Methods for meta-analysis

1. Study eligibility criteria

To get an overview of which studies should be included in the meta-analysis we defined the inclusion criteria by defining population, exposure, comparator, and outcome (PECO) [1]. Non-original research e.g., reviews, replies and meta-analyses were excluded.

In the *population* each pregnancy must only be included once in the meta-analyses to avoid bias of the weighted estimate and therefore the population in each study must be carefully considered. Especially information regarding the country and the time of which the population are recruited must be obtained and compared between the studies to avoid overlapping cohorts.

The *exposure* included any antidepressants in the first trimester. The risk of spontaneous abortion differs greatly within gestational age and therefore it is necessary to define the exposure window when examining studies of pregnant women [2]. Spontaneous abortion is defined as loss before the 22nd week of pregnancy, and in most cases it occurs before the 12th week [3]. Consequently the etiologically relevant window in the included studies must be between the conception and the 22nd week of pregnancy in case the pregnancy should be considered exposed to antidepressants. We therefore included studies that obtained information on antidepressant exposure in the 1st trimester.

The *comparator* group must be pregnancies that are not exposed to the antidepressant during the etiologically defined window. There are several options when defining this group. It could consist of unexposed pregnancy (regardless of depression diagnosis), pregnancies exposed to nonteratogens or pregnancies exposed to other antidepressants than the antidepressant concerned, a so-called active comparator group. Pregnant women with a diagnosis of untreated depression could be used to reduce confounding by indication and thereby increase the internal validity [2]. All comparison groups were considered relevant.

The *outcome* was spontaneous abortion defined as a record of pregnancy loss before the 22nd week of gestation.

2. Systematic literature search

Based on the above principles, we conducted a literature review applying the inclusion and exclusion criteria as follows. We searched the PubMed database and Embase in February 2021 using the terms shown in Table 1:

PubMed (all fields): (antidepressant OR TCA OR SNRI OR SSRI) AND (spontaneous abortion OR miscarriage OR pregnancy loss) Filters: Humans, Danish, English, from 2000 - 2021 (141 hits) Embase (all fields): ('antidepressant agent' OR TCA OR 'serotonin noradrenalin reuptake inhibitor' OR 'serotonin uptake inhibitor') AND ('spontaneous abortion' OR miscarriage OR 'pregnancy loss') AND [2000-2021]/py (382 hits)

Table 1 - Search fields used for the systematic literature search in PubMed and Embase

References listed in relevant articles were examined to make sure suitable articles were not overlooked. Two additional articles were found by this strategy (Einarson 2003[4], Sivojelozova 2005[5]). The filter “humans" was applied in the PubMed search since the aim was to look at human species. Original research articles in English or Danish language published between 2000 to 2021 were included. The inclusion criteria made it possible to look for suitable articles among those obtained by the specific search. This was done by reading the title and abstract of the articles and evaluating the relevance for the research question i.e. the association between antidepressant-use and spontaneous abortion. Two independent reviewers (Christine and Tobias) screened the articles making it less likely to overlook relevant articles. Articles not satisfying the inclusion criteria were excluded. Out of the 471 initially identified articles, 16 were selected for full-text review. Of those, 13 articles met the inclusion criteria and were included the meta-analysis *(figure 1).*

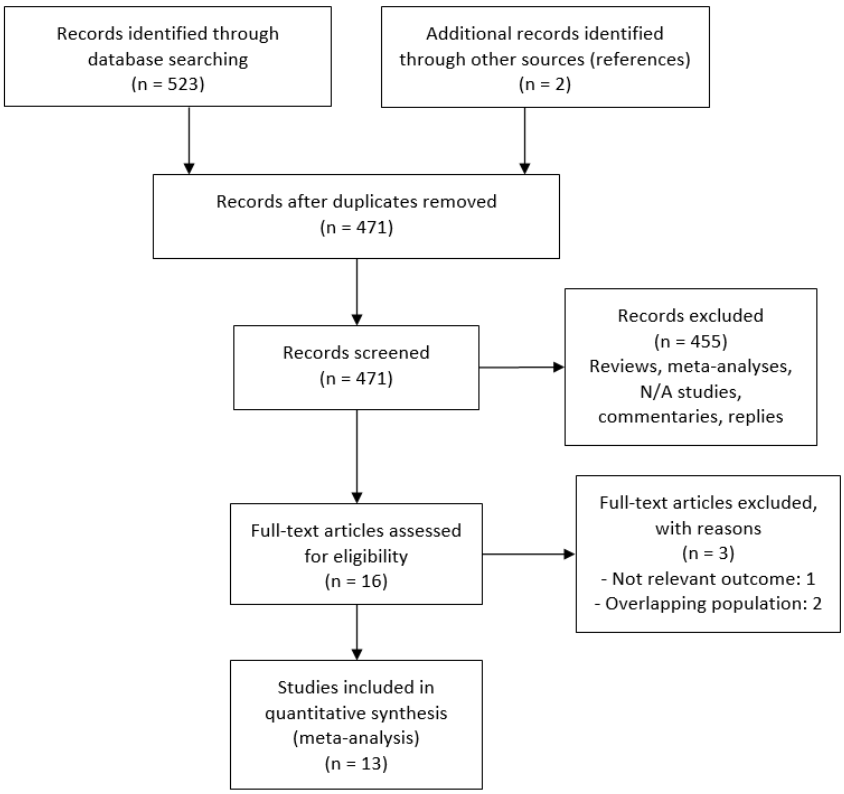

Figure 1 – Flowchart of included and excluded articles

Three of the identified records – all Danish registry-based studies – had overlapping populations (Andersen et al. Kjaersgaard et al. and Johansen et al [6-8]). All three articles adjusted for maternal age, income, and education [6-8]. Both Johansen et al. and Andersen et al. also adjusted for previous spontaneous abortion [6 8]. Johansen et al. further adjusted for self-reported mental illness, self-perceived health, use of alcohol, smoking, exercise, BMI, and if the pregnancy was planned [8]. We choose to include the study by Johansen et al. for the meta-analysis because it accounted for several confounders that the others did not.

3. Statistical methods

In the main meta-analysis, the aim was to estimate the effect of any antidepressant use in pregnancy and the risk of spontaneous abortion. This entailed different decisions on how the data should be analyzed. Firstly, some of the studies included in the meta-analysis had a second reference group of antidepressant users besides the non-teratogenic comparison-group. As this group used different antidepressant agents than the exposed group it was decided that data from the exposed group and the antidepressant exposed reference group could be combined. When this was the case the prevalence ratio, standard error and 95%-confidence interval were

calculated from the reported data by using Epibasic [9]. The prevalence ratios calculated were all from cross-sectional studies and are thus estimates of relative risk in cohort studies. Secondly, Almeida et al. had two comparison groups: women with and without the diagnosis of depression [10]. As the other studies did not differentiate their comparison group in depressed and non-depressed the two comparison groups were combined to one group. Thirdly, spontaneous abortion is a common outcome, with a prevalence of 15-20% [3]. This is important as the studies included in the meta-analysis used different measures of effect. Usually when it is a common outcome odds ratio and risk ratio cannot be assumed to be the same. However, there are exceptions to this. A nested control study included in our study used density sampling. The odds ratio can in this case be seen as the rate ratio without a rare disease assumption [11]. Furthermore, with the follow up time being short (pregnancy lasts about 9 months) the difference in person-time risk between cases and controls will be limited why the rate ratio can be seen as the relative risk. Finally, to estimate the combined effect both the random effects model and the fixed effect model were considered. Looking at similarities and heterogeneity, some of the studies included in the meta-analysis were alike in setup as several used the same teratology information service and way of collecting data. On the other hand, several studies were different regarding methods and even study design. Additionally, the composition of the antidepressant exposed group was different in the studies, which is why variation in the true effect for each study could be expected. As the random-effects model estimates the mean of the distribution of true effects, this model was chosen to estimate the combined effect [12]. Based on these decisions and calculations the combined effect was estimated using 'Episheet' software [13].

In addition to the main meta-analysis it was of interest and possible based on the studies included to make two separate sub-analyses. The first sub-analysis examined the association between SSRI use during pregnancy and spontaneous abortion. This sub-analysis was of interest as it allows to address the specificity of association, and because SSRIs are the most prevalent antidepressants used by Danish women of reproductive age (*Figure 2*). The second sub-analysis compared risk of spontaneous abortion in antidepressant-exposed pregnancies with pregnancies among women with untreated depression. This was done to reduce the risk of confounding by indication. Data for the two sub-analyses were extracted from the studies in which relevant information was directly reported or could be inferred from the publication.

The influence of a study result on a publication decision is called publication bias. As a result, published studies do not represent the real population of studies done. Large studies that show an association are more likely to be published than small or "null" studies [1]. To visualize this, a funnel plot is often used, with standard error on the vertical axis and natural logarithm to the effect size on the horizontal axis. This means larger studies generally will be grouped at the top of the funnel plot due to smaller standard error and the smaller studies at the bottom. An asymmetrical funnel plot is indicative of potential publication bias.

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