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Exposure to Faecal Contaminated Tap Water in Early Pregnancy: Incidence of Gestational Diabetes Mellitus and Subsequent Type 2 Diabetes

Ritva Kannisto[1,2]*; Maarit K Leinonen[3]; Jaana I Halonen[1]; Kati Tihtonen[4]; Mika Gissler[3,5]

[1]Finnish Institute for Health and Welfare, Department of Public Health, P.O. Box 30, 00271 Helsinki, Finland.

[2]Department of Internal Medicine, Mikkeli Central Hospital, South Savo Wellbeing Services County, Porrassalmenkatu 35-37, 50100 Mikkeli, Finland.

[3]Finnish Institute for Health and Welfare, Department of Data and Analytics, P.O. Box 30, 00271 Helsinki, Finland.

[4]Department of Obstetrics and Gynecology, Tampere University Hospital, Wellbeing Services county of Pirkanmaa, Elämänaukio 2, 33520 Tampere, Finland & Faculty of Medicine and Health Technology, Tampere University, Arvo Ylpön katu 34, 33520 Tampere, Finland.

[5]Region Stockholm, Academic Primary Health Care Centre, Solnavägen 1 E, 113 65 Stockholm, Sweden & Karolinska Institutet, Department of Molecular Medicine and Surgery, Anna Steckséns gata 53, 17176 Stockholm, Sweden.

***Corresponding Author(s): Ritva Kannisto**

Department of Internal Medicine, Mikkeli Central Hospital, Porrassalmenkatu 35-37, 50100 Mikkeli, Finland.

Tel: +358-403597556

Email: ritva.kannisto@etelasavonha.fi

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Abstract

Objective: In Finland a water supply accident exposed over 9000 inhabitants to a wide spectrum of human faecal microbes through contaminated tap water for two days in 2007. We hypothesized that the exposure equated with faecal microbiota transplantation. Since gut microbiome dysbiosis has been associated with metabolic disorders such as obesity and diabetes, our aim was to study the incidence of Gestational Diabetes Mellitus (GDM) and subsequent Type 2 Diabetes (T2DM) in women who were exposed to the contaminated water in early pregnancy or during six months before gestation. We hypothesised that the contaminated water would provide prevention against GDM and type 2 diabetes. In addition, our aim was to form a research cohort for further studies to examine the health of the children born from these pregnancies.

Methods: The study was a national register-based study (n=43 096). The contaminated water group included 100 parturients. Three comparison groups included unexposed parturients at the same stage of pregnancy living in the same town (n=170), in another municipality in the same region (n=244) and elsewhere in Finland (n=42 104).

Results: No statistically significant differences were observed in the incidence of GDM or subsequent T2DM between the exposed and the comparison groups.

Conclusion: Exposure to faecal microbiome contaminated tap water did not provide statistically demonstrable protection against GDM and T2DM. The cohort can be used for further studies to follow-up the metabolic health of the offspring and their health by the principles of the hygiene hypothesis.

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Introduction

The gut microbiome dysbiosis has been shown to be associated with obesity, metabolic syndrome (MetS), Type 2 Diabetes (T2DM), and Gestational Diabetes Mellitus (GDM) [1-3]. During pregnancy, the gut microbiota undergoes changes from first (T1) to third (T3) trimesters [4,5]. When transferred to germ-free mice,T3-microbiota induced metabolic changes resembling MetS [4]. Most studies have focused on late-pregnancy microbiota composition [5]. One study of 75 overweight and obese pregnants demonstrated that already in T1 the faecal microbiota differed in pregnants who developed GDM from those who did not [6]. Thus, the gut microbiota aberrations seemed to precede diagnosis of GDM [6]. Nutrition, antibiotics, and e.g., proton pump inhibitors (PPIs), metformin and selective serotonin reuptake inhibitors influence gut microbiome composition [7-9].

Extensive research has been conducted with the aim of finding targeted interventions to treat and prevent obesity by modifying the gut microbiome [10,11]. Faecal Microbiota Transplantation (FMT) is an established treatment for recurrent Clostridioides difficile infection [12]. The transplant material is made up of a healthy donor´s frozen or fresh stool and water as diluent and transplanted to the recipient´s gut through upper or lower gastrointestinal tract [13,14]. Evidence of weight gain and developing MetS after FMT is available from animal experiments and human case reports [15-17]. However, the results from human RCTs which have aimed weight loss and improving metabolic consequences of obesity are still inconclusive [15-20].

We investigated the incidence of GDM and subsequent T2DM in a cohort of parturients exposed to faecal microbiota via contaminated tap water prior to or during early pregnancy compared to unexposed controls. As secondary outcomes, we examined the incidence of other pregnancy complications that have been linked in prior studies to differences in microbial profile: gestational hypertension, preeclampsia, preterm birth, low birth weight and intrahepatic cholestasis of pregnancy [5,21]. Our hypothesis was that the incidence of the primary and the secondary outcomes is lower among the exposed compared to the controls. In addition, our aim was to form a research cohort for further studies to examine the health of the children born from these pregnancies.

Material and methods

A public water supply network became heavily contaminated in November 2007 in a Finnish town. During maintenance work, the wastewater network had been accidentally connected to the drinking water network and the sewage and tap water became mixed for two days. Tap water contained pathogens and a wide spectrum of human gut microbiome [22].

The town had about 30 000 inhabitants and contaminated tap water network area comprised a third of the population. In Finland tap water is almost exclusively used as drinking and household water for cooking, washing etc. Waterborne outbreak surveillance study showed that 98% of inhabitants of the contaminated area got their household water from public water supply network and during those two days they consumedan average of 4.9 glasses of non-boiled tap water per day as drinking water [22,23].

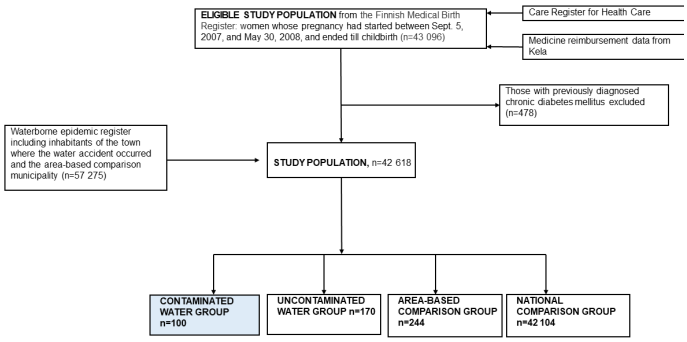

Figure 1: Flow Chart.

Formation of the study cohort

The study population was formed as shown in Figure 1. All pregnancies which started in Finland in the period from 5.9.2007 to 30.5.2008 were identified from the Medical Birth Register (MBR) maintained by the Finnish Institute for Health and Welfare. The period was defined so that the possible exposure to contaminated water occurred within six months before the start of pregnancy or during T1. This eligible study population comprised pregnancies of 43 096 parturients.

Next, pregnancies with maternal chronic diabetes were excluded as described in Appendix A. We used ICD-10 codes E10-E14 in MBR (2007-2008) and Care Register for Health Care (HILMO) (1998-2008), which includes hospitalizations and outpatient visits at specialist health care. The information on reimbursement codes and redeemed prescriptions for diabetes medicines were obtained from the Social Insurance Institution of Finland (Kela).

If diabetes was recorded during pregnancy by ICD-10 codes E10-E14, or if the special reimbursement for diabetes medication was granted during pregnancy, the diagnosis was defined as type 1 or 2 diabetes instead of GDM. After exclusions (478 parturients), the study population comprised 42 618 parturients.

The data on pregnancies was linked to a waterborne epidemic register which contains data on people who lived either in the contaminated (9 195 residents) or uncontaminated (20 821 residents) water network area, and people who lived in an area-based comparison municipality with similar living conditions (27 259 residents) at the time of water accident.

The final study population consisted of the cohort of exposed parturients who lived in the contaminated water area (n=100) and three comparison groups: unexposed parturients living in the uncontaminated water area of the same town (n=170), in the area-based comparison municipality (n=244), and elsewhere in Finland (n=42 104).

Definition and formation of the outcome variables

The primary outcomes were GDM and T2DM after the pregnancy until 2021.

GDM has been defined as glucose intolerance that was first recognized during pregnancy [24-26]. Latest definition of GDM is diabetes diagnosed in T2 or T3 that is not clearly overt diabetes prior to gestation. If diabetes is diagnosed in T1 it should be considered as previously undiagnosed diabetes [27,28]. An oral glucose tolerance test (OGTT) to diagnose GDM may be performed only in pregnants whose characteristics indicate an increased risk (targeted screening) or in all or most

pregnants (universal screening) [28-30]. In Finland in 2007, targeted screening was used and for BMI as a risk factor the limit was ≥25 kg/m² [30]. In 2008, the national Current Care Guidelines recommended to replace the targeted with universal screening, [28,29] as presented in Table 1.

An algorithm to define primary outcomes is described in detail in the Appendix A. Briefly, to define GDM we used ICD-10 codes O24.4 and O24.9 in MBR and HILMO. Additionally, information on pathological result of OGTT was obtained from MBR. To define T2DM, we used ICD-10 codes E11, E14 from HILMO and ICPC-2 code T90 from Register of Primary Health Care Visits (AvoHILMO), which holds data from all public primary care health centres since 2011. Additionally, we used information on reimbursement codes for diabetes medication issued between pregnancy end and December 31, 2021.

The secondary outcomes were gestational hypertension and preeclampsia/eclampsia, preterm birth, low birth weight (< 2500g) and intrahepatic cholestasis of pregnancy. Definitions of the secondary outcomes are presented in the Appendix B [31-34].

Other variables

From MBR, we received information on the following variables: maternal age at delivery, pre-pregnancy weight and height, parity, smoking habits, profession/socioeconomic status, specified risk factors and interventions relating to pregnancy (fertility treatment, OGTT performed, OGTT normal/pathological, insulin started), multiple pregnancy, mode of delivery, and best estimate of gestational age at delivery. Data on the offspring included date of birth, multiplicity, stillbirth/live birth, and birth weight.

The difference in full weeks between the onset of pregnancy and the time of exposure to sewage was also calculated.

By using the data on reimbursed medication, we identified the information on medical purchases according to the following periods: 1) three months before pregnancy or during T1, 2) during T2 or T3 or three months after delivery. The drugs we considered (ATC classification code): diabetes medication (insulins A10A, other A10B), systemic antibiotics (J01), gynaecological antimicrobial and antiseptic medication (G01), PPI´s (A02BC), antipsychotics (N05A), antidepressants (N06A), ursodeoxycholic acid (A05AA02).

Statistical analyses

All analyses were conducted using statistical software SAS 9.4. Differences between the study groups were calculated by using t-test, chi square test and the test of relative proportions.

Results

Baseline characteristics of the final study population are presented in Table 2.

Age: Higher age (>39 years) is one of the risk factors for GDM. The contaminated water group was slightly younger than the comparison groups. 35 years or older were 16.0% of parturients in the contaminated water group compared to the uncontaminated, area-based and the national comparison groups´, 20.6%, 20.9% and 17.8%, respectively.

Prevalence of obesity (BMI ≥ 30 kg/m²) was 15.0% in the contaminated water group, compared to 14.7%,12.7% and 10.8% in the comparison groups. Prevalence of normal or underweight (BMI < 25 kg/m²) was 62.0% vs 61.2%, 63.1% and 65.6%, respectively. However, the differences in pre-pregnancy mean BMI between the groups were statistically insignificant (Table 2). OGTT was performed for 47.0% of the parturients in the contaminated water group compared to 39.9%, 39.3% and 35.3% in the comparison groups.

Socioeconomic status appeared to be higher in the contaminated water group as there were more persons who were ranked as upper- or lower-white collar workers. However, missing data and unknown SES in this variable makes information uncertain. Slightly more were also *non-smokers.*

Parity was lower in the contaminated water group, two or more previous deliveries with 19.0% of the parturients vs 27.1%, 26.6% and 24.3% in control groups. *The mode of delivery* was comparable between the groups.

In the contaminated water group, 59.0% of the pregnancies started after the wastewater outbreak and 41.0% of the pregnancies were already ongoing at the time of the outbreak.

Use of medications that may affect the gut microbiota

Systemic antibiotics: 47% of the contaminated water group had no systemic antibiotic purchases during pregnancy, 3 months before pregnancy or 3 months after delivery compared to 51%, 47% and 53% in the comparison groups. In every group about 40% had made 1-2 purchases and at least 3 antibiotics purchases 13% in the contaminated water group vs 8%, 12% and 9% in the comparison groups (Table 3).

Gynaecological antimicrobial and antiseptic medication: purchases three months before pregnancy or duringT1 were rare (1-1.2%) in every group. During T2 or T3 or 3 months after delivery purchases were slightly more common: 4.0% in the contaminated water and 1.6-2.4% in the comparison groups.

Use of *diabetes medicines other than insulin* (ATC code A10B) was rare: only 0-1.8% had purchases 3 months before pregnancy or during T1, and 0-0.6% during T2 or T3 or 3 months after delivery, depending on the group.

Use of *antidepressants* was low and exact data cannot be presented due to small numbers. In the national comparison group 4.3%/3.4% (the first/the latter period) had made purchases. The use of *antipsychotics* and *PPIs* was even lower.

Gestational diabetes and subsequent type 2 diabetes in the final study population

The prevalence rates of GDM, subsequent T2DM and the secondary outcomes are presented in Table 4. Gestational hypertension was slightly more prevalent in the contaminated water group (7.0%) compared to the national comparison group (2.4%, p=0.002). Similar result was observed for preterm birth. For other outcomes we did not observe differences between the groups.

Table 1: 2008 guideline for universal screening for gestational diabetes using OGTT[1] in Finland.

Screening	Pregnancy weeks	Criteria
OGTT[1]	12-16	Previous GDM[2] diagnosis
		Pre-pregnancy BMI[3] ≥ 30 kg/m²
		Glucosuria in early pregnancy
		Family history of T2DM[4] (parents, grandparents, siblings, children)
		Polycystic Ovary Syndrome (PCOS)[5]
		Oral glucocorticoid medication
OGTT	24-28	Recommended to perform for all pregnants. Exceptions (no OGTT): - primiparous: < 25 years and pre-pregnancy BMI < 25 kg/m² and no family history of T2DM - multiparous: < 40 years and prepregnancy BMI < 25 kg/m² and no previous GDM diagnosis or macrosomic child
OGGT performed with 75 g glucose load limit values: ≥ 5,3 mmol/l (fasting), ≥ 10,0 mmol/l (1 h) and ≥ 8,6 mmol/l (2 h)		

[1]OGTT: Oral Glucose Tolerance Test; [2]GDM: Gestational Diabetes Mellitus; [3]BMI: Body Mass Index; [4]T2DM: Type 2 Diabetes Mellitus; [5]PCOS: Polycystic Ovary Syndrome.

Table 2: Baseline characteristics of the parturients in the study groups.

	Contaminated water parturients n=100	Uncontaminated water parturients n=170	Area-based comparison parturients n=244	National comparison parturients n=42 104
	n (%)	n (%)	n (%)	n (%)
Age at delivery, years				
< 25	16 (16.0%)	34 (20.0%)	25 (10.2%)	7 706 (18.3%)
25-29	41 (41.0%)	44 (25.9%)	75 (30.7%)	13 353 (31.7%)
30-34	27 (27.0%)	57 (33.5%)	93 (38.1%)	13 557 (32.2%)
≥ 35	16 (16.0%)	35 (20.6%)	51 (20.9%)	7 488 (17.8%)
mean/median (SD)	29.5/29.5 (4.8)	30.6/30.5 (5.4)	31.1/30.0 (5.0)	30.1/29.5 (5.4)
Prepregnancy BMI [1]				
< 18.5 kg/m²	6 (6.0%)	10 (5.9%)	9 (3.7%)	1 601 (3.8%)
18.5-24.9 kg/m²	56 (56.0%)	94 (55.3%)	145 (59.4%)	26 035 (61.8%)
25 -29.9 kg/m²	23 (23.0%)	41 (24.1%)	58 (23.8%)	8 747 (20.8%)
30 - 34.9 kg/m²	7 (7.0%)	17 (10.0%)	22 (9.0%)	3 097 (7.4%)
≥ 35 kg/m²	8 (8.0%)	8 (4.7%)	9 (3.7%)	1 437 (3.4%)
missing	0 (0.0%)	0 (0.0%)	1 (0.4%)	1 187 (2.8%)
mean/median (SD)	25.0/23.4 (5.3)	24.8/23.5 (5.0)	24.4/23.2 (4.6)	24.2/23.1 (4.7)
Parity				
0	42 (42.0%)	58 (34.1%)	90 (36.9%)	18 011 (42.9%)
1	39 (39.0%)	66 (38.8%)	89 (36.5%)	13 834 (32.9%)
2	12 (12.0%)	27 (15.9%)	35 (14.3%)	6 082 (14.4%)
≥ 3	7 (7.0%)	19 (11.2%)	30 (12.3%)	4 175 (9.9%)
Smoking in early pregnancy				
no	85 (85.0%)	141 (82.9%)	203 (83.2%)	3 4687 (82.4%)
yes	11 (11.0%)	18 (10.6%)	24 (9.8%)	6 270 (14.9%)
missing	4 (4.0%)	11 (6.5%)	17 (7.0%)	1164 (2.7%)
Socioeconomic status				
upper-white collar	19 (19.0%)	18 (10.6%)	37 (15.2%)	7 434 (17.7%)
lower-white collar	37 (37.0%)	54 (31.8%)	73 (29.9%)	13 486 (32.0%)
blue-collar	9 (9.0%)	20 (11.8%)	28 (11.5%)	5 133 (12.2%)
other	12 (12.0%)	16 (9.4%)	23 (9.4%)	4 869 (11.6%)
missing / unknown	23 (23.0%)	62 (36.5%)	83 (34.0%)	11 182 (26.6%)
Fertility treatment	3 (3.0%)	10 (4.1%)	17 (7.0%)	1 835 (4.4%)
Multifetal pregnancy	4 (4.0%)	5 (2.9%)	2 (0.8%)	623 (1.5%)
Mode of delivery				
vaginal	85 (85.0%)	143 (84.1%)	209 (85.7%)	35 319 (83.4%)
caesarean section	15 (15.0%)	27 (15.9%)	35 (14.3%)	6 772 (16.1%)
missing	0 (0.0%)	0 (0.0%)	0 (0.0%)	13 (0.03%)

[1]based on self-reported pre-pregnancy weight and height, but controlled during the first prenatal visit.

Table 3: Use of antibiotics in the study groups.

	Contaminated water parturientsn=100	Uncontaminated water parturients n=170	Area-based comparison parturients n=244	National comparison parturients n=42 104
Exposure to systemic antibiotics[1]				
In early pregnancy	29 (29.0%)	42 (24.7%)	72 (29.5%)	9 897 (23.5%)
In late pregnancy or postpartum	36 (36.0%)	61 (35.9%)	94 (38.5%)	14 633 (34.8%)
Number of antibiotic purchases				
0	47 (47.0%)	87 (51.2%)	114 (46.7%)	22 164 (52.6%)
1-2	40 (40.0%)	69 (40.6%)	101 (41.4%)	16 161 (38.4%)
3-4	10 (10.0%)	8 (4.7%)	25 (10.2%)	3 032 (7.2%)
≥ 5	3 (3.0%)	6 (3.5%)	4 (1.6%)	760 (1.8%)

[1]Exposure in early pregnancy defined as at least one antibiotic purchase from 3 months before pregnancy to the end of the first trimester. Late pregnancy and postpartum defined as at least one antibiotic purchase during the second or third trimester or three months after the delivery. A parturient may have had antibiotic purchases during both periods.

Table 4: Prevalence of the short-term and long-term outcomes.

	Contaminated water parturients n=100 Infants n=104	Uncontaminated water parturients n=170 Infants n=175	Area-based comparison parturients n=244 Infants n=246	National comparison parturients n=42 104 Infants n=42 734
	n (%)	n (%)	n (%)	n (%)
Gestational diabetes (GDM)[1]	11 (11.0%)	18 (10.6%) p-value[3] = 0.916	35 (14.3%) p-value = 0.408	4 664 (11.1%) p-value = 0.980
Gestational hypertension	7 (7.0%)	9 (5.3%) p-value = 0.566	10 (4.1%) p-value = 0.260	994 (2.4%) p-value = 0.002
Preeclampsia/ eclampsia	3 (3.0%)	4 (2.4%) p-value = 0.747	6 (2.5%) p-value = 0.775	822 (2.0%) p-value = 0.450
Intrahepatic cholestasis of pregnancy	3 (3.0%)	1 (0.6%) p-value = 0.113	7 (2.9%) p-value = 0.948	633 (1.5%) p-value = 0.220
Preterm birth, <37 weeks[2]	10 (9.6%)	16 (9.1%) p-value = 0.874	13 (5.3%) p-value = 0.115	2 435 (5.7%) p-value = 0.071
Low birth weight <2500 g[2]	3 (2.9%)	13 (7.4%) p-value = 0.118	13 (7.4%) p-value = 0.752	1 861 (4.4%) p-value = 0.490
Subsequent type 2 diabetes of those who had GDM	2 (18.2%)	3 (16.7%) p-value = 0.890	6 (17.1%) p-value = 0.789	620 (13.3%) p-value = 0.662

[1]Insulin was started for 27.3% in contaminated water group, 38.9% in uncontaminated water group, 20.0% in area-based comparison group and 18.2% in national comparison group.
[2]Numbers and percentages of infants.
[3]P-values in the table represent differences between exposed (contaminated water group) and each comparison group.

Discussion

We compared the incidence of GDM and subsequent T2DM in the group of parturients exposed to gut microbiota contaminated tap water during early pregnancy to unexposed parturients. Exposure to contaminated tap water was considered as a proxy for FMT that could be protective of the examined outcomes. We observed no differences in incidence of GDM or T2DM between groups. No clear differences were either observed for secondary outcomes, i.e., gestational hypertension and preeclampsia/eclampsia, preterm birth, low birth weight and intrahepatic cholestasis.

The amount and composition of the gut microbiome acquired by exposed individuals probably varied from person to person, and we had no accurate information on the consumption of contaminated water by the pregnant parturients who might be more careful with their drinking and eating habits than others. Accidental exposure to tap water contaminated by faecal microbiota provided a real-world intervention which would not be possible to conduct as research. Our established cohort is usable for future studies following up the offspring's health by the principles of the hygiene hypothesis.

The definition and classification of diabetes diagnosed during pregnancy has changed [24-27]. In our study, we interpreted that the person had unrecorded pre-pregnancy diabetes, if a diagnosis code of chronic diabetes appeared in registers first time during pregnancy. So, in our exclusion criteria defining pre-pregnancy diabetes we used the latest definition of GDM classification, though according to the definition at that time, every case of diabetes diagnosed during pregnancy would have been defined as GDM. That is possibly how it was mainly done in practical clinical work. In this study the pregnancies were from the year 2007 to the first months of the year 2009. Albeit universal screening for GMD was recommended in 2008, it can be assumed that mainly targeted screening existed during the study period as implementing changes in health care setting always takes time. In another Finnish study, the prevalence of GDM increased from 7.2% to 11.3% when comparing the years 2006-2008 to the years 2010-2012 [35]. In our study population the prevalence rates were slightly higher than those in general for 2006-2008: 11% in the contaminated watergroup, 11-14% in the comparison groups.

OGTT was performed more frequently in the contaminated water group, for 47%. The only risk factors indicative for targeted screening which we had in our data were age and pre-pregnancy BMI. However, the parturients in the contaminated water group were on average slightly younger and the prevalence of BMI ≥ 25 kg/m² (limit of BMI in targeted testing) was 38%. If they had other GDM risk factors for which they were tested, OGTT could have revealed more GDM cases in the contaminated water group compared to controls. It may be possible that exposure to faecal microbiota could have caused ben-

eficial changes in their microbiota that protected parturients from GDM.

A systematic review and meta-analysis of 20 studies and a total of 1 332 373 individuals assessed a nearly ten-fold risk of developing T2DM compared to those with a normoglycemic pregnancy and cumulative incidence was estimated to be around 16% for studies with more than ten years of follow-up [36]. In our study, the cumulative incidence of T2DM during the 12-13-year follow-up time was 13-18%, however, the numbers of the outcome events were low in the smaller groups.

Use of antidiabetics other than insulin (ATC code A10B) in the study population was very low. Most likely the use was metformin to improve fertility before the pregnancy, because during the study period, metformin was not yet recommended to be used for GDM and during pregnancy.

In the incidence of gestational hypertension, we found a statistically significant difference between the 7.0% of the contaminated water group and the 2.4% of the national comparison group (p=0.002), which may be one reason for the slightly higher incidence of preterm births, too. However, for prematurity the difference was statistically insignificant. Higher incidence of gestational hypertension could have caused higher prevalence of low birth weight (<2500 g). However, it was the lowest, 2.9%, in the contaminated water group and 4.3% in all children born alive in Finland in 2008 [37].

Limitations of this study are small sample size and outcome events in the study population which made the study statistically underpowered.

Conclusions

We observed no difference in the incidences of GDM or T2DM between parturients exposed to gut microbiome contaminated drinking water during early pregnancy compared to the unexposed parturients.The cohort will be used further to study health outcomes among the offspring.

Appendix: Algorithm to identify and categorize diabetes in the study. Definition of the secondary outcomes.

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Data availability: Data used in the analyses cannot be provided to third parties due to confidentiality reasons.

Ethical approval: This study was approved by the Ethics Committee of Finnish Institute for Health and Welfare. The study was register-based, and the subjects were not contacted personally. The original study collecting the data on waterborne epidemic was approved by the Ethics committee of Pirkanmaa Hospital District and the Institutional Review Board of the National Public Health Institute of Finland.

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Appendix A. Algorithm to identify and categorize diabetes mellitus in the study (in 2007-2021)

STEP 1: exclusion of the cases of pre-pregnancy diabetes mellitus (type 1, type 2 and other types of chronic diabetes)

Care Register for Health Care (HILMO): at least one inpatient or outpatient record with a diagnostic code for diabetes any time before the index pregnancy (ICD-10 codes E10-E14, ICD-9 code 250) or during the pregnancy (ICD-10 codes E10-E14)

OR

Finnish Special Refund Entitlement Register: reimbursement codes for diabetes medicines (103, 162, 171, 177, 215, 285, 295, 346, 358, 371, 382) recorded any time before or during the index pregnancy.

OR

At least one redeemed prescription for antidiabetic medication (ATC code A10) prior pregnancy; if A10B, at least one of the previous points must also be fulfilled[1].

STEP 2: identify gestational diabetes.

Medical Birth Registry variable for GDM (ICD-10 codes O24.4, O24.9)

OR

HILMO variable for GDM (ICD-10 codes O24.4, O24.9)

OR

Medical Birth Registry variable for pathological result in oral glucose tolerance test (OGTT)

STEP 3: identify subsequent type 2 diabetes mellitus.

HILMO and Register of Primary Health Care Visits (Avo-HILMO): at least one inpatient or outpatient record with a diagnostic code for diabetes (ICD-10 code E11, E14, IPCP-2 code T90) after delivery of the index pregnancy until the year 2021.

OR

Finnish Special Refund Entitlement Register: reimbursement codes 103, 162, 171, 177, 215, 285, 295, 346, 358, 371, 382 recorded any time after delivery of index pregnancy.

[1] because metformin is used for infertility without diabetes

Appendix B. Definition of the secondary outcomes

Chronic/essential hypertension: Arterial hypertension diagnosed before 20 weeks of pregnancy. Chronic hypertension was identified using ICD-10 codes I10-I15, O10-O11 in MBR or HILMO.

Gestational hypertension: Hypertension presenting after 20th week of pregnancy [31]. Gestational hypertension was identified using ICD-10 code O13 in MBR or HILMO.

Preeclampsia: Hypertension and proteinuria occurring after 20 weeks of pregnancy. Condition is called eclampsia when woman with pre-eclampsia develops convulsions [31]. Preeclampsia was identified using ICD-10 codes O14.0-14.9 and eclampsia using code O15.0 in MBR or HILMO.

Preterm birth: WHO recommends defining preterm birth as the delivery of an infant before 37^{+0} gestation weeks of pregnancy [32]. Data on gestational age at the time of delivery was obtained from the MBR.

Low birth weight: low birth weight (<2500 g) includes babies born preterm and babies with intrauterine growth restriction [32,33]. Data on birth weight were obtained from the MBR and used as a dichotomized measure (low birth weight; <2500 g).

Intrahepatic cholestasis of pregnancy (ICP): a liver disorder which occurs typically in the third trimester and is associated with significant perinatal risk. Ursodeoxycholic acid is commonly used as medication for ICP [34]. ICP was identified by ICD-10 code O26.6 in HILMO or MBR or redeemed medicine prescriptions of ursodeoxycholic acid (ATC code A05AA02) during the second or third trimester or three months after delivery.