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Cohort profile: the Swedish Maternal Microbiome project (SweMaMi) – assessing the dynamic associations between the microbiome and maternal and neonatal adverse events

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ABSTRACT

Purpose The Swedish Maternal Microbiome (SweMaMi) project was initiated to better understand the dynamics of the microbiome in pregnancy, with longitudinal microbiome sampling, shotgun metagenomics, extensive questionnaires and health registry linkage.

Participants Pregnant women were recruited before the 20th gestational week during 2017–2021 in Sweden. In total, 5439 pregnancies (5193 unique women) were included. For 3973 pregnancies (73%), samples were provided at baseline, and for 3141 (58%) at all three timepoints (second and third trimester and postpartum). In total, 31 740 maternal microbiome samples (vaginal, faecal and saliva) and 3109 infant faecal samples were collected. Questionnaires were used to collect information on general, reproductive and mental health, diet and lifestyle, complemented by linkage to the nationwide health registries, also used to follow up the health of the offspring (up to age 10).

Findings to date The cohort is fairly representative for the total Swedish pregnant population (data from 2019), with 41% first-time mothers. Women with university level education, born in Sweden, with normal body mass index, not using tobacco-products and aged 30–34 years were slightly over-represented.

Future plans The sample and data collection were finalised in November 2021. The next steps are the characterisation of the microbial DNA and linkage to the health and demographic information from the questionnaires and registries. The role of the microbiome on maternal and neonatal outcomes and early-childhood diseases will be explored (including preterm birth, miscarriage) and the role and interaction of other risk factors and confounders (including endometriosis, polycystic ovarian syndrome, diet, drug use). This is currently among the largest pregnancy cohorts in the world with longitudinal design and detailed and standardised microbiome sampling enabling follow-up of both mothers and children. The findings are expected to contribute greatly to the field of reproductive health focusing on pregnancy and neonatal outcomes.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The major strength of the Swedish Maternal Microbiome (SweMaMi) project is the large-scale and well-characterised longitudinal cohort of pregnant women with child follow-up planned until age 10.

⇒ The project includes comprehensive questionnaire data, microbiome sampling from three body sites and registry data, enabling analysis on pregnancy outcomes such as preterm birth with good statistical power.

⇒ Sample collection was performed in a longitudinal and standardised manner including both pregnant women and their offspring from all over Sweden.

⇒ One limitation is that the SweMaMi project is based on home sampling, therefore blood sampling and samples for metabolomics are lacking.

⇒ As in many research studies, participants with high socioeconomic status are overrepresented.

INTRODUCTION

Microbial communities (including bacteria, viruses and fungi) inhabit our bodies and actively contribute to the homeostasis and health of every individual.[1] The microbiome also seems crucial for a healthy pregnancy, both for the mothers and their offspring.[2–5]

The microbiome during pregnancy

There are specific microbiota in different anatomical niches, which could all affect our health. Regarding pregnancy and reproductive health, the vaginal microbiome is currently most studied.[6] In general, a low-diversity, *lactobacillus*-dominated vaginal microbiome is considered most healthy, while in the gut, a high-diversity gut microbiome (containing a large variety of species) is associated with health benefits.[7] The microbiome during pregnancy is dynamic and

behaves differently in each body site. In the vagina, the variation of microbes within a single sample (alpha diversity) and the variation of microbial communities between samples (beta diversity) are both reduced during pregnancy.[8] In the gut, alpha-diversity also appears to decrease, while the beta diversity, or distance between samples, increases.[9] Proinflammatory cytokine levels in faecal samples also seem to increase during pregnancy.[9] Yet, large studies have found that individual differences between women overrun any effect of gestational age[10] although distinct patterns in the gut microbiome can still be observed in association to pathological conditions.[11 12] Gingival inflammation is known to increase and saliva pH to decrease during pregnancy.[13] The gingival and saliva microbiome have been shown to differ significantly between pregnant and non-pregnant women, with an increase in evenness and total diversity among pregnant women.[14–16] Several demographic, lifestyle and health-related factors might impact on the maternal microbiome, including gynaecological conditions such as polycystic ovary syndrome (PCOS) and endometriosis, which are common causes of subfertility and pregnancy complications.[17–21] Obesity, excessive weight gain and gestational diabetes all leave an apparently distinct signature on the microbiome.[10 22–24] There seems to be a crosstalk between the host metabolism, microbiome and adipose tissue, which seems particularly important during pregnancy.[24–26]

The vaginal microbiome

Lactobacillus species (spp) are the most common colonisers of the vaginal tract in women of reproductive age.[8 27 28] Some women lack this *Lactobacillus* dominance in the vagina and have a diverse composition of other bacteria, including anaerobic bacteria, referred to as vaginal dysbiosis, which is asymptomatic in up to 50%.[29 30] During a healthy pregnancy, the *Lactobacillus* dominance in the vaginal microbiome becomes even more pronounced, likely due to the large increase in oestrogens.[8] For postpartum samples, when sex hormones drop again and lochia flows, the vaginal microbiome has been described to be more diverse compared with samples taken during the first and third trimester, with lower proportions of *Lactobacilli*.[31 32]

Vaginal dysbiosis, and particularly the presence of anaerobic bacteria, has been associated with higher risks of sexually transmitted infections,[33] poor fertility treatment outcome,[34] human papillomavirus infection and gynaecological cancers.[27 35] Vaginal dysbiosis has also been associated with miscarriage, preterm birth, preeclampsia, gestational diabetes and excessive gestational weight gain, which are all common gestational complications.[36–40] Some specific bacterial species linked to vaginal dysbiosis such as BVAB1, *Sneathia* spp, *Gardnerella vaginalis* and *Prevotella* spp, have been associated with increased risks of spontaneous preterm birth, yet may be linked to ethno-geographical differences.[37 38 41] Two recent studies (one with Chinese women and one mixed cohort with mostly Hispanic women) failed to identify associations between specific vaginal bacterial species and preterm birth highlighting the pathogenic complexity.[42 43] Yet, as our recent network meta-analysis on

preterm birth suggests, there may also be risk differences associated to the dominant *Lactobacilli* spp, with *L. crispatus* dominant vaginal microbiome presenting with the lowest risk.[44]

Prior studies investigating the vaginal microbiome and pregnancy outcomes are often inconclusive due to small sample sizes, cross-sectional designs and without sufficient adjustment for confounding factors such as comorbidities, prescribed drug use and lifestyle factors. Therefore, many research questions remain to be answered.

The gut and saliva microbiome

The gut and oral microbiota have also been shown to have systemic effects during pregnancy,[4 45] but the potential interaction with maternal and child health remains unclear. Specifically, the number of studies investigating the maternal gut microbiota in association with pregnancy outcomes are sparse.[4 10 46 47] There are claims of correlation between oral disease and the risk of preterm birth, including periodontal disease and the abundance of *Porphyromonas gingivalis* in subgingival plaque.[16 36] Interestingly, an intervention study providing pregnant women with xylitol chewing gum showed a reduction of periodontitis and lower incidence of preterm birth.[48] However, results from a recent meta-analysis on pregnancy and the oral microbiome were inconclusive and called for further studies of the topic.[16]

The maternal and infant microbiome

The maternal and early infant microbiome could also be associated with neonatal outcomes, such as fetal growth, metabolic acidosis, early metabolic or endocrine disturbances and outcome of neonatal intensive care.[49–51] There are also studies investigating infant microbiome and later disease, including allergies, asthma, type I diabetes, epilepsy and autism.[52–54] Still, large and longitudinal cohorts with high quality data are needed.

Summary of current literature and rational for the current cohort

In summary, research to date points at an association between the vaginal microbiome and poor pregnancy outcomes, but the results show large differences between countries and different ethno-geographical groups. The role of the gut and oral microbiome remains unclear. With increased knowledge, the microbiome could be considered as a possible treatment target for prevention of adverse pregnancy and child outcomes. The Swedish Maternal Microbiome (SweMaMi) project was initiated in 2017 to investigate the pregnancy microbiome and its association with pregnancy outcome and child health. In this cohort description, we give an overview of the SweMaMi study design, participants registered, and how the participants compare to the Swedish population.

Primary aims

The SweMaMi project was designed to deepen knowledge regarding the microbiome in reproductive health and pregnancy outcomes. The overall aim is to assess the association between the maternal vaginal, faecal and

oral microbiome during pregnancy and postpartum, and maternal and neonatal adverse events. The main research question is whether certain microbiome compositions during pregnancy are associated with an increased risk for adverse pregnancy outcomes, including preterm birth and pregnancy loss.

Other research questions regard characterisation of the maternal microbiome in pregnancies of women with risk factors for suboptimal pregnancy outcomes, such as PCOS, pre-eclampsia and endometriosis.[17] [18] In addition, we will investigate the infant microbiome, infant outcome and early-childhood diseases. The study design also allows for further clarification of the 'healthy' or 'normal' microbiome during pregnancy and postpartum and of the neonate.

COHORT DESCRIPTION
Study design and recruitment
The SweMaMi project enrolled participants between November 2017 and February 2021. The last babies of participating women were born at the end of 2021. All pregnant women (before gestational week 20) residing in Sweden with a personal identification number and understanding Swedish or English were eligible to participate. Pregnant women (primiparous and multiparous) were recruited through online advertisement on social media and in pregnancy-related mobile applications.

Information about the study was also shared via posters at Karolinska Institutet campus and in antenatal clinics in Sweden, especially in the greater Stockholm area. Since no healthcare visits were required to participate in the study, women all over Sweden could participate. Women could participate more than once if pregnant several times during the study enrolment period.

Informed consent was obtained from participants through the study webpage before answering the first online questionnaire and could be withdrawn at any time by contacting our team by email or telephone. In association with the home sampling of the infant, both parents (if applicable) signed a consent form. The study complied with the Declaration of Helsinki and the General Data Protection Regulation (GDPR) regulations.

Data collection
Data were collected at three different timepoints, starting with an online questionnaire, followed by home-sampling for microbiome assessment: (1) between pregnancy weeks 10 and 20; (2) pregnancy week 28 and 30 and (3) 5 and 8 weeks after expected delivery date: (including faecal sample from the infant) (see figure 1). Self-collected samples have previously been shown to match the quality of physician-collected samples.[55] [56]

Samples for microbiome analyses
Standardised microbiome sampling, processing and storage was performed to ensure comparability and quality of the samples.

Vaginal samples were collected by inserting a FLOQ swab (Copan, Italy) 2–3 cm into the vagina and rotating for 20–30 s. The tip of the swab was then broken off into a 1.9 mL FluidX tube (Brooks Life Sciences, Massachusetts, USA) which had 0.8 mL DNA/RNA shield (Zymo Research, California, USA).

The faecal samples were collected in DNA/RNA Shield-Faecal collection tube (Zymo Research, California, USA). Participants collected faecal samples from toilet paper with a spoon attached to the lid of the tube and put in the DNA/RNA shield.

Saliva samples were collected using a SalivaGene Collector (Invitek Molecular, Germany) tube according to the manufacturer's instructions.
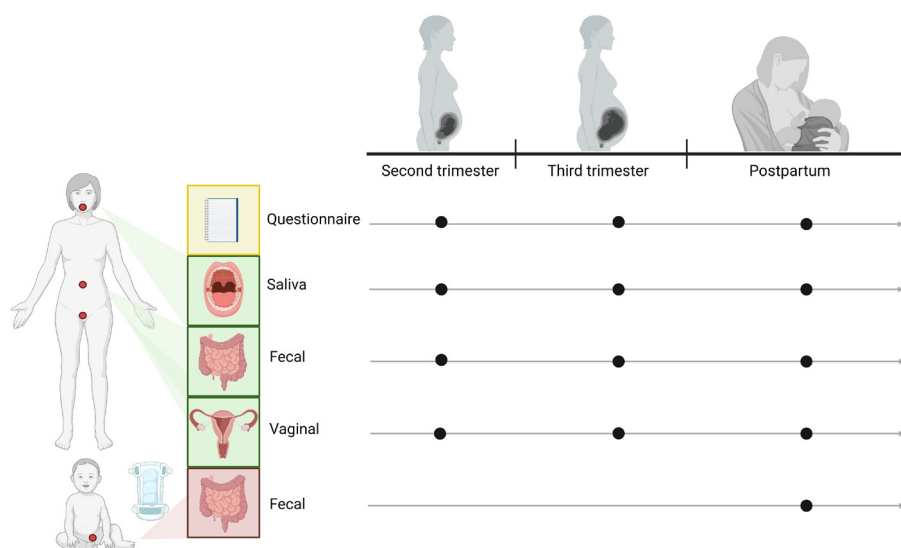
Figure 1 Overview of the SweMaMi study data and sample collection. SweMaMi, Swedish Maternal Microbiome.

All three sample collection kits ensure that the samples would be stable during transportation at room temperature from the participants and until registration and storage at −80°C until DNA extraction for at least 30 days (sent back by post).

Questionnaire data

The questionnaires were designed to gather detailed information on demographic characteristics (eg, age, educational level, body mass index (BMI) and country of birth), health characteristics (eg, detailed questions about menstruation before pregnancy, gynaecological and other comorbidities and characteristics, prescribed and non-prescribed drug use, pregnancy and delivery variables) and lifestyle (eg, diet and tobacco use).

The questionnaire also included standardised questions on mental health, alcohol use and vomiting: The Edinburgh Postnatal Depression Scale,[57] the Cohen Perceived Stress Scale,[58] the Alcohol Use Disorders Identification Test[59] and the 24-hour Pregnancy-Unique Quantification of Emesis Scale.[60] In addition, the Bristol Stool Chart, a frequently used measure in gastroenterology practice and research, was used to categorise stools into one of seven types[61] and Ferriman-Gallwey scoring was used to assess possible undiagnosed PCOS.[62]

All study information, questionnaires and sampling instructions were available both in Swedish and English.

Registry data

In addition, a registry linkage will be conducted to obtain more detailed information on the mothers and to be able to follow-up the health of the children. In Sweden, prenatal, delivery and early child healthcare at the Swedish child welfare centres are available to all residents, tax funded (free of charge), and participation is close to 100%.

Data from the following nationwide registries will be collected for the mother: The Medical birth registry (Medicinska födelseregistret), the In-Patient and Out-Patient registry (Patientregistret), the Prescribed Drug registry (Läkemedelsregistret), the Cause of death registry (Dödsorsaksregistret), the Swedish Quality Registry for caries and periodontal disease (SKaPa) and the Pregnancy Quality Register (Graviditetsregistret).[63–69] All these registries except the last, are nationwide and recording of information is compulsory for every Swedish resident. Although the pregnancy quality registry is not compulsory, coverage is high (about 90%).[69]

The Patient, Cancer, Death and Drug registries for the included children will be used to assess the health of the children up to the age of 10.[64 65 67 70]

Data analyses

Extraction and sequencing of microbial DNA

DNA extraction will be performed with standardised and automated pipelines as previously optimised by our group for vaginal,[71] saliva[72] and faecal samples.[73] The extraction method allows for the detection of all domains of life, namely bacteria, archaea, small eukaryotes including fungi and viruses. Longitudinal profiling of vaginal, faecal and oral microbiome samples will be performed at the Centre for Translational Microbiome Research (CTMR), Karolinska Institutet, Sweden, with a focus on microbial diversity and the correlation between the relative abundances of taxonomic groups with external factors. Taxonomical profiling will be based on metagenomic sequencing to identify important organisms on species/strain level as well as to characterise their functional information such as enzymes involved in metabolic pathways and antibiotic resistance. Additionally, unlike 16S rRNA gene sequencing, metagenomics can provide relative abundances viruses and fungi as well as bacterial species. Sequencing will be performed on MGI sequencers, MGI Tech Co., Ltd (G400 or T7 models) on paired 150 base pair reads, aiming for at least 20 million reads for faecal and saliva samples, or 60 million for vaginal samples due to the high amount of human DNA in vaginal swabs.[71]

Analyses of microbial sequencing data

Raw sequencing reads will be trimmed to remove low-quality basepairs, and human reads will be removed by mapping to the reference human genome. All sequencing data, depleted of human DNA, will be deposited in the European Nucleotide Archive. Generated sequencing data will be handled in accordance with Karolinska Institutet's commitments to secure handling of research data. Raw sequencing data will be primarily stored on the university's on-premises S3 system and will be downloaded to CTMR's private high-performance compute cluster Gandalf for bioinformatics processing and downstream bioinformatics and statistical analyses.

Reads will be taxonomically classified using Kraken V.2 or later on an appropriate database for each sample type, for example, the Human Oral Database[74] for saliva samples, OptiVag for vaginal samples[71] or Genome taxonomy database (GTDB)[75] for faecal samples. Since the Human Oral Database does not currently include viral or fungal sequences, these will be annotated on the GTDB as well.

Statistical methods

The associations between the microbiome, other risk factors and poor pregnancy outcomes will be investigated through comparisons of within-sample and between-sample diversity. Within-sample diversity will be estimated using standard ecological measures such as Simpson's and Chao1 indexes. Distances between samples will be estimated with non-metric, compositionality aware measures such as Bray-Curtis. We will also use evolutionary-aware measures, that is, weighted and unweighted Unifrac.

When we find community-level changes (ie, changes in diversity), we will identify differentially abundant organisms. All tests associating sequencing data to metadata will be conducted with tests that are compositionality-aware, such as beta-regressions. All tests will be corrected for multiple comparison with the Benjamini-Hochberg procedure. Additional confounders (such as maternal age, BMI, parity and prescribed drug use)

will be considered. Advanced statistical methods such as generalised linear modelling and mediation analysis will then be applied with identified environmental risk factors.

The planned work includes several substudies where sub-group and sensitivity analyses will be performed to ensure adequate use of confounding factors. The maternal microbiome composition will also be investigated as the outcome, with medical conditions (eg, PCOS, endometriosis) as the exposure. For other conditions, such as gestational diabetes, the associations could be hypothesised to be bidirectional. The maternal microbiome will also be used as the exposure variable for infant microbiome composition, while both the maternal and the infant microbiome will be investigated as the exposure for early childhood outcomes.

Outcomes
Pregnancy health characteristics and outcomes
The extensive questionnaire and registry data will be used to characterise the pregnancy health of the participating women and to associate these factors with microbial data. Information about the main outcome measure, preterm birth, defined as pregnancy shorter than 37 completed weeks of gestation, will be collected both from the postpartum questionnaire and from registry data.
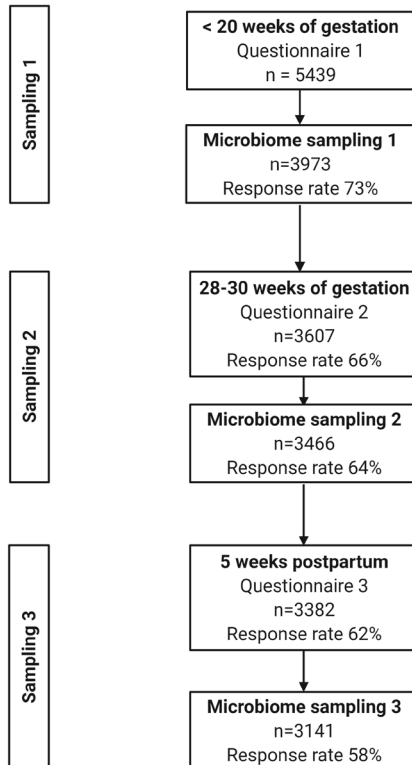
Figure 2 Flowchart showing the number of participants which completed each stage of the study with three questionnaires and three sample kits, with response rate as % of the women that answered questionnaire 1.

Infant and child outcomes
The SweMaMi project includes investigation of associations between the microbiome and neonatal outcomes, such as small or large for gestational age (babies who weigh significantly less or more than expected for the pregnancy length), intrauterine fetal death, low Apgar score, metabolic acidosis, early metabolic or endocrine disturbances and neonatal intensive care. Using the registry linkage, long-term follow-up of child outcomes will be applied, to investigate pregnant and infant microbial profiles, and potential associations with children's development and health conditions, defined as diagnostics of allergies, asthma, diabetes type I, epilepsy and autism.

Confounders
Possible confounders that may affect the outcomes will be checked using the above-mentioned registries and questionnaire data. Some data will be available from multiple sources, increasing the validity of the information. Those confounders include pregnancy related characteristics (eg, conception (natural or induced), duration of pregnancy, singleton or multiple pregnancy, parity and C-section or vaginal delivery) and maternal characteristics (eg, age, prescribed drug use, chronic comorbidities, mental health, lifestyle and socioeconomic-factors). Environmental factors such as unemployment, low income or poor mental health as well as reproductive health problems could impact on pregnancy outcome.[76] Low level of education and psychological stress have also been associated with a less optimal microbiome in pregnant women.[77 78]

Approximately 1/6 of the microbiome variation on population level can be attributed to the use of prescribed medications.[79] Therefore, we will also assess the role of maternal and early life intake of potential microbiome-modulating drugs including antibiotics, proton-pump inhibitors, metformin, anti-inflammatory drugs and others.[80]

Power analysis
A power calculation for preterm birth was performed prior to the start of the SweMaMi project based on the incidence of spontaneous preterm birth being 3% in Sweden. The initial power analysis indicated that a sample of 1300 participants, a possible difference of 2% in the proportion of preterm birth between women with different bacterial colonisation types could be detected (power 0.8, alpha 0.05). However, due to the planned use of subgroup analyses, and potentially large microbiome variation as well as dropouts over time we initially aimed for 2500 women. Moreover, to enable detection of small variations in the proportion of spontaneous preterm deliveries in different diversity/abundance groups, the inclusion was increased to reach a sample of at least 3300 women with full data.

Representativeness of SweMaMi cohort
To assess the representativeness of our study cohort, we compared it to the nationwide pregnancy data as obtained

Table 1 Overview of the Swedish Maternal Microbiome (SweMaMi) cohort

		SweMaMi n=5439	
		N	%
Maternal age, in years	≤19	6	0.1
	20–24	185	3.4
	25–29	1457	26.8
	30–34	2476	45.5
	35–39	1135	20.9
	≥40	180	3.3
Maternal body mass index, in kg/m^2 (weight before pregnancy)	<18.4 Underweight	131	2.4
	18.5–24.9 Normal weight	3586	65.9
	25.0–29.9 Preobesity	1113	20.5
	30.0–34.9 Obesity I	353	6.5
	35.0–39.9 Obesity II	104	1.9
	>40.0 Obesity III	43	0.8
Country of birth	Sweden	4917	90.4
	Other	497	9.1
Highest level of education	Primary school (förgymnasial)	82	1.5
	Senior high school (gymnasial)	963	17.7
	University/college (eftergymnasial)	4221	77.5
Family	In a relationship	5333	98.1
	Single	105	1.9
First pregnancy	Yes	1536	28.2
	No	3902	71.8
Number of previous pregnancies	0	1536	28.2
	1–3	3283	60.4
	>3	616	11.3
Parity	First child	2233	41.1
	Not first child	3204	58.9
Method of conception	Spontaneous	4892	90.0
	Any medically assisted reproduction	546	10.0
	IVF	343	6.3
	Hormonal treatment	128	2.3
	ICSI	109	2.0
	Insemination	79	1.5
	Surgically assisted	17	0.3
	Fertility treatment of the father	4	0.1
Number of previous miscarriages	0	3747	68.9
	1–2	1467	26.9

Continued

Table 1 Continued

		SweMaMi n=5439	
		N	%
	>3	225	4.2
Number of sexual partners in the last year	1	5211	95.8
	None or more than 1	221	4.1
Regular menstruation prior to pregnancy	Yes	4493	82.6
	No	946	17.4
Diagnosed endometriosis	Yes	208	3.8
Diagnosed polycystic ovary syndrome	Yes	438	8.1

ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilisation.

from The National Board of Health and Welfare for the same study period.[81] The most recent statistics for Sweden are from 2019 and the following characteristics will be compared: maternal age, parity, country of birth, highest level of education, BMI, tobacco use (smoking and snuff) and Swedish Region.

Basic functions in Excel and RStudio were used for all comparisons and overviews. Descriptive overview of the cohort was created with Excel, and a choropleth map of Sweden using R studio (V.1.3.1093), adjusted from.[81 82] χ^2 test was conducted to compare the SweMaMi cohort with the overall population in Sweden using R studio.

Patient and public involvement
The research team for this study on pregnancy has included pregnant women and mothers. Study participants has been encouraged to contact the team regarding any questions or comments on the study procedures. During the study design time period, the team also had close contact with different stake holders, including clinical staff and patient organisations.

FINDINGS TO DATE
Cohort description
In total, 5193 unique women (5439 pregnancies) registered for the study by answering the first questionnaire. For 3973 pregnancies (73%), samples were provided at baseline and for 3141 (58%) at all three timepoints (second and third trimester and postpartum). In total, 38 591 maternal microbiome samples (vaginal, faecal and saliva) and 3109 infant faecal samples were collected (total 41 700 samples) (figure 2).

The SweMaMi cohort characteristics are presented in table 1. On average, women were 31.7 (±4.3) years old at recruitment and the majority were born in Sweden (90%). Participants lived all over Sweden, with a majority residing in Stockholm (42%) (figure 3). Most of the women had a university level education (78%).
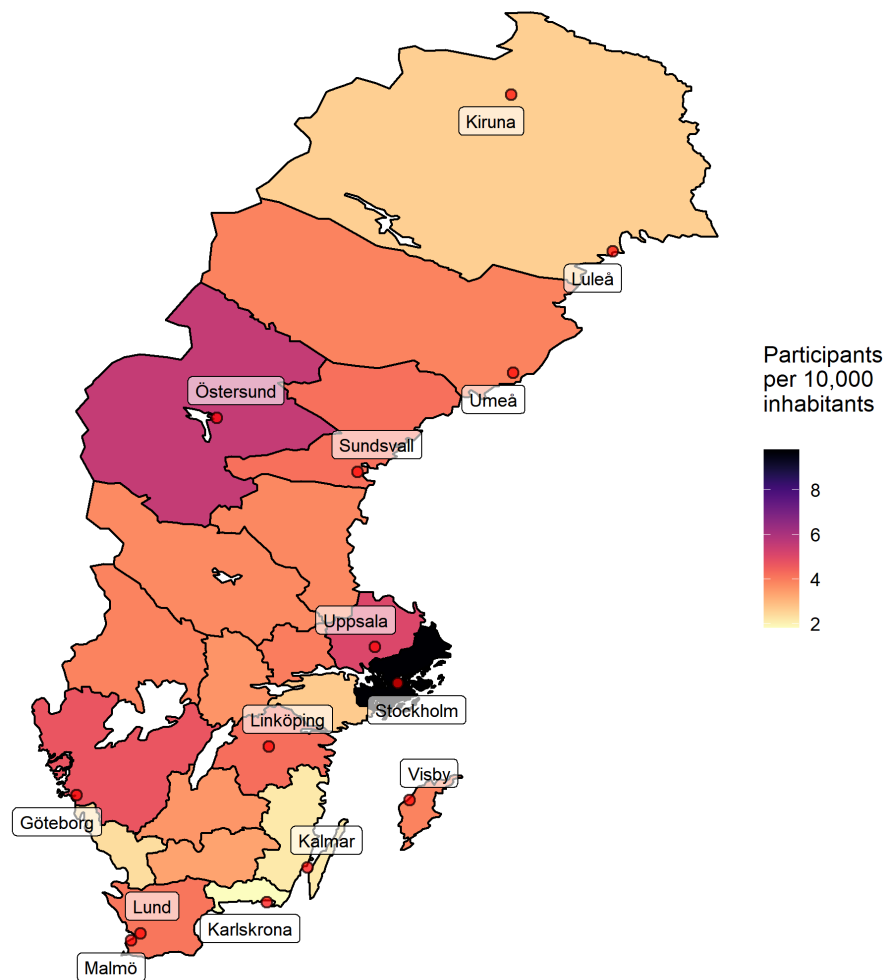
Figure 3 Choropleth map showing the number of SweMaMi participants per 10 000 inhabitants in each county of Sweden. Darker colour indicates higher number of participants per county. SweMaMi, Swedish Maternal Microbiome.

Most women (96%) reported having only one sexual partner in the previous year and were in a relationship (98%). For the majority of women (72%), this was not the first pregnancy. Most children were naturally conceived (90%), while 10% of women reported assisted reproductive technology (ART) use. Of those, in vitro fertilisation (IVF) was the most common (6%). Most women had no reported prior miscarriages (69%), but 4% had suffered recurrent pregnancy loss, defined as three or more consecutive miscarriages.[83]

Regarding gynaecological characteristics, 83% reported regular menstruation prior to conception, 4% had diagnosed endometriosis and 8% diagnosed polycystic ovary syndrome (PCOS).

Comparison to the Swedish population

Compared with the national pregnancy population in 2019 (see table 2), the SweMaMi participants were older (30.3% vs 41.9% younger than 30 years) and smoked less during early pregnancy (1.3% vs 3.9%). SweMaMi participants were more often normal weight (65.9% vs 51.8% with BMI between 18.5 and 24.9), university-level educated (77.5 vs 54.7%) and born in Sweden (90.5% vs 68.1%).

Strengths and limitations

The major strength of the SweMaMi project is the large-scale and well characterised cohort of pregnant women, including comprehensive questionnaire data, microbiome sampling from three body sites and registry data, enabling analysis on pregnancy outcomes such as preterm birth with good statistical power. In addition, sample collection was performed in a longitudinal and standardised manner including both pregnant women and their offspring from all over Sweden, with the possibility of a 10-year follow-up for the children. The project uses cutting-edge sequencing technology for metagenomic analyses of microbial DNA performs analysis on viral and fungal components and generates detailed genetic functional analysis of the microbiome, including metabolic pathways. The linkage to National registries is unique within the microbiome field, where Swedish registries are generally highly complete and up to date. The

Table 2 Characteristics of the Swedish Maternal Microbiome (SweMaMi) cohort, compared with all women giving birth in Sweden Swedish population in 2019

	SweMaMi (2017–2021) n=5439		All pregnant women giving birth in Sweden 2019* n=1 16 082		P value (X^2 test)
	N	%	N	%	
Maternal age, in years					<0.001
≤19	6	0.1	964	0.8	
20–24	185	3.4	10 787	9.5	
25–29	1457	26.8	35 972	31.6	
30–34	2476	45.5	40 488	35.6	
35–39	1135	20.9	20 499	18	
≥40	180	3.3	5106	4.5	
Mean	*31.7*		*30.7*		
Maternal body mass index, in kg/m^2 (weight before pregnancy)					<0.001
<18.4 Underweight	131	2.4	2783	2.4	
18.5–24.9 Normal weight	3586	65.9	59 384	51.8	
25.0–29.9 Preobesity	1113	20.5	29 636	25.9	
30.0–34.9 Obesity I	353	6.5	11 710	10.2	
35.0–39.9 Obesity II	104	1.9	3977	3.5	
>40.0 Obesity III	43	0.8	1439	1.3	
Mean	*24.1*		*25.3*		
Parity					<0.05
First child	2233	41.1	49 567	42.7	
Not first child	3204	58.9	66 515	57.3	
Country of birth					<0.001
Sweden	4928	90.5	78 033	68.1	
Other	498	9.1	34 497	30.1	
Highest level of education					<0.001
Primary school (förgymnasial)	82	1.5	11 303	10.5	
Senior high school (gymnasial)	963	17.7	37 501	34.8	
University/college (eftergymnasial)	4221	77.5	58 931	54.7	
Tobacco habits					<0.001
Smoking in early pregnancy	70	1.3	4527	3.9	
Use snus in early pregnancy	69	1.3	1393	1.2	
No tobacco in early pregnancy	5301	97.4	110 162	94.9	

*Data from the Swedish National Board of Health and Welfare https://www.socialstyrelsen.se/statistik-och-data/statistik/statistikamnen/graviditeter-forlossningar-och-nyfodda/.

questionnaire and the participant information were translated and provided in both Swedish and English to enable a broader recruitment.

The data collection also has limitations. The SweMaMi project is based on home sampling, and we therefore lack samples that were not feasible to collect at home or send by mail, such as blood samples or samples for bacterial culture. There would have been an advantage with more frequent sampling including a sample prior to pregnancy, but this was not feasible for this setup. We also lack samples for metabolomics to measure biochemical products produced on the microbiota in the mouth, gut and vagina, also because home sampling precludes samples that needs to stay frozen at all times. However, our protocol supports functional analyses of the microbiome, which will answer some questions regarding mechanistic pathways. In hindsight, the study would have benefited from even more detailed questionnaire data on diet and physical exercise. However, the number of questions had to be restricted to ensure that participants would agree to contribute and continue participation. In addition, as in many research studies, participants with high socioeconomic status are overrepresented.

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Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

Ethics approval The study protocol has been approved by the Regional Board of Ethics, Stockholm, Sweden (2017/1118-31).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. Register data cannot be shared, due to confidentiality.

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REFERENCES
1. Young VB. The role of the microbiome in human health and disease: an introduction for clinicians. *BMJ* 2017;356:j831.
2. Saadaoui M, Singh P, Al Khodor S. Oral microbiome and pregnancy: a bidirectional relationship. *J Reprod Immunol* 2021;145:103293.
3. Nuriel-Ohayon M, Neuman H, Koren O. Microbial changes during pregnancy, birth, and infancy. *Front Microbiol* 2016;7:1031.
4. Edwards SM, Cunningham SA, Dunlop AL, *et al*. The maternal gut microbiome during pregnancy. *MCN Am J Matern Child Nurs* 2017;42:310–7.
5. Gupta P, Singh MP, Goyal K. Diversity of vaginal microbiome in pregnancy: deciphering the obscurity. *Front Public Health* 2020;8:326.
6. Vinturache AE, Gyamfi-Bannerman C, Hwang J, *et al*. Maternal microbiome - A pathway to preterm birth. *Semin Fetal Neonatal Med* 2016;21:94–9.
7. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature* 2012;486:207–14.
8. Romero R, Hassan SS, Gajer P. The composition and stability of the vaginal microbiota of normal pregnant women is different from that of non-pregnant women. *Microbiome* 2014;2.
9. Koren O, Goodrich JK, Cullender TC, *et al*. Host remodeling of the gut microbiome and metabolic changes during pregnancy. *Cell* 2012;150:470–80.
10. Yang H, Guo R, Li S, *et al*. Systematic analysis of gut microbiota in pregnant women and its correlations with individual heterogeneity. *NPJ Biofilms Microbiomes* 2020;6:32.
11. Wang X, Liu H, Li Y, *et al*. Altered gut bacterial and metabolic signatures and their interaction in gestational diabetes mellitus. *Gut Microbes* 2020;12:1–13.
12. Altemani F, Barrett HL, Gomez-Arango L, *et al*. Pregnant women who develop preeclampsia have lower abundance of the butyrate-producer Coprococcus in their gut microbiota. *Pregnancy Hypertens* 2021;23:211–9.
13. Silva de Araujo Figueiredo C, Gonçalves Carvalho Rosalem C, Costa Cantanhede AL, *et al*. Systemic alterations and their oral manifestations in pregnant women. *J Obstet Gynaecol Res* 2017;43:16–22.
14. Paropkari AD, Leblebicioglu B, Christian LM, *et al*. Smoking, pregnancy and the subgingival microbiome. *Sci Rep* 2016;6:30388.
15. Kato S, Nagasawa T, Uehara O, *et al*. Increase in Bifidobacterium is a characteristic of the difference in the salivary microbiota of pregnant and non-pregnant women. *BMC Oral Health* 2022;22:260.
16. Jang H, Patoine A, Wu TT, *et al*. Oral microflora and pregnancy: a systematic review and meta-analysis. *Sci Rep* 2021;11:16870.
17. Roos N, Kieler H, Sahlin L, *et al*. Risk of adverse pregnancy outcomes in women with polycystic ovary syndrome: population based cohort study. *BMJ* 2011;343:d6309.
18. Farland LV, Prescott J, Sasamoto N, *et al*. Endometriosis and risk of adverse pregnancy outcomes. *Obstet Gynecol* 2019;134:527–36.
19. Evers JLH. Female subfertility. *Lancet* 2002;360:151–9.
20. Vander Borght M, Wyns C. Fertility and infertility: definition and epidemiology. *Clin Biochem* 2018;62:2–10.
21. Lv L-J, Li S-H, Li S-C, *et al*. Early-Onset preeclampsia is associated with gut microbial alterations in antepartum and postpartum women. *Front Cell Infect Microbiol* 2019;9:224.
22. Hasain Z, Mokhtar NM, Kamaruddin NA, *et al*. Gut microbiota and gestational diabetes mellitus: a review of Host-Gut microbiota interactions and their therapeutic potential. *Front Cell Infect Microbiol* 2020;10:188.
23. Stanislawski MA, Dabelea D, Wagner BD, *et al*. Pre-pregnancy weight, gestational weight gain, and the gut microbiota of mothers and their infants. *Microbiome* 2017;5:113.
24. Hu P, Chen X, Chu X, *et al*. Association of gut microbiota during early pregnancy with risk of incident gestational diabetes mellitus. *J Clin Endocrinol Metab* 2021;106:e4128–41.
25. Wu D, Wang H, Xie L, *et al*. Cross-talk between gut microbiota and adipose tissues in obesity and related metabolic diseases. *Front Endocrinol* 2022;13:908868.
26. Su Y, Chen L, Zhang D-Y, *et al*. The characteristics of intestinal flora in overweight pregnant women and the correlation with gestational diabetes mellitus. *Endocr Connect* 2021;10:1366–76.
27. Cheng L, Norenhag J, Hu YOO, *et al*. Vaginal microbiota and human papillomavirus infection among young Swedish women. *NPJ Biofilms Microbiomes* 2020;6:39.
28. Ravel J, Gajer P, Abdo Z, *et al*. Vaginal microbiome of reproductive-age women. *Proc Natl Acad Sci U S A* 2011;108:4680–7.
29. Joesoef MR, Schmid GP. Bacterial vaginosis. *Clin Evidence* 2005:1601.
30. van de Wijgert JHHM, Borgdorff H, Verhelst R, *et al*. The vaginal microbiota: what have we learned after a decade of molecular characterization? *PLoS One* 2014;9:e105998.
31. Nunn KL, Witkin SS, Schneider GM, *et al*. Changes in the vaginal microbiome during the pregnancy to postpartum transition. *Reprod Sci* 2021;28:1996–2005.
32. MacIntyre DA, Chandiramani M, Lee YS, *et al*. The vaginal microbiome during pregnancy and the postpartum period in a European population. *Sci Rep* 2015;5.

33 Edwards VL, Smith SB, McComb EJ, *et al*. The cervicovaginal Microbiota-Host interaction modulates Chlamydia trachomatis infection. *mBio* 2019;10:19. doi:10.1128/mBio.01548-19

34 Koedooder R, Singer M, Schoenmakers S, *et al*. The vaginal microbiome as a predictor for outcome of in vitro fertilization with or without intracytoplasmic sperm injection: a prospective study. *Hum Reprod* 2019;34:1042–54.

35 Brusselaers N, Shrestha S, van de Wijgert J, *et al*. Vaginal dysbiosis and the risk of human papillomavirus and cervical cancer: systematic review and meta-analysis. *Am J Obstet Gynecol* 2019;221:9-–18.

36 Dunlop AL, Mulle JG, Ferranti EP, *et al*. Maternal microbiome and pregnancy outcomes that impact infant health: a review. *Adv Neonatal Care* 2015;15:377–85.

37 Elovitz MA, Gajer P, Riis V, *et al*. Cervicovaginal microbiota and local immune response modulate the risk of spontaneous preterm delivery. *Nat Commun* 2019;10:1305.

38 Fettweis JM, Serrano MG, Brooks JP, *et al*. The vaginal microbiome and preterm birth. *Nat Med* 2019;25:1012–21.

39 Fox C, Eichelberger K. Maternal microbiome and pregnancy outcomes. *Fertil Steril* 2015;104:1358–63.

40 Grewal K, Lee YS, Smith A, *et al*. Chromosomally normal miscarriage is associated with vaginal dysbiosis and local inflammation. *BMC Med* 2022;20:38.

41 Downes KL, Ravel J, Gajer P, *et al*. 15: Gardnerella vaginalis and spontaneous preterm birth: new insights. *Am J Obstet Gynecol* 2018;218:S12–13. doi:10.1016/j.ajog.2017.10.426

42 Chan D, Bennett PR, Lee YS, *et al*. Microbial-driven preterm labour involves crosstalk between the innate and adaptive immune response. *Nat Commun* 2022;13:975.

43 Pace RM, Chu DM, Prince AL, *et al*. Complex species and strain ecology of the vaginal microbiome from pregnancy to postpartum and association with preterm birth. *Med* 2021;2:1027–49.

44 Gudnadottir U, Debelius JW, Du J, *et al*. The vaginal microbiome and the risk of preterm birth: a systematic review and network meta-analysis. *Sci Rep* 2022;12:7926.

45 Cobb CM, Kelly PJ, Williams KB, *et al*. The oral microbiome and adverse pregnancy outcomes. *Int J Womens Health* 2017;9:551–9.

46 Siena MD, Laterza L, Matteo MV, *et al*. Gut and reproductive tract microbiota adaptation during pregnancy: new insights for pregnancy-related complications and therapy. *Microorganisms* 2021;9:473.

47 Smid MC, Ricks NM, Panzer A, *et al*. Maternal gut microbiome biodiversity in pregnancy. *Am J Perinatol* 2018;35:24–30.

48 Aagaard KM, Valentine GC, Antony KM, *et al*. Lb 1: PPaX: cluster randomized trial of xylitol chewing gum on prevention of preterm birth in Malawi. *Am J Obstet Gynecol* 2022;226:S777. doi:10.1016/j.ajog.2021.12.015

49 Jiang L, Feng C, Tao S, *et al*. Maternal imprinting of the neonatal microbiota colonization in intrauterine growth restricted piglets: a review. *J Anim Sci Biotechnol* 2019;10:88.

50 Pammi M, Cope J, Tarr PI, *et al*. Intestinal dysbiosis in preterm infants preceding necrotizing enterocolitis: a systematic review and meta-analysis. *Microbiome* 2017;5:31.

51 Sanidad KZ, Zeng MY. Neonatal gut microbiome and immunity. *Curr Opin Microbiol* 2020;56:30–7.

52 Wan L, Yang G, Zhang S, *et al*. Investigation of the association between imbalance of the intestinal flora and infantile spasms: a pilot case-control study. *Transl Pediatr* 2021;10:819–33.

53 Kim E, Paik D, Ramirez RN, *et al*. Maternal gut bacteria drive intestinal inflammation in offspring with neurodevelopmental disorders by altering the chromatin landscape of CD4$^+$ T cells. *Immunity* 2022;55:145–58.

54 Groer MW, Gregory KE, Louis-Jacques A, *et al*. The very low birth weight infant microbiome and childhood health. *Birth Defects Res C Embryo Today* 2015;105:252–64.

55 Forney LJ, Gajer P, Williams CJ, *et al*. Comparison of self-collected and physician-collected vaginal swabs for microbiome analysis. *J Clin Microbiol* 2010;48:1741–8.

56 Hugerth LW *et al*. A comprehensive automated pipeline for human microbiome sampling, 16S rRNA gene sequencing and bioinformatics processing. *bioRxiv* 2018;286526.

57 Cox JL, Holden JM, Sagovsky R. Detection of postnatal depression. Development of the 10-item Edinburgh postnatal depression scale. *Br J Psychiatry* 1987;150:782–6.

58 Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. *J Health Soc Behav* 1983;24:385–96.

59 Babor TF, Higgins-Biddle JC, Saunders JB. *AUDIT: the alcohol use disorders identification test: guidelines for use in primary health care*. World Health Organization, 2001.

60 Ebrahimi N, Maltepe C, Bournissen FG, *et al*. Nausea and vomiting of pregnancy: using the 24-hour Pregnancy-Unique Quantification of Emesis (PUQE-24) scale. *J Obstet Gynaecol Can* 2009;31:803–7.

61 Amarenco G. [Bristol Stool Chart: Prospective and monocentric study of "stools introspection" in healthy subjects]. *Prog Urol* 2014;24:708–13.

62 Ferriman D, Gallwey JD. Clinical assessment of body hair growth in women. *J Clin Endocrinol Metab* 1961;21:1440–7.

63 The Swedish Medical Birth Registry - Statistics Sweden. Secondary The Swedish Medical Birth Registry - Statistics Sweden, 2017. Available: https://www.socialstyrelsen.se/statistik-och-data/

64 Ludvigsson JF, Andersson E, Ekbom A, *et al*. External review and validation of the Swedish national inpatient register. *BMC Public Health* 2011;11:450.

65 Wettermark B, Hammar N, Fored CM, *et al*. The new Swedish prescribed drug register-opportunities for pharmacoepidemiological research and experience from the first six months. *Pharmacoepidemiol Drug Saf* 2007;16:726–35.

66 Axelsson O. The Swedish medical birth register. *Acta Obstet Gynecol Scand* 2003;82:491–2.

67 Brooke HL, Talbäck M, Hörnblad J, *et al*. The Swedish cause of death register. *Eur J Epidemiol* 2017;32:765–73.

68 Mensah T, Tranæus S, Cederlund A, *et al*. Swedish quality registry for caries and periodontal diseases (SKaPa): validation of data on dental caries in 6- and 12-year-old children. *BMC Oral Health* 2021;21:373.

69 Stephansson O, Petersson K, Björk C, *et al*. The Swedish Pregnancy Register - for quality of care improvement and research. *Acta Obstet Gynecol Scand* 2018;97:466–76.

70 Barlow L, Westergren K, Holmberg L, *et al*. The completeness of the Swedish cancer register: a sample survey for year 1998. *Acta Oncol* 2009;48:27–33.

71 Hugerth LW, Pereira M, Zha Y, *et al*. Assessment of *vitro* and in *silico* protocols for sequence-based characterization of the human vaginal microbiome. *mSphere* 2020;5.

72 Bostanci N, Krog MC, Hugerth LW, *et al*. Dysbiosis of the human oral microbiome during the menstrual cycle and vulnerability to the external exposures of smoking and dietary sugar. *Front Cell Infect Microbiol* 2021;11:145.

73 Krog MC, Hugerth LW, Fransson E, *et al*. The healthy female microbiome across body sites: effect of hormonal contraceptives and the menstrual cycle. *Hum Reprod* 2022;37:1525–43.

74 Dewhirst FE, Chen T, Izard J, *et al*. The human oral microbiome. *J Bacteriol* 2010;192:5002–17.

75 Parks DH, Chuvochina M, Chaumeil P-A, *et al*. A complete domain-to-species taxonomy for bacteria and archaea. *Nat Biotechnol* 2020;38:1079–86.

76 Snelgrove JW, Murphy KE. Preterm birth and social inequality: assessing the effects of material and psychosocial disadvantage in a UK birth cohort. *Acta Obstet Gynecol Scand* 2015;94:766–75.

77 Dunlop AL, Knight AK, Satten GA, *et al*. Stability of the vaginal, oral, and gut microbiota across pregnancy among African American women: the effect of socioeconomic status and antibiotic exposure. *PeerJ* 2019;7:e8004.

78 Hantsoo L, Jašarević E, Criniti S, *et al*. Childhood adversity impact on gut microbiota and inflammatory response to stress during pregnancy. *Brain Behav Immun* 2019;75:240–50.

79 Falony G, Joossens M, Vieira-Silva S, *et al*. Population-level analysis of gut microbiome variation. *Science* 2016;352:560–4.

80 Brusselaers N. Prescribed drugs and the microbiome. *Gastroenterol Clin North Am* 2019;48:331–42.

81 Socialstyrelsen. Statistik om graviditeter, förlossningar och nyfödda. Secondary Statistik om graviditeter, förlossningar och nyfödda, 2021. Available: https://www.socialstyrelsen.se/statistik-och-data/statistik/statistikamnen/graviditeter-forlossningar-och-nyfodda/

82 Lacey M. Statistics for the COVID-19 outbreak in Sweden. statistics for the COVID-19 outbreak in Sweden, 2021. Available: http://lacey.se/c19/

83 Pillarisetty LS, Gupta N. *Recurrent pregnancy loss*. Treasure Island, FL: StatPearls, 2021.