Prenatal nitrosatable prescription drug intake, drinking water nitrate, and the risk of stillbirth: a register- and population-based cohort of Danish pregnancies, 1997–2017

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Abstract

Background: Nitrosatable drugs commonly prescribed during pregnancy can react with nitrite to form N-nitroso compounds which have been associated with an increased risk of stillbirth. Whether maternal residential drinking water nitrate modifies this association is unknown. We investigated, if household drinking water nitrate was associated with stillbirth, and if it modified the association between nitrosatable prescription drug intake and the risk of stillbirth.

Methods: We conducted an individual-level register- and population-based cohort study using 652,810 women with the first recorded singleton pregnancy in the Danish Medical Birth Registry between 1997 and 2017. Nitrosatable drug exposure was recorded by use of the Danish National Patient Registry defined as women with a first redeemed prescription of a nitrosatable drug the first 22 weeks of pregnancy. The reference group was women with no redeemed prescription of a nitrosatable drug in this period. The average individual drinking water nitrate concentration level (mg/L) was calculated in the same period. We categorized nitrosatable drugs as secondary amines, tertiary amines, and amides. Cox hazard regression was used to estimate crude and adjusted hazard ratios with 95% confidence intervals for stillbirth stratified into five categories of nitrate concentrations: ≤ 1 mg/L, > 1–≤ 2 mg/L, > 2–≤ 5 mg/L, > 5–≤ 25 mg/L, and > 25 mg/L.

Results: Drinking water nitrate exposure in the population was not associated with the risk of stillbirth. Among 100,244 women who had a nitrosatable prescription drug redeemed ≤ 22 weeks of pregnancy, 418 (0.42%) had a stillbirth compared to 1993 stillbirths (0.36%) among 552,566 referent women. Women with any nitrosatable prescription drug intake and > 1–≤ 2 mg/L nitrate concentration had an increased risk of stillbirth [adjusted hazard ratio 1.55 (95% confidence interval, 1.15–2.09)] compared with referent women. In the stratified analyses, the highest risk of stillbirth was found among women with secondary amine intake and > 25 mg/L nitrate concentrations [adjusted hazard ratio 3.11 (95% CI, 1.08–8.94)].

Conclusions: The association between nitrosatable prescription drug intake and the risk of stillbirth may depend on the level of nitrate in household drinking water. Evaluations of the effect of nitrosatable drug intake on perinatal outcomes might consider nitrate exposure from drinking water.

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Background
An estimated 15–24% of U.S. and Danish women use a nitrosatable drug at some point in time during pregnancy, including common drugs such as antibiotics, asthma medications and antiemetics [1, 2]. This broad range of medications contain compounds such as secondary amines, tertiary amines, and amides. Nitrate (NO₃⁻) from drinking water and other nitrate sources is converted to nitrite (NO₂⁻) in the human body and subsequently can react with amines and amides in the gastrointestinal tract to form teratogenic N-nitroso compounds (NOCs) under highly acidic environments as in the stomach. NOCs are formed to a greater extent, if the concentration of nitrosating agent is high [3, 4]. NOC formation in the maternal stomach and the subsequent transplacental transmission to the fetus is the most likely source of prenatal exposure to NOCs [5–7]. The teratogenic effect of NOCs may be caused by abnormal development through DNA alkylation [8].

While prenatal nitrosatable drug exposure may cause congenital malformations and preterm birth, few studies have examined the relation between nitrosatable drugs and stillbirth [2, 9–13]. Stillbirth is a rare adverse pregnancy outcome, in Denmark and in other high-income countries, with an estimated rate of 2–5 per 1000 births [14]. The etiology of stillbirth is mainly unknown, but impaired placental function is as a possible major underlying risk factor. Other risk factors include advanced parental age, primiparity, high body mass index (BMI), and maternal smoking during pregnancy, infections, and diseases such as diabetes and hypertension [15–19]. Our results from an earlier study indicated that nitrosatable prescription drug intake (e.g. use of penicillin for infection or terbutaline for asthma) during the first 22 weeks of pregnancy may increase the risk of stillbirth. However, we lacked data on sources of NO₃⁻ exposure that may modify the association between nitrosatable drug exposure and the risk of stillbirth, including data from drinking water sources [2].

Danish tap water is based completely on groundwater with farming contributing to NO₃⁻ contamination [20]. Roughly 5% of the Danish population is exposed to tap water concentrations > 25 mg/L NO₃⁻, with levels having decreased during the last decades, because of national agricultural nitrogen regulation, local action plans, and infrastructural changes [21]. To protect against infant methemoglobinemia, European Union regulatory limits have been set to 50 mg/L NO₃⁻ in drinking water and a similar level has been set in the U.S. (44 mg/L NO₃⁻). NO₃⁻ exposure below the current regulatory limits has, however, been associated with miscarriage, intrauterine growth restriction, stillbirth, premature birth, and childhood cancer [22–26].

In this study, we examined whether NO₃⁻ in maternal residential drinking water was associated with the risk of stillbirth, and if it modified the association between prenatal nitrosatable prescription drug intake and the risk of stillbirth. Our hypothesis was that the risk of stillbirth would be higher among women who had a nitrosatable prescription drug redeemed during pregnancy and whose residential water NO₃⁻ concentrations were > 25 mg/L compared to those, who did not redeem a nitrosatable prescription drug.

Methods
Study design and population
We conducted an individual-level register- and population-based cohort study using data from the Danish Civil Registration System, the Danish Medical Birth Registry, and the Danish National Prescription Register [27–29]. All women with a registered singleton and first recorded pregnancies in Denmark between 1997 and 2017 were identified from the Danish Medical Birth Registry (n = 707,467), regardless of their previous pregnancies before the study period. After excluding 54,657 births with coding errors on gestational length, maternal drinking water estimates from private wells, or missing drinking NO₃⁻ data, our study population included a total of 652,810 mother-child dyads.

Data sources
The registers utilized in this study offer an exceptional resource with their depth and breadth of data collected. The unique civil personal registration (CPR) number, which has been assigned to nearly all (> 99%) Danish residents since 1968, was used as key identifier to link data between the national registries. In addition, the Danish Civil Registration System holds weekly updated information on date of birth, sex, and residential history [27].

The Danish Medical Birth Registry has information on all births in Denmark from 1973 onwards, including the CPR number of the child, the mother, and the registered father. It also holds information on gestational age based mainly on ultrasound measures and occasionally on last menstrual period, height and weight at birth, birth date, birth outcome (liveborn or death and cause of death), sex, parental age, and number of previous births [28].

Keywords: Nitrosatable drug, Amine, Amide, N-nitroso compounds, Stillbirth, Drinking water nitrate, Cohort study
The Danish National Prescription Register holds information on redemption of prescribed medication. This register has individual-level data on all prescribed drugs dispensed at any pharmacy in Denmark since 1995 [29]. Pharmacies are required to register all redeemed prescriptions, which are coupled with the reimbursement of expenses from the state. This ensures highly accurate prescription data and completeness has been estimated to be >97% [30].

Nitrosatable drug assessment
Detailed procedures for classifying as nitrosatable and for categorizing them based on their functional groups and indications have been discussed in detail elsewhere [1]. We used nitrosatable medicinal compounds lists from published literature to classify nitrosatability, and we included the medication reported to be used between 1997 and 2017 [1, 4, 31]. We included only the medication reported to be used in our population in the study period. All drugs were categorized on the basis of the presence of secondary amine, tertiary amine, and amide functional groups. These categories are not mutually exclusive.

We identified the Anatomical Therapeutic Chemical codes for the categorized drugs and matched these with codes in the Danish National Prescription Register, which also contained information on the date of redemption of the prescription (see Supplementary Table S1 for a complete list of included nitrosatable drugs). All analyses are based on the first nitrosatable prescription drug redeemed from the estimated first day in the last menstrual period (week 0) until the end of week 22 of pregnancy. Women with no redeemed nitrosatable prescription drug during this period were defined as the unexposed comparison.

Drinking water NO₃⁻ assessment
The Danish national geodatabase Jupiter holds drinking water monitoring data [32]. The maternal residential drinking water NO₃⁻ estimates build on the approach developed and described by Schullehner et al. [33]. In short, annual drinking water production volume weighted average NO₃⁻ concentrations were calculated for public water supply areas and spatio-temporally linked with the geocoded residential history of every person registered in the Danish Civil Registration System. For this study, 107,821 NO₃⁻ samples in 3635 public waterworks between 1993 and 2017 were extracted. NO₃⁻ levels were imputed by interpolation for missing years, if a NO₃⁻ sample was available within 3 years, otherwise the year was assigned as missing NO₃⁻. Individual exposure for each woman was computed as the time-weighted average concentration during pregnancy taking into account changes in residence during pregnancy [34]. The average NO₃⁻ concentration level (mg/L) for each woman was calculated in the period from the estimated first day in the last menstrual period (week 0) until the end of week 22. As private wells are not monitored to the same degree as public supplies, users of private wells (approximately 3% of Danish residents) were excluded from the study population.

Stillbirth assessment
Before 1 January 2004, stillbirth in Denmark was defined as the birth of a non-vital fetus after ≥28 completed weeks of pregnancy. In 2004 the cut point changed to 22 completed weeks, as in most other countries [35]. To harmonize the study population, we extracted all registered stillbirths born at 22 weeks of pregnancy or later between 1997 and 2017. Pregnancy period was based on the child’s date of birth and gestational age at birth in days.

Statistical analyses
We investigated, if drinking water NO₃⁻ in the population was associated with stillbirth by using Cox proportional hazards model with gestational age (days) as the underlying time scale. We estimated crude and adjusted hazard ratios (aHRs) and corresponding 95% confidence intervals (CIs) for stillbirth in relation to five categories of residential drinking water NO₃⁻ concentrations defined a priori according to those previously described cut-off in Coffman et al. 2021 [25]. The first category consists of exposures below the uppermost limit of detection at 1 mg/L. The four other categories were categorized as following: >1-≤2 mg/L, >2-≤5 mg/L, >5-≤25 mg/L and >25 mg/L.

We also examined whether the association between any nitrosatable prescription drug intake and stillbirth was modified on the multiplicative scale by drinking water NO₃⁻ stratified into the five categories of NO₃⁻ concentrations. In a secondary analysis, secondary amine, tertiary amine, and amide intake were also stratified into the five categories of NO₃⁻ concentrations to further examine effect medication by drinking water nitrate in these functional groups.

Adjusted analyses included the following covariates identified a priori using existing literature and directed acyclic graphs and obtained from the Danish Medical Birth Registry: parity (continuous), urbanicity of maternal address at birth (five categories), maternal age at delivery (continuous), smoking during pregnancy (yes/no), pregestational BMI (continuous), and highest level of maternal education (six categories) and maternal occupation (seven categories), which were based on the International Standard Class of Occupation and Education codes (ISCO-88 and ISCED) obtained from Statistics Denmark
The proportional hazard assumption and the linearity of covariates were accepted. All analyses were performed using Stata, version 15.0 (StataCorpLP, College Station, TX, USA) and R 4.0 (R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria).

Results

Our study included 652,810 women with a singleton and first recorded pregnancy leading to birth from 1997 to 2017, regardless of their previous pregnancies before the study period. Among these women, 100,244 (15.3%) had at least one redeemed prescription of a nitrosatable drug during the first 22 weeks of pregnancy. Among those prescribed, the most commonly redeemed drugs were phenoxymethylpenicillin (38.5%), metoclopramide (22.0%), and terbutaline (7.4%) (data not shown). Women, who had a nitrosatable prescription drug intake were generally younger, had a higher BMI, were parous, smoked, were less educated, were more often unemployed, and lived more often in rural and provincial towns compared with referent women, irrespective of NO₃⁻ (Table 1).

We found no association between NO₃⁻ exposure levels in the population and risk of stillbirth in crude and adjusted analyses (Table 2). Among the 100,244 women who had a nitrosatable prescription drug intake during the first 22 weeks of pregnancy, 418 (0.42%) had a recorded stillbirth, while among the 552,566 unexposed women 1993 (0.36%) stillbirths were recorded. Women with any nitrosatable prescription drug intake >1–2 mg/L NO₃⁻ category had a higher risk of stillbirth [aHR 1.55 (95% CI, 1.15–2.09)] compared with referent women, irrespective of NO₃⁻ (Table 1). We conditioned our analyses on survival until 22 weeks of pregnancy on the risk of stillbirth was strongest among women within the >1–2 mg/L NO₃⁻ category, whereas comparatively lower, although still higher, risks were observed with higher exposure categories. This may be attributed to survival bias where pregnancy loss or inability to conceive among women in the highest category of exposure may obscure our results. In stratified analyses, the highest risk of stillbirth was found among women with a secondary amine intake in the >25 mg/L NO₃⁻ concentration category [aHR 3.11 (95% CI, 1.08–8.94)], however, this finding should be interpreted cautiously due to the low number of cases.

In our previous study, where we found an increased risk of stillbirth with nitrosatable prescription drug intake during the first 22 weeks of pregnancy, we adjusted for municipalities as a proxy measure for drinking water NO₃⁻. Other birth outcomes studies have focused on nitrosatable drug exposure in conjunction with dietary NO₃⁻ intake and risk of adverse birth outcomes finding that nitrosatable prescription drug intake coupled with dietary NO₃⁻ intake higher than 1.93 and 3.20 mg/day increased the risk of preterm birth and neutral tube defects [38, 39]. Based on what is known about the endogenous formation of NOCs, and our most recent findings, it is important to take drinking water NO₃⁻ into account when assessing the potential harmful effects of nitrosatable drugs on the risk of adverse perinatal outcomes.

Discussion

To the best of our knowledge, this is the first study to examine potential effect measure modification by household NO₃⁻ tap water on the association between prenatal nitrosatable prescription drug intake and the risk of stillbirth. While drinking water NO₃⁻ concentrations were not associated with the risk of stillbirth, we found that the association between nitrosatable prescription drug intake may be modified by maternal residential drinking water NO₃⁻.

Overall, we observed that the potential effect of any nitrosatable prescription drug intake during the first 22 weeks of pregnancy on the risk of stillbirth was stronger among women within the >1–2 mg/L NO₃⁻ category, whereas comparatively lower, although still higher, risks were observed with higher exposure categories. This may be attributed to survival bias where pregnancy loss or inability to conceive among women in the highest category of exposure may obscure our results. We conditioned our analyses on survival until 22 weeks and the most susceptible fetuses to nitrosatable drug exposure in conjunction with higher levels of NO₃⁻ concentrations may not survive until that gestational age. If survival was influenced by the synergy of the two exposures, the magnitude of our effect may be incorrect, but likely in the correct direction [37]. In stratified analyses, the risks were slightly less than those within >1–2 mg/L NO₃⁻ category (Table 3).

We found similar results to the main analysis, when we examined secondary amines, tertiary amines, and amides stratified into the five categories of NO₃⁻ concentrations. Overall, the highest risk of stillbirth [aHR 3.11 (95% CI, 1.08–8.94)] was found among women with a secondary amine intake and >25 mg/L NO₃⁻ concentrations compared with referent women. Among women with tertiary amine or amide intake and >25 mg/L NO₃⁻ exposure, the risk of stillbirth was higher with an estimated [aHR 1.79 (95% CI, 1.18–2.69)] and an [aHR 1.45 (95% CI, 1.02–2.06)] compared to referent women (Table 4).

The associations with each of the most common drugs as exposures (phenoxymethylpenicillin, metoclopramide, terbutaline), stratified into the five categories of NO₃⁻ concentrations, were similar to our main analysis (data not shown).
Table 1  Characteristics of the cohort of women who redeemed, and who did not redeem a nitrosatable prescription drug during the first 22 weeks of pregnancy, 1997–2017, Denmark

| Characteristic                                | Redeemed nitrosatable drug during the first 22 weeks of pregnancy |
|-----------------------------------------------|-------------------------------------------------------------------|
|                                               | No                  | Yes                  |
|                                               | (n = 552,566)       | (n = 100,244)        |
| Age, y, n (%)                                 |                     |                     |
| <19                                           | 5706 (1.0)          | 1266 (1.3)          |
| 19–24                                         | 66,476 (12.0)       | 14,666 (14.6)       |
| 24–29                                         | 193,577 (35.0)      | 34,355 (34.3)       |
| 29–34                                         | 189,329 (34.3)      | 32,348 (32.3)       |
| > 34                                          | 96,972 (17.5)       | 17,541 (17.5)       |
| Missing                                       | 506 (0.0)           | 68 (0.0)            |
| Body mass index, kg/m², n (%)                 |                     |                     |
| <18                                           | 14,607 (2.6)        | 2336 (2.3)          |
| 18–25                                         | 192,003 (34.7)      | 27,979 (27.9)       |
| 25–30                                         | 55,658 (10.1)       | 10,271 (10.2)       |
| > 30                                          | 29,124 (5.3)        | 7361 (7.3)          |
| Missing                                       | 261,174 (47.3)      | 52,297 (52.2)       |
| Parity, n (%)                                 |                     |                     |
| Nulliparous                                   | 422,921 (76.5)      | 68,909 (68.7)       |
| Primiparous                                   | 76,403 (13.8)       | 17,930 (17.9)       |
| Multiparous                                   | 43,918 (7.9)        | 11,570 (11.5)       |
| Missing                                       | 9324 (1.7)          | 1835 (1.8)          |
| Smoking during pregnancy, n (%)               |                     |                     |
| No                                            | 401,154 (72.6)      | 66,726 (66.6)       |
| Yes                                           | 82,775 (15.0)       | 19,259 (19.2)       |
| Missing                                       | 68,637 (12.4)       | 14,259 (14.2)       |
| Maternal level of education, n (%)            |                     |                     |
| Higher education or PhD                       | 58,747 (10.6)       | 6820 (6.8)          |
| Middle education                              | 124,762 (22.6)      | 18,744 (18.7)       |
| Basic education                               | 37,321 (6.8)        | 5834 (5.8)          |
| High School or vocational                     | 213,658 (38.7)      | 40,075 (40.0)       |
| Primary school                                | 101,265 (18.3)      | 26,277 (26.2)       |
| Unknown                                       | 13,584 (2.5)        | 2171 (2.2)          |
| Missing                                       | 3229 (0.6)          | 323 (0.3)           |
| Maternal occupation, n (%)                    |                     |                     |
| Owner of business                             | 10,267 (1.9)        | 1606 (1.6)          |
| Chief executive or employee with high income  | 70,443 (12.7)       | 9270 (9.2)          |
| Employee with middle income                   | 106,673 (19.3)      | 16,453 (16.4)       |
| Employee with low income                      | 153,572 (27.8)      | 30,148 (30.1)       |
| Employee unspecified income                   | 81,095 (14.7)       | 14,403 (14.4)       |
| Pensioners                                    | 2301 (0.4)          | 1087 (1.1)          |
| Unemployed                                    | 127,184 (23.0)      | 27,231 (27.2)       |
| Missing                                       | 1031 (0.2)          | 46 (0.0)            |
| Urbanicity of maternal address at birth, n (%)|                     |                     |
| Rural areasa                                  | 130,413 (23.6)      | 26,497 (26.4)       |
| Provincial townb                              | 143,405 (26.0)      | 28,956 (28.9)       |
| Provincial cityc                              | 84,114 (15.2)       | 13,289 (13.3)       |
| Suburb of capital                             | 63,944 (11.6)       | 12,403 (12.4)       |
| Capital                                       | 121,507 (22.0)      | 18,205 (18.2)       |
| Missing                                       | 9183 (1.7)          | 894 (0.9)           |
However, as patients have to pay a portion of the cost of their redeemed prescriptions, our estimates likely reflect actual drug intake. An additional concern is use of over-the-counter nitrosatable drugs because these are not covered by registry data. However, intake of over-the-counter drugs would result in misclassification of exposed as unexposed and likely bias our findings to the null. We only included drugs according to ATC codes independent of administration route. Topical applied drugs such as nicotine replacement will not lead to formation of NOCs as in the acidic environment in the stomach which would lead to misclassification of unexposed as exposed and likely further bias our findings to the null.

Furthermore, although our classification of nitrosatable drugs is based on the most extensive reviews and up-to-date resources available, there is still the possibility that some nitrosatable drugs have been missed as the...
components have not been tested for the formation of genotoxic-carcinogenic N-nitroso derivatives.

Moreover, nitrosamine impurities have been found in drugs such as Valsartan and Varenicline (https://www.fda.gov/drugs/drug-safety-and-availability/information-about-nitrosamine-impurities-medications, https://www.ema.europa.eu/en/human-regulatory/post-authorisation/referral-procedures/nitrosamine-impurities). However, we did not include these drugs in our exposure, because of the low level of nitrosamines concentrations. These drugs are not classified as nitrosatable drugs, and we doubt that including them in the exposure would bring new insights of importance, and if we did so more drugs should also be considered.

It is expected that exposure misclassification due to consumption of bottled water could also be present. However, we do not consider this to be a large source of bias, because annual bottled water consumption in Denmark is among the lowest in Europe (~26 L per person) [40]. We were not able to quantify the amount of water a woman consumed and therefore assumed equivalent consumption for all pregnancies.

### Table 4

| Household NO3- average (mg/L) | Secondary amine intake during the first 22 weeks of pregnancy | No stillbirth | Stillbirth | HR (95% CI) |
|-----------------------------|-------------------------------------------------------------|--------------|-----------|-------------|
|                             | Unadjusted Adjusted a                                      |              |           |             |
| ≤ 1                         | No                                                          | 146,486      | 594       | 1           | 1           |
|                             | Yes                                                         | 4947         | 22        | 1.12 (0.74–1.72) | 1.22 (0.64–2.30) |
| > 1–≤2                      | No                                                          | 192,737      | 682       | 1           | 1           |
|                             | Yes                                                         | 6044         | 31        | 1.50 (1.05–2.15) | 1.39 (0.78–2.49) |
| > 2–≤5                      | No                                                          | 207,954      | 723       | 1           | 1           |
|                             | Yes                                                         | 6666         | 26        | 1.16 (0.78–1.72) | 1.20 (0.65–2.20) |
| > 5–25                      | No                                                          | 61,126       | 234       | 1           | 1           |
|                             | Yes                                                         | 2095         | 6         | 0.78 (0.35–1.76) | 0.38 (0.05–2.76) |
| > 25                        | No                                                          | 21,700       | 87        | 1           | 1           |
|                             | Yes                                                         | 644          | 6         | 2.36 (1.03–5.40) | 3.11 (1.08–8.94) |
| Household NO3- average (mg/L)| Tertiary amine intake during the first 22 weeks of pregnancy| No stillbirth | Stillbirth | HR (95% CI) |
|                             | Unadjusted Adjusted a                                      |              |           |             |
| ≤ 1                         | No                                                          | 141,881      | 583       | 1           | 1           |
|                             | Yes                                                         | 9552         | 33        | 0.85 (0.60–1.21) | 0.55 (0.27–1.12) |
| > 1–≤2                      | No                                                          | 187,809      | 668       | 1           | 1           |
|                             | Yes                                                         | 10,972       | 45        | 1.18 (0.87–1.59) | 1.79 (1.18–2.69) |
| > 2–≤5                      | No                                                          | 201,622      | 700       | 1           | 1           |
|                             | Yes                                                         | 12,998       | 49        | 1.11 (0.83–1.49) | 1.34 (0.85–2.12) |
| > 5–25                      | No                                                          | 58,941       | 221       | 1           | 1           |
|                             | Yes                                                         | 4280         | 19        | 1.21 (0.76–1.94) | 1.61 (0.74–3.53) |
| > 25                        | No                                                          | 20,950       | 87        | 1           | 1           |
|                             | Yes                                                         | 1394         | 6         | 1.07 (0.47–2.44) | 1.54 (0.54–4.35) |
| Household NO3- average (mg/L)| Amide intake during the first 22 weeks of pregnancy          | No stillbirth | Stillbirth | HR (95% CI) |
|                             | Unadjusted Adjusted a                                      |              |           |             |
| ≤ 1                         | No                                                          | 134,846      | 550       | 1           | 1           |
|                             | Yes                                                         | 16,587       | 66        | 0.98 (0.76–1.27) | 0.79 (0.50–1.27) |
| > 1–≤2                      | No                                                          | 178,988      | 622       | 1           | 1           |
|                             | Yes                                                         | 19,793       | 91        | 1.34 (1.08–1.67) | 1.45 (1.02–2.06) |
| > 2–≤5                      | No                                                          | 191,278      | 668       | 1           | 1           |
|                             | Yes                                                         | 23,342       | 81        | 1.01 (0.80–1.27) | 1.07 (0.72–1.59) |
| > 5–25                      | No                                                          | 55,633       | 202       | 1           | 1           |
|                             | Yes                                                         | 7588         | 38        | 1.40 (0.99–1.98) | 1.50 (0.79–2.86) |
| > 25                        | No                                                          | 19,854       | 83        | 1           | 1           |
|                             | Yes                                                         | 2490         | 10        | 0.98 (0.51–1.90) | 0.90 (0.32–2.54) |

*a Adjusted for parity, urbanicity and maternal age, body mass index, smoking, education and occupation*
Confounding by indication is of concern, as in most non-randomized observational studies. Drugs are prescribed for a medical purpose, and the estimated association could be caused by this underlying condition rather than the drug. We presume however, that if this bias was a major problem, we would have observed variation in associations by drug type according to their functional groups, which we did not. We found similar increased risk for all nitrosatable drug types and the variation in magnitude of the risk between functional groups was small. We did adjust for confounders available from the Danish registries including maternal age, parity, and lifestyle factors; however, we had no information on maternal infections and comorbidities, and therefore residual confounding is possible.

We also did not have information on individual dietary sources of NO$_3^-$ and NO$_2^-$ (i.e. green vegetables and processed meat), antioxidant supplementation, or vitamin C. Processed red meat products, may also contain low preformed NOCs [41]. However, while high drinking water NO$_3^-$ is consistently associated with endogenous capacity, dietary NO$_3^-$ is less likely to increase nitrosation due to the presence of nitrosation inhibitors such as polyphenols and vitamin C in vegetables, which are the largest contributor to dietary NO$_3^-$ intake [42, 43]. Further, dietary factors vary by socioeconomic status and by urbanicity. To partly control for NO$_3^-$ intake from dietary sources, we adjusted for maternal education and occupation and urbanicity in the analyses.

Our study had several strengths, including individual-level estimates of NO$_3^-$ in maternal drinking water based on measurements performed by certified laboratories. We were able to obtain and link these measurements even as maternal residence changed throughout pregnancy instead of relying on residence at birth. We had a large population and used longitudinal register data, thereby reducing the risk of selection bias and eliminating the risk of recall bias; information on nitrosatable drug exposure was recorded before and independently of the outcome. The classification of stillbirth was based on registry data, with records for inpatient and outpatient treatment in a free of charge antenatal program, which almost all Danish pregnant women take part in [44].

**Conclusions**

Drinking water NO$_3^-$ was not associated with risk of stillbirth, however, we found some evidence that the association between prenatal nitrosatable prescription drug intake and the risk of stillbirth may depend on the level of NO$_3^-$ in household drinking water. As nitrosatable prescription drug intake is common during pregnancy and the contamination of groundwater by NO$_3^-$ is ubiquitous, it may be important to consider levels of drinking water NO$_3^-$, when examining the effect of nitrosatable drug exposure on the risk of perinatal outcomes. Large multicenter studies examining fetal death should be conducted in order to make recommendations to prescribers and regulators.

**Abbreviations**

NO$_3^-$: Nitrate; NO$_2^-$: Nitrite; NOCs: N-nitroso compounds; CIs: Confidence intervals; HRs: Hazard ratios; aHRs: Adjusted hazard ratios.

**Supplementary Information**

The online version contains supplementary material available at [https://doi.org/10.1186/s12940-021-00805-z](https://doi.org/10.1186/s12940-021-00805-z).

**Additional file 1. Table S1.** List of included nitrosatable drugs with Anatomical Therapeutic Chemical (ATC) codes.

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Not applicable.

**Authors’ contributions**

Thomsen had full permission to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Concept and design: Thomsen, Olsen, and Liew. Acquisition, analysis, or interpretation of data: Thomsen, Schullehner, Olsen, Ramlau-Hansen, Ebdrup, Liew, and Coffman. Drafting the manuscript: Thomsen, Olsen, Ramlau-Hansen, Coffman and Ebdrup. Critical revision of the manuscript for important intellectual content: All authors. Statistical analysis: Thomsen and Schullehner. Obtained funding: Olsen and Liew. Administrative, technical, or material support: Ramlau-Hansen and Schullehner. Supervision: Olsen, Ramlau-Hansen, Schullehner, and Liew. The author(s) read and approved the final manuscript.

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**Availability of data and materials**

The analyses for this study were performed at Statistics Denmark within a strict privacy rules where only researchers who received a signed permit were allowed to do analyses within a secured environment.

**Declarations**

**Ethics approval and consent to participate**

In Denmark, the Act on Processing of Personal Data does not require ethical committee permission or obtained consent for anonymized register studies on existing data. The present study was registered at Aarhus University’s Data Inventory (2020-051-000001-1897).

**Consent for publication**

All the authors have reviewed and approved the manuscript for publication.

**Competing interests**

The authors declare no conflict of interest.

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