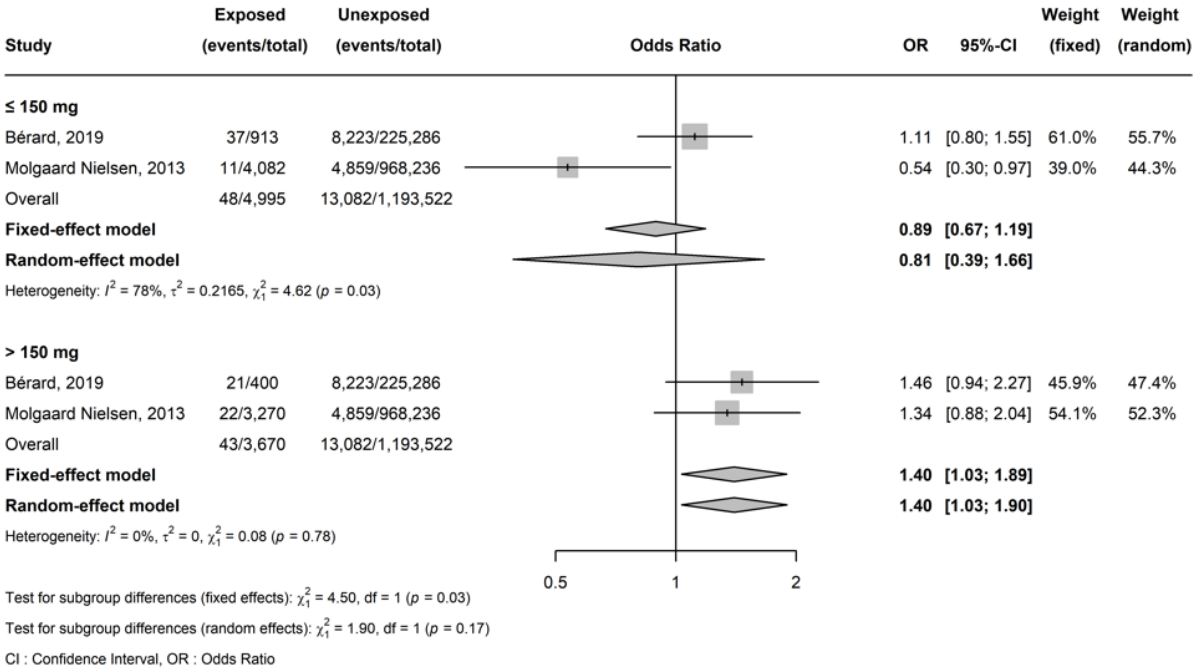


eFig. 9 Pooled odds ratios (OR) for major musculoskeletal malformation associated with exposure to any dose of fluconazole (9a crude OR) and to cumulative dose of fluconazole (≤ 150 mg; >150 mg) (9b crude OR)

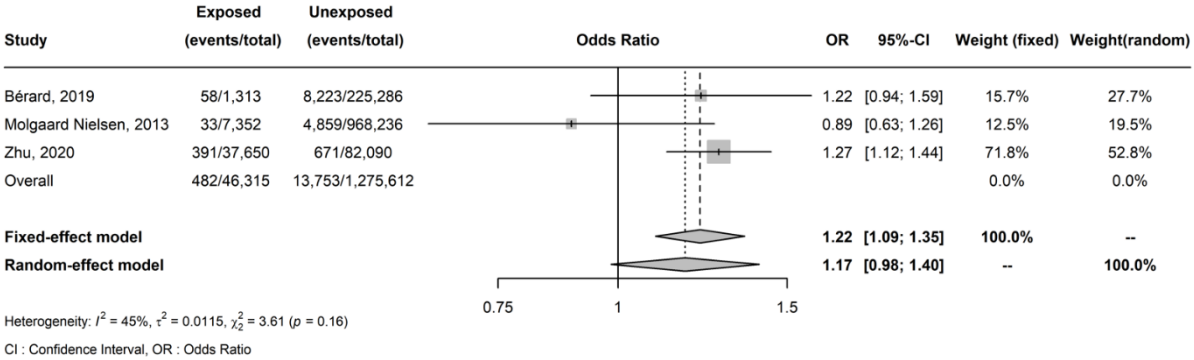
10a: Sensitivity analyse for musculoskeletal MCM Any dose / Definition of MCM (crude estimates)



10b: Sensitivity analyse for musculoskeletal MCM Cumulative dose / Definition of MCM (crude estimates)



10c: Sensitivity analyse for musculoskeletal MCM Any dose / Reference group (crude estimates)



eFig. 10 Sensitivity analyses for major musculoskeletal congenital malformation associated with exposure to fluconazole according to: the definition of MCM (10a any dose, crude OR; 10b cumulative dose, crude OR) and to the reference group (10c any dose, crude OR)

Appendix

Appendix 1 Comparison between the protocol registered in PROSPERO and what was done and is presented in the article

Appendix 2 Search algorithm for each database

Appendix 3 List of confounders identified a priori during the extraction of data

Appendix 4 Recalculation of endpoints for specific subgroups of malformation

Appendix 5 Comparison of the different subgroups of malformation according to the data provided in the selected articles

Appendix 1 Comparison between the protocol registered in PROSPERO and what was done and is presented in the article

Topic	PROSPERO	Presented in the article	Justification
Exposition	To distinguish between low dose and high dose regimen of fluconazole treatment.	Analyses are made according to "any dose" of fluconazole and 2 categorisations of the cumulative dose (≤ 150 mg and > 150 mg).	Wide heterogeneity in terms of exposure group definition.
Outcome mcm	Additional outcome of the study: minor congenital malformations in the offspring (including live births, stillborn and medical terminations of pregnancies), following the definition proposed by the EUROCAT.	Not presented.	None of the studies specifically described the risk of mcm associated with fluconazole use in the first trimester of pregnancy.
Outcome miscarriage	Additional outcome of the study: miscarriages defined as early miscarriages (loss of pregnancy before 14 GW) or late miscarriages (loss of pregnancy between 14 and 22 GW) because this outcome could be related to a congenital malformation.	Miscarriages without distinction according to the term of pregnancy.	Only one study evaluated specifically miscarriages before 12 GW (data furnished by the contacted author). The other studies did not distinguish between early or late miscarriages.
Subgroups analysis	We planned a priori subgroup analyses according to relevant study level covariates (single dose versus repeated low-dose treatment), category of term of exposure (before 4 GW, between 4 and 10 GW, after 10 GW), type of unexposed group (no treatment, placebo, local fluconazole). Random-effects meta-regression will be used to evaluate the effect of the cumulated dose.	Subgroups analysis according to relevant study level covariates and category of term of exposure not presented. Meta-regression not presented.	Lack of information in the selected studies.
Sensitivity analysis	The following sensitivity analyses to assess the robustness of the results will be performed: - A sensitivity analysis based on risk of bias - A sensitivity analysis considering only adjusted OR	Sensitivity analysis based on risk on bias: not presented. Sensitivity analysis considering only adjusted OR: presented in the main analysis. Sensitivity analysis according to the definition of MCM in the selected studies, to the control group and excluding HR are presented.	All studies evaluating the risk of malformation were considered at serious risk of bias, except one that was in abstract form and for which the risk of bias was "no informative". Besides, there was only one study evaluating the risk of miscarriage that was at moderate risk of bias. We performed the main analysis by separating crude OR and adjusted OR. Sensitivity analysis have been added considering the characteristics of the studies
GRADE evaluation	Not planned	Added	To rate the certainty of the evidence of our results.

Legend: GW = gestational weeks

Appendix 2 Search algorithm for each database

Database	Search algorithm
PubMed	**Pregnancy** #1 pregnancy [mh] #2 pregnant women [mh] #3 pregnancy trimester, first [mh] #4 pregnancy [tiab] #5 pregnant [tiab] #6 gestation [tiab] #7 “first trimester” [tiab] **#8 #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7** ((((((pregnancy [mh]) OR (pregnant women [mh])) OR (pregnancy trimester, first [mh])) OR (pregnancy [tiab])) OR (pregnant [tiab])) OR (gestation [tiab])) OR ("first trimester" [tiab]) **Fluconazole** #9 fluconazole [mh] #10 fluconazole [tiab] #11 TRIFLUCAN [tiab] #12 DIFLUCAN [tiab] #13 Fluconazol [tiab] #14 ELAZOR [tiab] #15 Fungata [tiab] **#16 #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15** ((((((fluconazole [mh]) OR (fluconazole [tiab])) OR (TRIFLUCAN [tiab])) OR (DIFLUCAN [tiab])) OR (Fluconazol [tiab])) OR (ELAZOR [tiab])) OR (Fungata [tiab]) **Total** **#17 #8 AND #16** **(((((((pregnancy [mh]) OR (pregnant women [mh])) OR (pregnancy trimester, first [mh])) OR (pregnancy [tiab])) OR (pregnant [tiab])) OR (gestation [tiab])) OR ("first trimester" [tiab])) AND (((((((fluconazole [mh]) OR (fluconazole [tiab])) OR (TRIFLUCAN [tiab])) OR (DIFLUCAN [tiab])) OR (Fluconazol [tiab])) OR (ELAZOR [tiab])) OR (Fungata [tiab]))**
EMBASE	**Pregnancy** #1 'pregnancy'/exp #2 'first trimester pregnancy'/exp #3 'pregnant woman'/exp #4 pregnancy:ab,ti #5 'first trimester pregnancy':ab,ti #6 pregnant:ab,ti #7 gestation:ab,ti

	#8 #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 **Fluconazole** #9 'fluconazole'/exp #10 fluconazole:ab,ti #11 triflucan:ab,ti #12 diflucan:ab,ti #13 fluconazol:ab,ti #14 elazor:ab,ti #15 fungata:ab,ti #16 **#9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15** **Total** **#17 #8 AND #16** **('pregnancy'/exp OR 'first trimester pregnancy'/exp OR 'pregnant woman'/exp OR pregnancy:ab,ti OR 'first trimester pregnancy':ab,ti OR pregnant:ab,ti OR gestation:ab,ti) AND ('fluconazole'/exp OR fluconazole:ab,ti OR triflucan:ab,ti OR diflucan:ab,ti OR fluconazol:ab,ti OR elazor:ab,ti OR fungata:ab,ti)**
Cochrane (CENTRAL and Reviews)	**Pregnancy** #1 (pregnancy):ti,ab,kw #2 (pregnant):ti,ab,kw #3 (first trimester):ti,ab,kw #4 (gestation):ti,ab,kw **#5 #1 OR #2 OR #3 OR #4** **Fluconazole** #6 (fluconazole):ti,ab,kw #7 (diflucan):ti,ab,kw #8 (fluconazol):ti,ab,kw **#9 #6 OR #7 OR #8** **Total** **#10 #5 and #9**
Clinical trial.gov	Pregnancy (condition or disease) Fluconazole (other terms)
WHO (ICTRP)	“fluconazole and pregnancy” Fluconazole (intervention) Pregnancy (condition)

Appendix 3 List of confounders identified a priori during the extraction of data

Cofounders identified a priori	
Evaluation of the risk of congenital malformation	**Evaluation of the risk of miscarriages**
Maternal age	Maternal age
Body mass index	Body mass index
Pregestational diabetes	Pregestational diabetes
Personal and familial history of malformation	Personal history of spontaneous abortion
Smoking	Smoking
Alcohol consumption	Alcohol consumption
Exposure to other medication	Exposure to other medication
Illicit drug consumption	Illicit drug consumption
Maternal comorbidities	Maternal comorbidities
Socio-economic aspects	Socio-economic aspects
Paternal characteristics (age, comorbidities, exposure to medication, drug consumption…)	Paternal characteristics (age, comorbidities, exposure to medication, drug consumption…)
Other cofounders taken into account by the authors	Other cofounders taken into account by the authors

Appendix 4 Recalculation of endpoints for specific subgroups of malformation

Reconstructed groups of malformations (concerned studies)	Justification
Transposition of great vessels (Howley 2016, Zhu 2020)	In 60% of the cases the TGV are isolated. dTGA is the most frequent, LTGA is very rare. - In Howley: there is no LTGA described in the malformations. We considered LTGA n=0 and added the numbers 0 + dTGA to find TGV. - Zhu described TGV.
Cleft lip and or palate (Howley 2016, Zhu 2020)	- For Howley: we added the numbers of cleft lip, cleft palate and cleft lip with palate. - For Zhu: we added the numbers of cleft palate, cleft lift and cleft palate with lip.
Poly or syndactyly (Jick 1999, Molgaard-Nielsen 2013)	- Jick described poly or syndactyly. - For Molgaard-Nielsen 2013: we added the numbers of polydactyly and syndactyly.
Musculoskeletal (Bérard 2019, Molgaard-Nielsen 2013, Zhu 2020)	This group includes limb anomalies, cranial anomalies of the face (i.e. without clefts), poly or syndactyly. We took into account the definition of malformations and the coding used by the authors in each study and the number of patients presented). - We started from the group as described in Bérard's article and reconstructed this group for Molgaard Nielsen 2013 and for Zhu 2020. - For Molgaard-Nielsen 2013: we added the numbers of limb defect (which includes limb reduction defect), other cranial defect and craniosynostosis. - For Zhu 2020: we added the numbers of limb defect and musculoskeletal.
Genito-urinary (Bérard 2019, Zhu 2020)	This group includes genital and urinary malformations. - For Bérard and Zhu, to reconstruct this group we added the numbers of "genital" and "urinary". - Bérard provided us the numerical data for this reconstructed group.

Appendix 5 Comparison of the different subgroups of malformation according to the data provided in the selected articles

Subgroup of malformations	Number of references (authors)	Codes/definition	Comment
Nervous	N=2 (Bérard, Zhu)	Bérard: ICD-9 codes 740-759 excluding codes of minor congenital malformations or chromosomal abnormalities: 743.6, 744.1-744.4, 744.8, 744.9, 747.0, 747.5, 750.0, 752.4, 752.5, 754.6, 755.0, 755.1, 757.2-757.6, 757.8, 757.9, 758.4. ICD-10 codes Q00-Q99, excluding codes of minor malformations or chromosomal abnormalities: Q08- Q10, Q162, Q17-Q19, Q250, Q270, Q381, Q515, Q516, Q20-Q53, Q664-Q666, Q689, Q70, Q81-Q84, Q94-Q95. Zhu: Central Nervous System 740.xx-742.xx.	**Equivalent**
Eyes	N=2 (Bérard, Zhu)	Bérard: ICD-9 codes 740-759 excluding codes of minor congenital malformations or chromosomal abnormalities: **743.6** (congenital anomalies of eyelids lacrimal system and orbit), 744.1-744.4, 744.8, 744.9, 747.0, 747.5, 750.0 (tongue tie), 752.4 (congenital anomalies of cervix vagina and external female genitalia), 752.5 (undescended and retractile testicle), 754.6 (congenital valgus deformities of feet), 755.0 (polydactyly), 755.1 (syndactyly), 757.2-757.6, 757.8, 757.9, 758.4. ICD-10 codes Q00-Q99, excluding codes of minor malformations or chromosomal abnormalities: Q08- Q10, Q162, Q17-Q19, Q250, Q270, Q381, Q515, Q516, Q20-Q53, Q664-Q666, Q689, Q70, Q81-Q84, Q94-Q95. Zhu: Eye Anomalies 743.xx (exclude if only 743.6x and **743.8x** = other specified anomalies of eye)	**Zhu excluded more malformations**

Subgroup of malformations	Number of references (authors)	Codes/definition	Comment
Cardiac	N=5 (Bérard, Zhu, Jick, Molgaard-Nielsen 2013)	Bérard: ICD-9 codes 740-759 excluding codes of minor congenital malformations or chromosomal abnormalities: 743.6, 744.1-744.4, 744.8, 744.9, **747.0 (patent ductus arteriosus), 747.5 (absence or hypoplasia of umbilical artery)**, 750.0, 752.4, 752.5, 754.6, 755.0, 755.1, 757.2-757.6, 757.8, 757.9, 758.4. ICD-10 codes Q00-Q99, excluding codes of minor malformations or chromosomal abnormalities: Q08- Q10, Q162, Q17-Q19, Q250, Q270, Q381, Q515, Q516, Q20-Q53, Q664-Q666, Q689, Q70, Q81-Q84, Q94-Q95. Zhu: Cardiovascular Anomalies: Conotruncal malformations 745.0x, 745.1x, 745.2x ; Tetralogy of Fallot 745.2x ; Transposition of great vessel 745.1x ; d-Transposition of great arteries 745.10 ; Single ventricle defects 745.3x ; Ventricular septal defect 745.4x ; Secundum atrial septal defect 745.5x AND no preterm ; Atrioventricular septal defect 745.6x ; Right-sided defects 746.00, 746.01, 746.09, 746.1x, 746.2x, 746.83, 747.3x AND no preterm, 746.02 AND no preterm ; Left-sided defects 747.1x, 747.2x, 746.3x, 746.5x, 746.7x, 746.81, 746.82, 746.84 ; **Patent ductus arteriosus 747.0x AND no preterm** ; Persistent pulmonary hypertension of the newborn (416.0x or 747.83) AND no preterm ; Pulmonary venous return 747.4x ; Other cardiac defects 745.7x, 745.8x, 746.8, 746.85-746.87, 746.89 ; Cardiac not otherwise specified 745, 745.9, 746, 746.9x (exclude if only 746.99), 747. Molgaard-Nielsen 2013: Heart defects overall: Q20-Q26, **excluding: Q211C, Q250** (if gestational age < 37) ; **Q211 = Atrial septal defect, Q250 = Patent ductus arteriosus** Tetralogy of Fallot Q213 Pulmonary artery hypoplasia Q257F Ventricular septal defects Q210 Hypoplastic left heart Q234 Jick: “heart defect” present at birth that resulted in surgery or other treatment for functional or cosmetic reasons (no more precision).	**Zhu included 747.0 (patient ductus arteriosus) and no preterm, Molgaard-Nielsen did the same, whereas Bérard excluded patient ductus arteriosus.** **No detail for Jick.**
Digestive	N=2 (Bérard, Zhu)	Bérard: ICD-9 codes 740-759 excluding codes of minor congenital malformations or chromosomal abnormalities: 743.6, 744.1-744.4, 744.8, 744.9, 747.0, 747.5, **750.0 (tongue tie)**, 752.4, 752.5, 754.6, 755.0, 755.1, 757.2-757.6, 757.8, 757.9, 758.4. ICD-10 codes Q00-Q99, excluding codes of minor malformations or chromosomal abnormalities: Q08- Q10, Q162, Q17-Q19, Q250, Q270, Q381, Q515, Q516, Q20-Q53, Q664-Q666, Q689, Q70, Q81-Q84, Q94-Q95. Zhu: Gastrointestinal 750.xx-751.xx (exclude if only **750.0x, 750.1x, 750.50, 751.0x**)	**Zhu excluded more malformations**

Subgroup of malformations	Number of references (authors)	Codes/definition	Comment
Genito-urinary	N=3 (Bérard, Zhu, Jick)	Bérard: ICD-9 codes 740-759 excluding codes of minor congenital malformations or chromosomal abnormalities: 743.6, 744.1-744.4, 744.8, 744.9, 747.0, 747.5, 750.0, **752.4** (congenital anomalies of cervix vagina and external female genitalia), **752.5** (undescended and retractile testicle), 754.6, 755.0, 755.1, 757.2-757.6, 757.8, 757.9, 758.4. ICD-10 codes Q00-Q99, excluding codes of minor malformations or chromosomal abnormalities: Q08- Q10, Q162, Q17-Q19, Q250, Q270, Q381, Q515, Q516, Q20-Q53, Q664-Q666, Q689, Q70, Q81-Q84, Q94-Q95. Zhu: Genital 752.xx (exclude if only: **752.42** Imperforate hymen, **752.52** Retractile testis); do not count 752.5x if preterm Urinary 753.xx (exclude if only 753.7x Anomalies of urachus) Jick: "genito urinary" present at birth that resulted in surgery or other treatment for functional or cosmetic reasons (no more precision).	**Almost equivalent.** **No detail for Jick.**
Musculoskeletal	N=3 (Bérard, Zhu, Molgaard-Nielsen 2013)	Bérard: "musculoskeletal" ICD-9 codes 740-759 excluding codes of minor congenital malformations or chromosomal abnormalities: 743.6, 744.1-744.4, 744.8, 744.9, 747.0, 747.5, 750.0, 752.4, 752.5, **754.6 (congenital valgus deformities of feet), 755.0 (polydactyly), 755.1 (syndactyly)**, 757.2-757.6, 757.8, 757.9, 758.4. ICD-10 codes Q00-Q99, excluding codes of minor malformations or chromosomal abnormalities: Q08- Q10, Q162, Q17-Q19, Q250, Q270, Q381, Q515, Q516, Q20-Q53, **Q664-Q666**, Q689, Q70, Q81-Q84, Q94-Q95. Zhu: "musculoskeletal" + "limb defect" Musculoskeletal = 754.0, 754.1, 756.0, 754.4x, 754.5x, 754.6x, 754.7x, 754.2, 756.1x, 756.4, 756.5x, 756.3, 754.8, 754.89, 754, 756, 756.9, 756.8x Limb defect = **755.xx** (exclude if only **755.65= Macrodactylia of toes**) Molgaard-Nielsen 2013: "limb defect" (including limb reduction defect) + "other cranial defect" + "craniosynostosis" (ICD-10 codes) Limb defect: **Q66-Q74** (excluding: **Q662-Q669**, Q670-Q678, Q680, Q682A, Q683-Q685, Q740G) Other cranial defects: Q183, Q188, Q755, Q758, Q759 Craniosynostosis: Q750	**Molgaard-Nielsen excluded Q65 (congenital deformities of hip) whereas Bérard included Q65.** **Zhu included (755.XX) whereas Bérard excluded 755.1 (syndactyly)**