Objective: To determine the pattern of gut microbiota in first and third trimester among Malay pregnant women.

Design: This is a prospective observational study.

Setting: In two tertiary level hospitals.

Sample: Twelve pregnant Malay women without any endocrine disorders and not on antibiotic or probiotics within 4 weeks prior to recruitment.

Methods: Participants’ basic demographic details and anthropometric measurement were obtained. Stool samples in first and third trimester were collected and prepared for 16S ribosomal ribonucleic acid metagenome analysis. All statistical analyses were carried out using SPSS version 22. Comparative metagenomics analysis was performed using METAGENassist. Statistical significance was defined as a P-value <0.05.

Main outcome measures: Taxonomic distribution of gut microbiota in first and third trimester of pregnancy.

Results: The most abundant phylum during the first and third trimester were Bacteroidetes, Firmicutes, Proteobacteria and Actinobacteria. There were compositional differences of gut microbiota at the genus level between first trimester and third trimester. Fifteen genera were identified as important contributors to the clustering of microbiota composition. Abundances of Eubacterium and Brevundimonas in first trimester were 2.95 and 2.44 folds higher than in third trimester, respectively. There were compositional differences of gut microbiota at the genus level between women with different body mass index (BMI) group. Women with higher BMI had lower Bacteroidetes and higher Proteobacteria.

Conclusion: There were different gut microbiota composition at genus level between first and third trimester, and between women with different BMI group.
THE REPRODUCTIVE TRACT MICROBIOME IN PRETERM PRELABOUR RUPTURE OF FETAL MEMBRANE (PPROM)

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Background: PPROM precedes 30% of spontaneous preterm birth cases. Prior to PPROM, there is a shift from Lactobacillus spp. dominance of vaginal microbiota towards highly diverse compositions. It remains unclear if this is reflected in the upper gestational tissues. In this pilot study, we explore this by using metataxonomics to compare matched vaginal, fetal membrane (FM) and placental bacterial compositions in women presenting with PPROM.

Methods: Matched vaginal swabs taken close to delivery by caesarean section, placenta tissue and FM tissue (distal, midway and adjacent to rupture site) were collected from women presenting with PPROM (n=15). Negative swab controls were also collected and processed. MiSeq-based sequencing of 16S rRNA gene amplicons was performed to profile bacterial composition.

Results: A total of 7/15 (47%) of women with PPROM had highly diverse vaginal microbiota compositions deplete in Lactobacillus spp.. The remainder (8/15, 53%) were dominated by Lactobacillus spp.. Despite G. vaginalis being readily detected in 6/15 (>2% relative abundance) vaginal samples, it was detected in only 3/60 (0.05%) FM and placenta samples. Similarly, vaginal L. iners dominance was poorly correlated with FM or placental composition. Codetection of Sneathia spp. was observed in 3 matched patient sample sets.

Interpretation: Our data indicate that vaginal microbiota composition is not always reflected in upper gestational tissue composition at delivery supporting the notion that ascending infection leading to PPROM is limited to specific species. Validation is undergoing in a larger cohort, as are investigations into the functional significance of bacterial composition at these different body sites.
Objective: The maternal microbiome plays an important role in fetal susceptibility to infection, and may correlate with type I interferon (IFN) signaling at the level of the placenta.

Study Design: To study this, we conducted two infectivity experiments. First, germ-free, conventional, and adult-colonized pregnant Swiss Webster mice were inoculated with Zika Virus (ZIKV), an example of a fetal infectious agent. Second, germ-free pregnant Swiss Webster mice were adult-colonized with *Fusobacterium nucleatum* mixed with *Lactobacillus reuteri* (jointly referred to as FNLR), examples of gut microbes. Sterile caesarean section was performed just prior to delivery.

Results: For the ZIKV group, decreased splenic IFN alpha (p=0.01) and beta (p=0.001) transcripts were associated with similar proportions of placental ZIKV detection (40% vs 50%; p=ns) in germ-free versus conventional animals, but increased splenic IFN alpha (p=0.01) transcripts were associated with even more increased ZIKV titers ($10^7$ vs $10^2$ log viral copies/g RNA) in adult-colonized versus conventional animals. Complementarily, in the FNLR group, increased placental IFN alpha (10.2 vs 2.1 fold change mRNA, p<0.001) and beta (3.9 vs 2.0 fold change mRNA, p=0.0194) transcripts were associated with FNLR adult-colonized inoculation versus germ-free controls.

Conclusions: Taken together, these results indicate that timing of the mother’s acquisition of microbes is important in modulating the IFN balance of the placenta. We speculate that aberrant type I IFN signaling, rather than a simplistic “too high” or “too low” mentality, may be associated with increased susceptibility to infection in the fetus.
Brain-Gut Axis and Its Influence on Gestational Weight Gain in African American Women

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Background: Excessive gestational weight gain (GWG) is a significant predictor of adverse obstetric outcomes and lifelong health risks for the woman and infant. In this study I investigated the role of the maternal gut microbiome to maternal obesity.

Sample and Design: This was a prospective, longitudinal study of 27 pregnant women enrolled in a larger study of the microbiome in pregnancy (1R01NR014800). In this sub-study (1 F31 NR015722-01A1), the participants were enrolled for one additional study visit. Analyses included means, descriptive statistics, ANOVA testing and linear regression modeling to predict the key outcome variables of weight and composition change during the pregnancy.

Results: The difference in the change in ratio of FTB (Firmicutes to Bacteroidetes) showed a negative correlation with the ratio at the first time point (r = -.98, p < .001). Also, the category of weight gain at mid-gestation was associated with change in FTB ratio (f = 3.48, p = .05), with significant difference in FTB ratio between the inadequate versus excessive gainers. A linear regression model examining the variables of FTB at time 1 and the Adverse Childhood Experience score explained 25 percent of the variance in the initial weight (Adjusted R square = .25, F (2, 26) = 5.33, p = .01).

Conclusion: Although interval and total GWG clearly impact obstetric outcomes, and although in non-pregnant populations the gut microbiome and weight gain are closely linked, little research to date has explored the association between the gut microbiome and interval or total GWG.
The BASIC-Study: Alterations of Inflammation And HPA-Axis Markers In Perinatal Depression

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The BASIC study is aimed at investigating biomarkers of health, mental health in particular, in pregnant women and have been recruiting pregnant women 2009-2018 in Uppsala, Sweden. The participants have filled out questionnaires at two times during pregnancy as well as at six weeks, six and 12 months postpartum. Blood samples for biomarkers as well as for genetic analyses have been collected.

Previous results from the BASIC study have shown that:

- Women with antenatal depression or on SSRI treatment during pregnancy had lower levels of inflammatory markers in comparison to healthy pregnant controls (i.e. TRAIL, CSF-1, CX3CL1, VEGF-A, and IL-15Ra)
- Among women with depressive symptoms postpartum, five inflammatory markers (TRANCE, HGF, IL-18, FGF-23, and CXCL1) were elevated in comparison to healthy postpartum controls.
- Comparing pregnant and postpartum women without depressive symptoms, levels of 41 markers were lower and nine markers higher in the postpartum period.
- High corticotrophin-releasing hormone (CRH) levels in mid-pregnancy were associated with the use of antidepressants and the development of postpartum depressive symptoms.
- Postpartum evening cortisol levels were associated with depressive symptoms, but
- Cortisol awakening response did not differ between pregnant women with or without depressive symptoms.

Since 2016, 683 women have participated in a sub-study with microbiota samples (vaginal, fecal and saliva samples) at pregnancy week 20 and 32 as well as six weeks postpartum. Analysis will be performed during the fall 2019.
Title: IMPACT OF MATERNAL DIET ON GUT MICROBIOTA DURING PREGNANCY

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Background: An adequate microbial colonization early in life is crucial for supporting human health. Maternal microbiota forms the first inoculum for the neonate and, therefore it is important to consider which factors could affect gut microbiota composition during pregnancy in order to avoid the transference of altered maternal microbiota to the offspring. Diet is one of the most powerful tools to shape microbiota, however little is known about the impact of specific dietary components during pregnancy on maternal gut microbiota.

Objective: To determine the effect of diet during pregnancy on the gut microbiome of healthy mothers.

Methods: A total of 86 healthy pregnant women were included in the study. Dietary information was recorded using FFQ and gut microbiota profiling was carried out by 16S rRNA Illumina sequencing from maternal stool samples collected at birth.

Results: Maternal diet during pregnancy shaped the gut microbiota at birth. We identified two gut microbiome clusters characterized by higher abundance of Prevotella for cluster I and Ruminococcus for cluster II. Higher dietary fibres, animal protein and omega-3 fatty acids intakes were observed for cluster II. We also showed a significant pattern that associated positively plant based nutrients with members of the Christensellaceae family, Dehalobacterium and Eubacterium and negatively with Dialister and Campylobacter.

Conclusions: Maternal microbiota is shaped by diet during pregnancy which may have significant effects on early neonatal microbiota composition and thereafter on the immunological and metabolic development with short and long-term effects on infant’s health.
Background: Preterm birth (PTB) is one of the leading causes of neonatal mortality. The causes for spontaneous PTB are multifactorial and remain often unknown. In this study, we tested the hypothesis that human milk oligosaccharides (HMOs), prebiotic, anti-infective and immunomodulatory glycans in blood and urine, modulate the maternal urinary and vaginal microbiome, and influence the risk for PTB. We analyzed the vaginal and urinary microbiome of a cross-sectional cohort of women with and without preterm labor and correlated our findings with measurements of HMOs in urine and blood.

Results: We identified several microbial signatures associated with short cervix, PTB and/or preterm contractions such as *Lactobacillus jensenii*, *L. gasseri*, *Ureaplasma sp.* and *Gardnerella sp.* Additionally, we observed associations between sialylated HMOs, in particular 3'-sialyllactose, with PTB, short cervix and increased inflammation. Specific HMOs, such as 2'-fucosyllactose and lactodifucotetraose positively correlated with members of microbial communities in vagina and urine, particularly, with *Gardnerella* signatures, confirming an influence of HMOs on the microbiome profile.

Conclusion: Identifying serum and urinary HMOs and several key microorganisms associated with PTB, our findings point at two distinct processes modulating the risk for PTB. One process seems to be driven by sterile inflammation, characterized by increased concentrations of sialylated HMOs in serum. Another process might be microbiome-mediated, potentially driven by fucosylated HMOs in urine. Our results support current efforts to improve diagnostics and therapeutic strategies.
Human Milk Oligosaccharides (HMO) are Present in Midgestation Amniotic Fluid (AF) and Associated with a Sparse but Consistently Present AF Microbiome

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HMOs comprise a diverse group of indigestible complex glycans in sialylated (SL) or fucocylated (FL) forms thought only be found in human milk. HMOs play a role in infant gut health by favoring proliferation of beneficial bacteria, acting as “decoys” for pathogens, and establishing the gut epithelial barrier. During a full spectrum metabolomics screen on n=731 midgestation AF samples, we identified a peak consistent with a glycan oligosaccharide. Hypothesizing that this spectral peak was an HMO, AF samples were analyzed on the 1290 Infinity (6545 Q-TOF in positive/negative ion modes). Lowess normalization enabled deconvolution, and correction for multiple comparisons employed an FDR=0.1 with significance set a priori at q<0.001. Validation/quantification of HMOs in a subgroup (n=279) occurred via oligosaccharide extraction over C18/carbograph microcolumns, followed by fluorescent labeling with 2-aminobenzamide, and analysis via amide-80 HPLC with separation and annotation by ion trap mass-spectrometry. DNA was extracted from AF samples with controls and subjected to 16S V4 and shotgun metagenomic sequencing (Illumina & BGI). Results isolated 3’SL HMO in AF at 589.34±18.65 ng/ml (Fig.1). Metagenomic sequencing revealed an association with microbiome taxonomy and phylogenetic diversity (Fig.2). We report for the first time that 3’SL HMOs are found in midgestation AF at physiologic levels, albeit log-lower than breast milk. Parallel metagenomics analysis demonstrated microbiome composition and functional variation in a sparse community distinct from contamination controls. Collectively, these findings suggest both a known beneficial bacterial substrate (3’SL HMO) and a sparse but consistently present microbiome can be detected in AF from midgestation pregnancies.
Figure 1. A) Linear Regression of 3’ SL concentration and Gestational Age (n=279), and B) Histogram of 3’ SL concentrations (n=279) demonstrating that variations in 3’ SL in midtrimester amniotic fluid follow a normal distribution and could not be explained by any maternal baseline characteristics nor pregnancy outcome.

Figure 2. A) Alpha Diversity (Chao1, Shannon, Simpson) plots versus 3’SL (n=279). B) Unweighted UniFrac PCA colored by 3’ SL concentrations (n=279). C) Genus level heatmap with ward dendrogram clustering of DMM groups 2-4 depicting relative reciprocal abundance demonstrating differential abundance of beneficial keystone taxa with 3’SL HMO in the amniotic fluid.
009

001 - Pregnancy and Pregnancy Complications

Restoration of the Vaginal Microbiome and the Microbial Ecology Following Obstetrical Fistula Repair in Lilongwe, Malawi

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The vaginal microbiome is a mediator of reproductive health/disease, with well documented fluctuations in ecology and dysbiosis as a result of pregnancy, infection, and/or antibiotics. However, community restoration following disruptive events have not been previously studied. We hypothesized that a longitudinal analysis of the vaginal microbiome community following surgical repair of chronic obstetrical fistula would reveal informative results regarding vaginal microbiome resiliency. Women (n=15) undergoing delayed repair of their obstetrical VVF/RVF consented an observational, prospective cohort at the Bwaila Hospital Fistula Care Centre (Lilongwe, Malawi). Surgical, medical, dietary and other metadata were collected alongside daily vaginal, urine & rectal microbiome specimens from pre-op (day 0) through discharge (d9-28), and 6-week post-operative. DNA was extracted from all samples with controls and subjected to 16S V4 & shotgun metagenomic sequencing (Illumina) followed by a customized longitudinal bioinformatic analysis pipeline. Following obstetric fistula repair, the vaginal community was restored and differentiated from the rectal and urine communities (Fig.1A). Each woman demonstrated near daily changes in vaginal ecology, being more similar to herself than to others (Figs.1B-2). Community microbial dynamics were shaped by several keystone genera, including lactobacilli and bifidobacteria (Fig.2C). All women with a successful fistula repair demonstrated microbial restoration within 6 weeks, despite months to years of symptomatic VF. We report for the first time restorative vaginal community ecology in n=15 subjects undergoing delayed obstetrical fistula repair. While larger cohorts are needed to understand potential microbial predictors of successful obstetrical VF repair, these findings illustrate the remarkable resilience
of the vaginal microbiome.

Figure 1. A) Beta diversity plot as measured by Bray-Curtis dissimilarity of samples from all body sites. Rectum samples maintained a distinct bacterial composition compared to vaginal (posterior fornix, introitus) samples. B) Beta diversity plot as measured by Bray-Curtis dissimilarity in only vaginal samples, colored according to patient ID. Each woman appeared to remain more similar to herself than to others in the cohort.

Figure 2. Phylogenetic diversity and taxonomic changes occur in the vaginal microbiome post obstetrical-fistula repair, stabilizing within 14 days postoperative. A) Alpha diversity (Shannon) volatility plot by body site and within subject over time, B) Order level taxa bar plot sorted by patient and week postoperative, and c) heatmap (Class) level. These findings mean that the vaginal microbiome is restored after obstetrical fistula repair, following the recolonization of several key vaginal taxa (e.g., bifidobacterial and lactobacilli).
The vaginal microbiota (VMB) has been implicated in both healthy and adverse pregnancy outcomes, and research has suggested links between commensal microorganisms and various genital-tract infections. This ongoing study aims to investigate the VMB characteristics and possible associations with *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), *Trichomonas vaginalis* (TV), and *Mycoplasma genitalium* (MG) genital infections among pregnant and post-delivery women in Pemba Island, Tanzania. Vaginal swabs were collected in eNAT buffer two times during pregnancy and once after delivery. Detection of genital infections was performed using PCR and investigation of VMB using Intergenic Spacer profiling assay. VMB was clustered into community state type (CST). VMB alpha diversity was compared longitudinally, and Wilcoxon rank sum test was used to determine the statistical significance of the changes (p<0.05). To date, 44 women were longitudinal tested. The most common CSTs were *Lactobacillus* dominant ones (89%) antenatal and non-*Lactobacillus* dominant ones (58%) postdelivery. The Shannon diversity was comparable intrapartum and postdelivery, whereas a significant increase in VMB richness (p=0.03) has been observed postdelivery. Before delivery, 59% of women maintained the same CST, whereas post-delivery the CSTs shifted in 92% of women. This study aims to be one of the few investigating longitudinally the prevalence of curable genital infections and VMB characteristics in pregnant women across different time points during pregnancy and post-delivery. All of the genital infections were present in the tested women. Additional analysis with the cohort characteristics, and the association of VMB dysbiosis with genital infections and with pregnancy outcomes will be presented.
Introduction: High circulating estrogen levels during pregnancy favor a *Lactobacillus* spp. enriched vaginal microbiota (VMB) seen in pregnant women regardless of ethnicity. Increased microbial diversity and richness have been linked to preterm birth. The composition and possible changes in VMB in late pregnancy at term or post-term, and just before parturition, however, is yet uncharted.

Materials and Methods: We sampled 335 Finnish women between 37 0/7 and 42 1/7 weeks of gestation at the time of elective cesarean section, admittance to labor ward due to contractions or during induction of labor due to postmaturity. We collected an extensive background questionnaire and vaginal swabs for microbiota analysis. For bacterial community profiling, we used Illumina MiSeq platform to sequence hypervariable V3-4 regions of the 16S rRNA gene.

Results: We have just started to analyze the data. The preliminary analysis has identified interesting associations between parity and the vaginal microbiota as well as the effects of contractions and gestational weeks on the microbiota composition. The final results and conclusions will be presented at the conference.
Progesterone increases Bifidobacterium relative abundance during late pregnancy


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Gestation is accompanied by alterations in the microbial repertoire, however the mechanisms driving these changes are unknown. Here we demonstrate a dramatic shift in the gut microbial composition of women and mice during late pregnancy including an increase in the relative abundance of Bifidobacterium. Using in vivo transplanted pellets, we found that progesterone, the principal gestation hormone, affects the microbial community. The effect of progesterone on the richness of several bacteria species including Bifidobacterium was also demonstrated in vitro, indicating a direct effect. Altogether, our results delineate a model in which progesterone promotes Bifidobacterium growth during late pregnancy.
Formation of primary gut microbiota is extremely important in developing balance between health and disease. The fetal gut microbiome has not been robustly investigated, despite recent demonstrations of intracellular microorganisms with diverse metabolic and immune regulatory functions. Until now, the course of pregnancy was believed to be a sterile environment. However, recent studies have suggested that microbial populations are present in the fetal also prior to birth. Our results have identified first signature of microbial presence during embryo development, showing that first colonization most likely occurs prenatally. However, direct evidence of utero transfer of microbiota is still lacking. Therefore, improving our knowledge on factors, physiological mechanisms and modifiers involved in shaping the first formation of the gut is crucial for assessing the early life microbiome and their potential role in early life infant health. Furthermore, the influence of the maternal gut microbiome on the future risk of diseases of the fetus and the offspring is still to be determined.

Present study has for the first time looked at the origin of fetal gut microbiota, and indicated its exact route of transmission. These innovative studies have provided new knowledge on factors enabling the microbiota formation, maintenance, prevalence its stability, and modulation its resources, which is particularly important for disease predisposition and treatment, and prevention prognosis. In the future, these results will allow identifying novel probiotics. Progress in this area can open a new window into clinical research and bring a potential treatment for curing many infections by restoring gut microbiota homeostasis.
Gestational diabetes treatment is associated with changes in gut and saliva microbiome composition
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Aim: To address how gestational diabetes (GDM) treatment alters the gut and saliva microbiomes.

Methods: We obtained stool and saliva samples for 252 pregnant women, previously recruited for a randomized trial of very tight versus less tight glycaemic targets in women with GDM: 209 women with GDM and 38 women without GDM (control group). Women with GDM were randomized to 2 groups per target glycaemic levels: GDM1 (tight glycaemic targets, fasting blood glucose <5.1 mmol/L and <7.0 mmol/L postprandial, N=106) and GDM2 (less tight glycaemic targets, <5.3 mmol/L and <7.8 mmol/L, respectively, N=103). Stool and saliva samples were collected within 1-2 weeks after recruitment (28.8±3.6 weeks) and at 35-36 weeks of gestation (T3). 16S rRNA gene sequence analysis was carried out after sequencing on a MiSeq platform.

Results: During second trimester (T2) there were no differences in the gut and saliva microbiomes between GDM1, GDM2 and controls. T3 saliva microbiomes of GDM women had greater bacterial richness compared to control (p=0.04). Stool T2-T3, saliva T2-T3 and stool T3 samples within a group (GDM1, GDM2 or control) were more similar to each other than they were to samples in other groups (p=0.03, 0.05 and 0.03 respectively). In T3 stool we found a two-fold increase in the relative abundance of Firmicutes and a 3-fold increase in relative abundance of Ruminococcus in GDM relative to control.

Conclusion: We found differences in saliva and gut microbiomes of GDM women compared to healthy women after 1 month of treatment.
001 - Pregnancy and Pregnancy Complications

Oral Ursodeoxycholic Acid for Treatment of IHCP Alters the Metagenomic Composition of the Placenta with Changing Bile Acid Profile

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Introduction: In intrahepatic cholestasis of pregnancy (IHCP), exogenous ursodeoxycholic (UDCA) bile acid displaces high levels of primary and (microbial derived) secondary bile acid derivatives, and accumulates in the placenta. Since microbial taxonomical makeup and bile acid composition are interdependent in the gut, we sought to determine if the metagenomic taxonomical profile of the placenta is altered in association with changing bile acids.

Methods: Placentas from IHCP patients, with (n 30) and without (n 10) ursodeoxycholic acid treatment, and control (n 10) were profiled by quantitative MRM mass spectrometry, and the taxonomical composition of a subset profiled by whole genome shotgun (WGS) sequencing.

Results: Of bile acids detected, 9 of 11 bile acids increased in placenta in IHCP (p<0.05). Hierarchical clustering revealed their composition to structure in association with IHCP and UDCA treatment (A). BA Group 4 structured around increased UDCA and its derivatives, which correlated to the time since diagnosis/treatment (36 days, p=0.01). Beta diversity analysis of the placental microbiome revealed clustering by virtue of treatment with UDCA which became more significant when alternately stratified by detection of UDCA and derivatives (B, p<0.05). LEfSe revealed enrichment of numerous taxa including Actinomyces oris (p<0.05). Taxonomical beta diversity correlated very strongly to the concentration of UDCA and its derivatives (C, p<0.002).

Conclusions: The apparent dynamic taxonomic changes detected in placenta in coordination with bile acids that are bacterial metabolites and agonists, suggests that the low abundance metagenomic signal in the placenta originates from an adapting live community, and cannot be explained by contamination.
DEPRESSIVE SYMPTOMS IN THE THIRD TRIMESTER OF PREGNANCY ARE ASSOCIATED WITH MATERNAL POSTPARTUM MICROBIOTA DIVERSITY IN A BRAZILIAN COHORT

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Objective: Evaluate whether maternal depression in the third trimester of pregnancy (baseline: 28th-35th weeks) is associated with differences in alpha and beta diversity of the maternal gut microbiota of postpartum women (30 days postpartum) enrolled in a cohort developed in Rio de Janeiro – Brazil.

Methods: In a prospective cohort with 151 mother-infant pairs, maternal stool microbiota from 53 mothers were sequenced at 30 days postpartum using 16S rRNA gene sequencing (MiSeq). Depressive symptoms was assessed at baseline using the Edinburgh Postnatal Depression Scale (EPDS; cut-off ≥ 11; \[n_{\text{depressed}}=17, n_{\text{not depressed}}=36\]). Alpha diversity indices included Shannon, Faith-PD, and Observed OTUs. Beta diversity metrics included Bray-Curtis dissimilarity, weighted and unweighted UniFrac distance. QIIME2 was used to analyze alpha and beta diversity using rarefaction plots, PCoA analysis and distance boxplots. Statistical analyses included Mann-Whitney U Test and permutational multivariate analysis of variance (PERMANOVA).

Results: Based on the Bray-Curtis dissimilarity, and UniFrac distances, microbiota beta diversity was significantly different in depressed versus not depressed women (PERMANOVA: Bray-Curtis, pseudo-F=2.985860, p=0.001; Unweighted UniFrac pseudo-F= 1.708088, p=0.018; Weighted UniFrac pseudo-F= 4.149247, p=0.005). Alpha diversity based on the Shannon index tended to be lower in depressed women but did not reach statistical significance (MannWhitney U Test: p=0.069).

Conclusion: At 30 days postpartum, the beta diversity of the maternal gut microbiota is significantly different in depressed versus not depressed women.
For the last century it was assumed that the intrauterine environment is sterile and microbial colonization starts during delivery. In 2014 it was proposed that the placenta harbors a unique microbiome. Researchers are still debating if colonization starts during birth (the sterile womb paradigm) or already intrauterine (in utero colonization hypothesis).

This study further investigates if there is a placental and intrauterine microbiome in healthy full-term pregnancies. Comprehensive microbiome sampling (placenta, vernix, amniotic fluid, oral, vaginal and fecal) was performed on 50 women undergoing elective cesarean sections and 26 women with vaginal deliveries. The placental samples were analyzed with gene sequencing and qPCR of the 16S rRNA for the quantification of bacterial DNA. qPCR analysis of placenta tissues (fetal, intermediate, maternal site) showed low levels of bacteria in the maternal side only. Amplicon sequencing found no evidence for the presence of bacteria in placenta tissues above background contamination as confirmed by qPCR levels under the detection limit of gene sequencing. qPCR analysis of vernix did result in no detectable bacterial DNA in the samples from babies delivered by cesarean section, but low copy numbers of bacterial genes were in vernix of the babies born vaginally, most likely due to contamination during delivery. Culture experiments of placenta tissue did result in large numbers of colonies from placentas after vaginal delivery but no or only single colonies from placentas after cesarean sections.

Our findings do not support the existence of an intrauterine microbiome in women with normal uncomplicated pregnancies.
002 - Birth Mode

Mapping intestinal neuro-immune-microbiota communications at birth, and their lifelong immunological impact

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The infant gut undergoes massive microbial colonization at birth, which is crucial for proper development of intestinal and systemic immunity. Recent studies suggest that alterations to gut microbiota composition in early life, are linked to disease development later in life. However, the molecular mechanisms that facilitate proper host-microbiota interactions at birth remain mostly unknown.

To analyze these interactions, we have developed an intestinal organ culture system that preserves the physiologic tissue structure and cellular complexity. This advantage facilitates experimentations which cannot be reliably performed in-vivo, and has already led us to discover some unexpected roles for enteric neurons in mediating microbiota-induced effector and regulatory T-cells (Treg) development.

Based on these findings, we hypothesize that the enteric nervous system mediates immune-microbiota interactions at birth, and that these early neuro-immune-microbiota communications impact T-cells development and disease susceptibility later in life. To address this hypothesis, we utilize the gut organ culture system to systemically map the intestinal neuro-immune responses to neonatal microbiota at birth, and to determine whether alterations to neonatal microbiota composition in mice and humans disrupt early-life intestinal responses.

Our preliminary work established the use of the gut organ culture system as a valuable tool for dissecting host-microbiota interactions at birth. We expect that this transformative experimental approach will provide novel insights into neuro-immune-microbiota interaction at birth, and their long-term immunological impact.
DEVELOPMENT MODE IS NOT ASSOCIATED WITH DIFFERENCES IN DIVERSITY OF THE INFANT GUT MICROBIOTA AT APPROXIMATELY ONE MONTH POSTPARTUM IN RIO DE JANEIRO

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Objective: Evaluate whether delivery mode (vaginal or cesarean) is associated with differences in alpha and beta diversity of the gut microbiota of infants aged 25-48 days enrolled in the Rio de Janeiro.

Methods: Of 151 mother-infant pairs enrolled in the Rio de Janeiro, infant stool microbiota from 56 infants (25-48 days postpartum) were sequenced using 16S rRNA gene sequencing (MiSeq). Delivery mode was recorded as vaginal (n=41) or cesarean (n=15) for each mother-infant pair. Beta diversity metrics evaluated included Bray-Curtis dissimilarity, weighted and unweighted UniFrac distance. Alpha diversity indices included Shannon, Faith-PD, and Observed OTUs. QIIME2 was used to analyze alpha and beta diversity using rarefaction plots, PCoA analysis, and distance boxplots. Statistical analyses included Mann-Whitney U Test and permutational multivariate analysis of variance (PERMANOVA).

Results: Based on the Bray-Curtis dissimilarity, and UniFrac distances, infant gut microbiota beta diversity was not significantly different in infants born vaginally versus those born by cesarean section after the first month of life. Similarly, alpha diversity based on Shannon, Faith-PD, and Observed OTUs indices, was not significantly different in infants born vaginally versus those born by cesarean section.

Conclusion: Differences in alpha and beta diversity of the infant gut microbiota after the first month of life are not significantly different between infants born vaginally compared to infants born via cesarean section.
Birth Mode

A synbiotic mixture of scGOS/lcFOS and Bifidobacterium breve M-16V is able to restore the delayed colonization by Bifidobacterium in C-section delivered infants

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C-section born infants have a compromised gut microbiota at birth. Epidemiological data indicates associations between C-section and the development of immune and metabolic disorders.

The objective of this study was to determine the effect of a specific synbiotic mixture in restoring the delayed colonization by *Bifidobacterium* in C-section delivered infants.

In a multi-country double-blind, controlled study, 153 infants born by elective C-section were randomised to receive (1) an infant formula supplemented with prebiotics (scGOS/lcFOS) and *B. breve* M-16V, or (2) a formula with scGOS/lcFOS, or (3) a control formula from birth until age 4 months. Thirty infants born vaginally were studied in parallel. Stool samples were collected at d3, d5, w4, w8, w12, w16, and w22 (6 weeks post-intervention). Fecal counts of total bifidobacteria, different *Bifidobacterium* species including the probiotic strain, and other bacterial groups were determined with molecular tools. pH and SCFA were also measured in the stool samples.

We confirmed the delayed colonization by *Bifidobacterium* in C-section delivered infants. Synbiotic supplementation resulted in higher means of total *Bifidobacterium* gene counts from day 3/5 (P<0.0001) till week 12 (P=0.032) and a reduction of Enterobacteriaceae from day 3/5 (P=0.002) till week 12 (P=0.016) compared to the control group. This was accompanied with higher acetate and a lower fecal pH.

We demonstrated how infant formula supplemented with specific synbiotics restores colonization by *Bifidobacterium sp.* in C-section born infants. We are currently leveraging a multi-omics approach to determine effects of synbiotic supplementation on gut microbiome function.
It is known that birth mode, among other factors as gestational age or antibiotics, shapes the initial neonatal microbiota. However, other potential environmental factors, as homebirth, would affect neonatal microbial colonization. Nevertheless, studies showing the impact of hospital vs homebirth on this process are not still uncovered. Thus, this study was aimed to investigate the influence of place and mode of delivery on the early colonization during the first month of life and its implication on intestinal function.

Gut microbiota composition of neonates born by C-section (CS, n=13), vaginal delivery at hospital (HVAG, n=21) and homebirth (HB, n=15) was determined by sequencing of 16S rRNA gene. Gut barrier function and innate immune response were assessed using a triple co-culture system composed by a simulated intestinal epithelium and macrophage-like cells exposed to fecal supernatants from neonates, mimicking neonatal gut.

CS born infants had a shifted microbial colonization pattern, characterized by depletion in *Bifidobacterium* and *Bacteroides* genus. Cells exposed by HB infant’s fecal supernatant showed a better gut barrier function, with higher transepithelial electrical resistance across the monolayer. These supernatants also induced a higher immune response in epithelial and monocytic cell lines than those from CS infants.

Mode and place of delivery had an influence on the microbial colonization patterns. These would have a key impact on the gut barrier function and immune system development in the neonate. Our results highlight the importance of host-microbial contact during the first month of life for the immune system and gut barrier development.
Breast milk influences metabolic and immunologic health of neonates. It contains a diverse population of bacteria, but little is known about the vertical transfer of factors influencing the milk microbiome and the potential impact of microbes on infant health. Variation in carbohydrate content of maternal diet during lactation time may influence the biological role that milk microbiome could potentially play for human health.

The aim of this study is to investigate influence of prebiotic lactulose, on selected bacteria of mother’s milk microbiome and production of gases during its fermentation.

In order to investigate in vitro fermentation of lactulose by the human milk microbiota, selected bifidobacteria and lactobacilli, isolated from human milk in different lactation stage were anaerobically incubated in media with 0.5 % lactulose. The compositions of the milk microbiota samples were observed before and after 72 h of incubation time using DGGE. The changes in biochemical parameters and gas production before and after fermentation were monitored by MicroOxymax respirometer.

Lactulose as a prebiotic for maternal diet has positive effect on the growth of *Bifidobacterium* and *Lactobacillus* of breast milk microbiome. In vitro fermentation of lactulose by probiotic bacteria, selected from human milk, showed a bifidogenic effect, since decrease in pH of media and cumulative production of CO$_2$ were observed.
The Neonatal Period

Speculating gut microbiota pattern of very low birth weight infants from the birth to 1 month of life in Korea

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Introduction: To speculate the status of fetus gut microbiome environment, we collected meconium and the consecutive series of stools from very low birth weight infants (VLBWIs). We designed this prospective study to evaluate the short term clinical outcomes according to microbial commensalism patterns.

Methods: The included patient subjects were preterm babies whose birth weight was less than 1,500 g in Kyung Hee University Hospital. The very first meconium was obtained and additional 3 more consecutive stool samplings were done in every 7 days. The obtained samples were frozen immediately under -72°C. Ilumina MiSeq platform was used to analyze V3 and V4 regions of 16s rRNA sequence of commensal bacteria in the samples. The infants clinical variables were collected and principal coordinated analysis (PCoA) was performed.

Results: Thirty five stool samples from seven babies were analyzed. The first week’s median OTU, Shannon, and Simpson index were 179(33-377), 4.91(1.04-6.59), and 0.91(0.49-0.98). The second week’s were 73(35-356), 1.83(0.68-4.86), and 0.64(0.19-0.95). The third week’s were 63(29-314), 1.84(0.12-2.71), and 0.51(0.02-0.79). PCoA analysis showed that Enterobacter cloacae dominant 2 patients showed longer hospital stay, more sepsis episode, bronchopulmonary dysplasia(BPD), retinopathy of prematurity(ROP). Enterococcus faecalis dominant 4 babies showed shorter hospital stay, no ROP, 1 BPD, 1 sepsis episode only. They accomplished faster full feeding establishment. No IVH of greater than grade 3 was observed.

Conclusion: We observed increased diversity in the very first meconium of VLBWIs. Their consecutive stool commensalism showed decreased diversity. According to dominant species of microbiome, VLBWIs’ short term outcome differs.
Human milk contains many bioactive compounds including, among many others, a diverse population of bacteria. This milk microbiota is transferred to the infant through breastfeeding. The origin of milk microbiota is controversial but, at least partly, comes from the maternal gut. On the other hand, mother’s maternal postnatal psychosocial distress may alter gut microbiota. In this context, the objective of this study was to explore whether maternal psychosocial distress was related to milk bacterial composition and diversity during the first three months postpartum. Fifty-one healthy women collected breast milk samples at 2, 6, and 12 weeks postpartum and filled in mood questionnaires on experienced stress, anxiety and depression at 6 weeks postpartum. A metataxonomic (16S rRNA gene sequencing (region V3 and V4) using Illumina MiSeq technology) approach was used to assess bacterial diversity and abundance. Firmicutes was the most frequent and abundant phylum, but its proportion decreased progressively with time, while Proteobacteria and other less abundant phyla increased during the first 3 months of breastfeeding. No significant differences in the relative abundance of major bacterial genera were detected between selected women with high (n=13) and low (n=13) psychosocial distress. However, progressive and distinct changes in the bacterial profile of milk samples of women with low psychosocial distress were observed. Regarding bacterial diversity, high maternal psychosocial distress was related to significantly lower bacterial diversity in milk at 3 months post-delivery, compared to low maternal psychosocial distress. In conclusion, this study suggests a potential relation between maternal psychosocial distress and milk microbiota.
An altered gut microbiota establishment in early life is linked with the development of atopic dermatitis

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Gut microbiota maturation in early life is a dynamic process that is still incompletely understood. It has been hypothesized that inadequate microbiota maturation can cause immune deregulations linked to manifestation of non-communicable diseases, such as atopic dermatitis, later in life.

We investigated the gut microbiota maturation of children from birth up to school-age in association to the subsequent development of atopic dermatitis (AD) in a deeply phenotyped cohort.

Fecal samples from 440 children were collected at ages 5, 13, 21, 31 weeks and 6-12 years and profiled by amplicon sequencing of the 16S rRNA V3 gene region.

Our findings show that the complexity of the microbiota gradually increases over time. Changes in complexity and composition were mostly driven by the duration of breast feeding (p=0.001) and the age of introduction of solid food (p=0.003), whereas the impact of environmental and genetic factors were less pronounced.

Next, we introduced the application of a joint model that enables the use of repeatedly collected infant fecal samples (longitudinal analysis) and link its variations with the probability to develop AD at a given age (survival analysis). The results show a decreased microbial richness (Shannon index p=0.0001) to be associated with an increased risk to develop AD. Furthermore, several bacterial genera were associated with a reduced risk of AD, including *Lachnobacterium* and *Faecalibacterium*.

Altogether our findings suggest that adequate gut microbiota maturation during the first year of life is crucial to reduce the risk of AD later in life.
The Neonatal Period

Intestinal Dysbiosis in Preterm Infants Receiving High Enteral Iron Supplementation

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Objective: All very low birth weight (VLBW, birth weight <1500 g) infants require iron supplementation for iron deficiency but the optimum dosage is not well defined. Oral iron supplementation has been associated with intestinal dysbiosis, increase in enteric Proteobacteria, decrease in beneficial bacteria from Firmicutes and Actinobacteria, and lower bacterial diversity, in term infants and school-aged children. Similar studies are needed in preterm infants. We hypothesize that oral iron supplementation is associated with intestinal dysbiosis in preterm infants.

Methods: Weekly stool samples from VLBW infants, who were given 5 mg of Fe daily in addition to fortification in breast milk, were analyzed for microbiome using 16S rRNA V4-5 region. Bacterial composition was evaluated in relation to the supplemental iron mg/kg/day dose using linear mixed-effects models and other methods.

Results: We analyzed the data on 342 stool samples from 80 infants with birth gestational age and weight of 28.1 ± 2.4 weeks and 1103 ± 210 grams. The iron dose was 4.8 ± 1.1 (range 3.4-8.3) mg/kg/day. Iron dose correlated with increase in Proteobacteria, decrease in Firmicutes and lower bacterial species (Figure 1). Infants receiving ≥6 mg/kg/day of supplemental iron had higher relative abundance of Proteus, lower in Bifidobacteria, and lower Shannon index after controlling for influential perinatal variables (Figures 2).
Conclusions: High dose of oral iron supplementation was associated with intestinal dysbiosis in VLBW infants. Identifying the optimum dose of oral iron in preterm infants is a research priority and individualized iron therapy may be necessary.
003 - The Neonatal Period

Very Low Birth Weight Infant Gut Microbiome Richness and Diversity is Associated with Neurodevelopment at 2 Years of Age

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The purpose of this study was to explore associations between infant gut microbiome diversity and toddler neurodevelopment. We followed 78 low birth weight infants (<1500 g) through the first 6 weeks of their hospital stay, and followed up 20 infants as two year old children. Stool microbiome was collected each week and at home visits. From DADA2-generated ESVs, OTUs were used to calculate Shannon, Simpson, Inverse Simpson, and Chao indices. Infants had typical hospital courses, with length of stay of about 80 days, and various illnesses and conditions associated with prematurity. Their health, growth and development was measured at home visits. The Battelle Neurodevelopmental Screening instrument was completed by the children, as well as several other inventories. The richness of the gut microbiome (number of observed OTUs) of the infant significantly positively influenced adaptive (r=.69, p=.002), personal social (r=.60, p=.01), motor(r=.53, p=.03) and total scores(r=.73, p=.001). Shannon diversity was correlated with the adaptive subscale (r=.49, p=.045) and total score (r=.51, p=.02). These infants had a very high abundance of gammaproteobacteria and low diversity in the NICU. By 2 years of age the gut microbiome approached composition of the mothers’, but still retained enterobacteriaceae ESVs. There were intercorrelations with gut microbiome richness and diversity, gestational age and Battelle scores. The contribution of the gut microbiome to neurodevelopment was confounded by gestational age, and in this small sample was not able to be disentangled. Nevertheless, the data compel more granular examination of microbes that might influence long term brain development.
Characterising the Gastric and Faecal Proteome to Unravel Gastrointestinal Function and Maturation in Preterm Infants

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The nutritional requirements of preterm infants are unique and challenging to meet in neonatal care, yet crucial for their growth, development and health. As such, it is relevant to increase understanding of how dietary inputs are being processed by the immature and developing gastrointestinal tract of preterm infants. In this study, we therefore investigated gastrointestinal function and maturation during early life of preterm infants, including functioning of the gut microbiota.

Gastric aspirates (n = 40 infants) and faecal samples (n = 10 infants) were collected during the first two and six postnatal weeks of life respectively, and analysed with metaproteomics through LC-MS/MS.

Differences in the gastric proteome were mainly driven by the percentage of human milk in enteral feeding (22.7%, p = 0.002) and sample pH (11.8%, p = 0.002). Proteins involved in digestive and immune functioning were significantly more abundant at times of human milk-predominated feeding. Composition of the faecal proteome was associated with gestational and postnatal age (10.4% and 6.3% respectively, p = 0.002). Specific human digestive enzymes in faeces were more abundant with increasing postnatal age. Bacterial digestive enzymes in faeces were analysed across gestational and postnatal age.

In conclusion, our data provides insights in the gastric and faecal proteome of preterm infants including digestive functioning of the gut microbiota. Deeper understanding of gastrointestinal maturation and functioning in preterm infants, might contribute to the improvement of current nutrition support strategies.
Loss of Npas2 liver expression during fetal & neonatal development alters the gut microbiome at light/dark timepoints in mice

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In utero exposure to a maternal high fat diet (HFD) results in the targeted epigenetic reprogramming and disruption of circadian clock genes. To examine the relationship between entraining the circadian clock and the gut microbiome, we conditionally knocked out (cKO) the neuronal PAS domain protein 2 (Npas2) gene in the liver during fetal or neonatal developmental timepoints, hypothesizing that it would significantly alter the offspring gut microbiome at light/dark intervals. Stool samples were collected at 4-hour increments over 24-hour light/dark cycles in neonatal Npas2 cKO (n=63), neonatal control (WT, n=62), fetal Npas2 cKO (n=70), and fetal control (WT, n=70) mice fed an HFD. DNA was sequencing using 16S-V4 on Illumina and analyzed for ASV taxonomy & pathway abundance prediction via DADA2 & PICRUSt2. Comparisons were subjected to ANOVA and Posthoc Tukey-Kramer (multiple), or Welch’s T test (2-way) with 95% CI. Neonatal cKO mice had a significantly increased relative abundance of Firmicutes (p = 0.038) and Proteobacteria (p = 0.009) at 3AM reflecting a phase shift in the rhythmic abundance of Firmicutes, whereas the Fetal cKO mice had no phase shift (Fig.1). We also observed light cycle phase-shifts in the pathway abundances among neonatal cKO mice (q< 0.01), and a universal dampening of responses in the fetal cKO mice when compared to WT, demonstrating lack of entrainment & adaptability when Npas2 is deleted in utero (Fig.2). Taken together, our findings suggest that disruptions to Npas2 hepatic expression significantly
alters the gut microbiome which may play a role in disrupting metabolic adaptation.

Figure 2. Neonatal (top) and Fetal (bottom) Npas2 cKO mouse gut microbiome is significantly altered at the dark to light transition timepoint. Relative abundance of gut microbiome at the phylum level in fetal Npas2 cKO (black) and WT (grey) at 4-hour intervals over a 24-hour period. * = p < 0.05.
Fig. 2 Heatmap comparisons reporting statistically significant predicted pathway abundances between light and dark in the 8) WT light (n=38) vs dark (n=48), and C)Fetal cKO light (n=20), vs dark (n=22), and D) Neo cKO light (n=18) vs dark (n=33) using Welch’s t-test (2-way) (p < 0.05, NTST > 2, min. sample freq. = 100)
Gut microbiota is composed of microorganisms located in the intestine, and plays important role in whole body homeostasis. Pectin is a prebiotic dietary fiber, mainly from apples and citruses, which affects the gut microbiota showing local intestinal and systemic effects. However, the prebiotic significance is poorly understood in regard to their fermentation profiles and redox milieu for health effects.

The aim of this research is to investigate significance of apple pectin in infant nutrition, their impact on selected bacteria of infant’s gastrointestinal microbiome and total antioxidant capacity by electrochemical determination of changes in redox potential.

To investigate the in vitro fermentation of pectin by the infant stool microbiota, we anaerobically incubated the selected bifidobacteria and lactobacilli, isolated from infant’s feces collected from term vaginally delivered infants, at 3 day of life, with 0.5 % pectin. The compositions of the stool microbiota samples were observed before and after 72 h of incubation time using DGGE. The changes in redox capacity of media before and after fermentation were electrochemically determined by cyclic voltammetry and differential pulse voltammetry.

Pectin has positive effect on the growth of Bifidobacterium and Lactobacillus, of infant’s gastrointestinal microbiome. In vitro fermentation of pectin by probiotic bacteria selected from infant’s stool showed a bifidogenic effect, decrease in pH and an increase in redox capacity of media. In vitro electrochemical antioxidant test can quickly determine the changes during fermentation of pectin and can be used to monitor biochemical effects of fermentation, and as a growth indicator of selected species.
The infant gut microbiome is established in the first days of life, and several primary sources of microbial organisms contribute to this process. Previous studies (Asnicar et al 2017; Korpela et al 2018; Ferretti et al 2018) showed by strain-level metagenomic profiling the important role of the mother in seeding the infant gut. However, although breastfeeding is supposedly an important route for microbial transmission, experimental challenges have hindered the investigation of microbial transmission from breast milk with metagenomics. To characterize the influx of breast milk strains in the infant, we used a longitudinal hybrid approach by sequencing isolates from the breast milk of 13 mothers sampled between one day and one year postpartum and surveying the presence of those strains in the corresponding infant stool metagenomes. We isolated common breast milk microbes, including Lactobacilli, Bifidobacteria, Streptococci, and Staphylococci, and less expected taxa, such as Enterococci, Actinomyces, Kocuria rhizophila, and Acinetobacter radioresistens. However, only a limited fraction of these species were shared at the strain level between breast milk and infant gut microbiome, and strain transmission was mainly limited to those species commonly associated with breast milk, suggesting that other taxa might be only transiently present on the breast skin of the mother during breastfeeding and are not transmitted to the infant. By combining cultivation-based and cultivation-free methods, we are showing that breast milk is an important source of microbial colonizers. The identification of strains naturally transmitted through breastfeeding could be a potential source of probiotics for non-physiological nutrition regimes.
003 - The Neonatal Period

Changes in early intestinal microbiota induced by transfers of vaginal, milk-associated and fecal microbiota from pregnant mothers do not program intestinal microbiota in adult rats

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Programming of intestinal microbiota (IM) composition may contribute to the developmental origin of health and disease (DOHaD) since it greatly affects host physiology far beyond the digestive area and its neonatal set-up is particularly sensitive to perinatal environment including birth mode. Thus, IM could act as a mechanistic relay between early environment and adult health, subject that early modifications long-last during entire life. However, although durable shaping of IM during neonatal period is assumed, whether early alterations in gut microbiota actually persist until adulthood is still uncertain.

Therefore, we followed up IM composition by 16s-sequencing throughout the growth of rats which were submitted to transfers of vaginal, fecal and milk-associated microbiota in the neonatal period. Vaginal, fecal and milk-associated microbiotas were collected during gestation and lactation in Sprague-Dawley dams prone (OP) or resistant (OR) to obesity and used as inocula. They were given to Fischer pups orally, every day, from birth to D15, thus constituting 3 experimental groups (F-OP, F-OR and controls which received only the vehicle).

Transfers of these distinct inocula progressively modulate pups IM that showed significant differences in indexes of a- and b-diversities and some families abundances at weaning. However none of these markers were significantly affected in rats at adulthood.

Our data therefore do not support the hypothesis of a programmation of the adult IM but query the influence of the transfer conditions (type of inocula, time window,...) or the subsequent diet and the consequences of the early IM changes on the programming of host physiology.
Human milk is generally accepted as the best nutrition for newborns and has been shown to support the optimal microbiota, growth and development of infants. Milk from women who deliver prematurely differs from that of women who deliver at term. For example, premature milk is richer in glucosaminoglycans (GAG), which appear to act as decoys providing binding sites for pathogenic bacteria to prevent adherence to the enterocyte, than term milk. A dose of mother’s own milk > 50 ml/kg/d decreases the risk of newborn hospital readmission rate. In this study we examined breast milk samples of 30 mothers of preterm infant, in their raw and different storage and supplementation stages. Tested samples were colostrums, transitional milk, mature milk (PBM), in raw stage, and in different stage of pasteurization and storage pasteurized mature milk, milk after 48h storage at 4C, milk after 7d storage at -20C, milk after 7d storage at -20C and pasteurized, milk after 30d storage at -20C, milk after 30d storage at -20C, and pasteurized; PBM supplemented with fortifier (PBM+FF) and infant formula for premature infants (IF PRE). It is well known that antioxidants may influence microbiome. According to measured total antioxidant capacity the best solution for premature babies nutrition is preterm mature milk with fortifier.
003 - The Neonatal Period

Impact of Medication, Nutrition and Probiotics in the First Three Weeks of Live: Comparison of Preterm Infants’ Gut Microbiomes in Three Neonatal Intensive Care Units

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The first weeks in life are crucial for the development of the infant’s microbiome. For research on necrotizing enterocolitis (NEC), the microbiome development is of great interest, as no specific cause of this disease has been identified yet and its anamnesis is most likely linked to a microbial component.

For preterm-born infants spending their first days in hospital, factors shaping their microbiome are mainly the neonatal intensive care unit’s environment including parents’ microbiome, nutrition and medication. Therefore, the influence of these factors was compared in a cohort of 56 infants born in three Austrian hospitals which follow different NEC prophylaxis regimes regarding medication, probiotic administration and nutrition (details available at: Kurath-Koller et al., 2017):

<table>
<thead>
<tr>
<th>Hospital</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probiotics</td>
<td>Lactobacillus</td>
<td>Bifidobacterium</td>
<td>None</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>Gentamycin</td>
<td>None</td>
<td>Gentamycin</td>
</tr>
<tr>
<td>Antimycotics</td>
<td>Nystatin</td>
<td>Fluconazole</td>
<td>Nystatin</td>
</tr>
<tr>
<td>Feeding</td>
<td>Mainly breastmilk</td>
<td>Mainly formula milk</td>
<td>Mainly breastmilk</td>
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The development of the infants’ gut microbiomes during the first three weeks of life was investigated by 16S rRNA gene amplicon sequencing in overall 388 samples.

Our results indicate, that the infants’ microbiomes are more similar in the first days but diverge with increasing age, shaping three significantly distinguishable clusters respective of the three hospitals. Even after correction for the differences in the probiotic regime, the microbiome profiles remained indicative for the hospital.

This study shows that the hospitals’ regimes have major impact on the formation of the neonatal gut microbiome. For a better understanding of the anamnesis of NEC in this consideration, further investigations on these aspects need to be performed.
035

003 - The Neonatal Period

Maternal gut and breast milk microbiota affect infant gut antibiotic resistome and mobile genetic elements

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Background: The fecal bacterial microbiota of infants harbor diverse resistomes, distinct from those of adults. Antibiotic resistance genes are generally more abundant in infants, but their origins are largely unknown as they can be found even in infants without antibiotic exposure, although some of the genes are likely to be transmitted from the mother.

Objectives: Our objective was to determine the effects that maternal microbiota has on infant resistome and microbiome.

Methods: We studied the taxonomic composition, resistome and mobile genetic elements of the infant gut as well as maternal gut and breast milk microbiomes using shotgun metagenomic sequencing.

Results: Infant gut resistomes were largely shaped by bacterial phylogeny with Escherichia coli being highly correlated with resistance gene abundance. The resistance gene and mobile genetic element profiles of infants were more similar to their own mothers’ gut microbiota than to the microbiota of unrelated mothers. Interestingly, the same phenomenon was observed with the mobile genetic elements found in breastmilk. In addition, we observed that termination of breastfeeding and intrapartum antibiotic prophylactic treatment of mothers were linked to higher abundances of specific antibiotic resistance genes and mobile elements. We show that resistance genes and mobile genetic elements can be transferred from the mother to the infant gut directly via breast milk and indirectly from the maternal gut. Our results suggest that infants inherit the legacy of past antibiotic consumption of their mothers, but that the gut microbiota composition yet has a major impact on the overall resistance load.
MATERNAL PRE-PREGNANCY BODY MASS INDEX (BMI) IS NOT ASSOCIATED WITH DIFFERENCES IN INFANT GUT MICROBIOTA DIVERSITY AT APPROXIMATELY ONE MONTH POSTPARTUM

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Objective: To evaluate whether pre-pregnancy BMI is associated with differences in alpha and beta diversity of the gut microbiota of infants aged 25-48 days enrolled in Rio de Janeiro.

Methods: Prospective cohort with 151 mother-infant pairs enrolled in the study to date, infant stool microbiota from 57 infants (25-48 days postpartum) were sequenced using 16S rRNA gene sequencing (MiSeq). Maternal pre-pregnancy BMI was computed using the subject’s self-assessed pre-pregnancy weight and measured height. BMI values were categorized as underweight (n=1), normal weight (n=31), overweight (n=17), and obese (n=8). Beta diversity metrics evaluated included Bray-Curtis dissimilarity, weighted and unweighted UniFrac distance. Alpha diversity indices included Shannon, Faith-PD, and Observed OTUs. QIIME2 was used to analyze alpha and beta diversity using rarefaction plots, PCoA analysis, and distance boxplots. Statistical analyses included Mann-Whitney U Test and permutational multivariate analysis of variance (PERMANOVA).

Results: Based on the Bray-Curtis dissimilarity, and UniFrac distances, infant gut microbiota beta diversity was not significantly associated with different maternal pre-pregnancy BMI categories. Similarly, alpha diversity of the infant gut microbiota based on Shannon, Faith-PD, and Observed OTUs indices, was not significantly different based on maternal pre-pregnancy BMI.

Conclusion: Alpha and beta diversity of the infant gut microbiota after the first month of life were not significantly different based on maternal pre-pregnancy BMI.
Background Habitual supplementation of fish oils, rich in n-3 polyunsaturated fatty acids, during lactation is promoted as having beneficial effects on infant physiology; however, the effects of fish oil supplements on infant bacteriome establishment and immune development remains unclear.

Methods A six-month observational, prospective cohort study was conducted whereby 47 out of 91 Canadian women self-administered fish oil supplements during breastfeeding and 44 women chose not to supplement. Infant stool and mothers’ breast milk was collected once per month from birth until 6-months of age. Gas chromatography for breast milk lipid profiles and high-throughput sequencing of fecal microbiota in infants were carried out. Immune markers and parent-reported questionnaires were used to assess infant immune development at 1 week, 1 month, and 5 months of age and health outcomes up to 2 years.

Results We show that fish oil increased breast milk eicosapentaenoic acid (EPA) and decreased secretory IgA in lactating women, despite no changes to breast milk docosahexaenoic acid (DHA). High-throughput sequencing revealed an increase in *Bifidobacteria* and *Lactobacillus* spp. in infants suckling on fish oil supplementing mothers. Additionally, the predicted high-level phenotypes of the infants’ microbiome suggest that fish oil may lower commensal traits involved in pathogen colonization resistance. Despite indications that fish oil decreases defensive inflammation, there were no differences in reported sickness incidence in toddlers.

Conclusion This study shows that women supplementing with fish oil associates with decreases in defensive inflammatory responses and corresponding infant fecal microbiome that has anti-inflammatory potential and reduced immune priming.
Early development of the gut bacteriome and virome and their interactions

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Early gut microbiome development influences health outcomes later in life. Though recent studies have focused on the bacterial composition of the microbiome, the viral composition remains largely ignored. We thus aimed to investigate the role of virome and bacteriome in the early development of the gut ecosystem. We use Lifelines-NEXT, a prospective birth cohort in the Northern Netherlands consisting of, till date, 500 pregnant mothers (aimed 1500), their newborns and partners. Repeated sampling of maternal stool commences at 12 weeks of gestation and stool collection from babies occurs at 7 time points from birth until at least 12 months. Various other biomaterials like blood, placental biopsies and breast milk, and data on environmental and medical factors are collected.

In a Lifelines-NEXT pilot consisting of 30 mother-infant pairs that were longitudinally sampled (n=217) during pregnancy and after birth, we performed metagenomic sequencing of DNA from virus-like particles and total microbiome. In a principal coordinate analysis, the gut bacteriome of mothers and infants formed significantly different clusters (p<0.001). Shannon diversity was significantly higher in mothers than in infants (p<2.16X10⁻¹⁶) with infants showing greater diversity with increasing age. Infants had significantly greater abundances of various species of *Escherichia* and *Bifidobacterium* whereas *Alistipes putredinis* and *Faecalibacterium prausnitzii* were most abundant in mothers. The strain of *Bifidobacterium breve* was found exclusively in infants and remained stable for the first 3 months after birth. The virome analysis and its relation with bacteriome is ongoing and will be presented at the meeting.
003 - The Neonatal Period

PROPERTIES OF TRANSIENT MILK FROM MOTHERS OF PRETERM INFANTS WHICH CAN INFLUENCE MICROBIOTA OF INFANTS

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Transient milk from mothers of preterm infants is the most abundant milk used in banks of human milk given the capacity of preterm infants to directly consume it and the amount of milk produced. Milk banks collect, pasteurize, and freeze/store human milk. The processing might alter properties of milk which can influence microbiota of infants, but the effects have not been fully examined. Compared with colostrum and mature milk of mothers of preterm infants transient milk has lower ORP value indicating better total antioxidant capacity (TAC) than colostrum or mature milk. Main component of TAC in transient milk is vitamin C. Ascorbic acid in human milk reverts oxidation reactions because of its affinity to prevent the initiation of lipid oxidation by neutralization of singlet oxygen, or by regenerating the tocopheroxyl radical that can be produced during the lipid oxidation cascade to its native form. Milk was obtained from twenty mothers of preterm infants (gestational age 28-36 weeks; birth weight 900-2,470 g). Milk samples were obtained from 4th day postpartum to two weeks (transient). The mothers were asked to express milk between 8:00 and 10:00 AM. The main effect of transient milk processing is decrease of vitamin C and increase of urate. Our results indicate that whey of transient milk of mothers of preterm infants may be the safest fraction for preservation and very suitable for addition of fortifiers to achieve optimal effect on microbiota in preterm infant nutrition.
003 - The Neonatal Period

The P4 study: postpartum maternal and infant faecal microbiome 6 months after hypertensive versus normotensive pregnancy

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Introduction: The evidence linking the microbiome to human health, including hypertension and cardiovascular health is increasing. The microbiome changes and its affect on pregnancy outcomes including hypertensive disorders (HDP) is still unclear, and postpartum data is lacking.

Objective/hypothesis: To explore differences in faecal microbiome six months post-partum between women and their infants who had normotensive pregnancies (NP) versus those who had gestational hypertension or preeclampsia (HP).

Methods: Stool samples from 18 mother-infant pairs 6 months postpartum after NP or HP. 16S V3V4 amplicons were used to study their faecal microbiome. Samples were aliquoted and stored at -80°C. All samples were then extracted using a PSP AllPrep DNA extraction kit, and 16S sequencing analysis performed.

Results: Ten NP and eight HP (Six preeclampsia, two gestational hypertension) mother-infant pairs participated.

Sequencing results: The HP mothers gut microbiome were not significantly different compared with the NP mothers after six months of giving birth on both a and b diversity. However, we observed that HP’s baby a diversity was significantly lower than NP mothers’ baby, indicating potential risk of HP on healthy develop of baby’s gut microbiome. The most influential confounding factors for baby’s microbiome in this cohort were mode of birth, antibiotic use and feeding methods. Furthermore, bacteria belong to genera Bifidobacterium, Bacteroides, Streptophyta and Sutterella were found to be reduced in babies belonging to HP mothers. The results obtained in this study demonstrated that HP may not have an influence on mum microbiome at six months but significantly affected baby’s gut microbiome development.
The Neonatal Period

Effects of oropharyngeal administration of human colostrum in fecal microbiota of premature newborns.

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The objective of the present study was to evaluate the establishment of intestinal microbiota in preterm infants undergoing colostrum therapy, as well as the microbial composition of breast milk administered. This study included preterm infants submitted to colostrum therapy at an public Hospital in São Paulo, Brazil. The study has two groups: premature infants submitted to raw colostrum (RC, n=12) and premature infants submitted to pasteurized colostrum (PC, n=13) determined according to medical recommendations. Four stool samples were collected at different times: first evacuation, stool sample of the 7th, 22nd and 37th day of life. The intestinal microbiota was characterized by 16S rRNA gene sequencing and real time PCR analysis for total bacteria and Bifidobacterium. The stool samples, we observed a high abundance of Proteobacteria in the first 20 days of life and abundance of Firmicutes in the last sample. However, PC group had higher abundance of those Phyla compared with RC group. At genera level, the RC group had greater prevalence of Bacteroides compared to the PC group. Even in low quantity, all the infants submitted to colostrum therapy, both PC and RC group, had the presence of Bifidobacterium, an important genus for the initial establishment of the intestinal microbiota. The oropharyngeal administration of pasteurized colostrum or raw colostrum was able to modulate the preterm intestinal microbiota in different ways. In addition, the administration of pasteurized colostrum was also able to provide the prevalence of genus Bifidobacterium in early life, which is an important genus for infant intestinal microbiota.
The Neonatal Period

Bile acids metabolism by infants gut microbiota matures in four stages over three years

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Bile acids (BA) metabolism is a co-metabolism between host and gut microbiota and regulates a lipid, carbohydrate and energy metabolism in host. The principal metabolisms of BAs by intestinal bacteria are deconjugation, dehydroxylation, and epimerization, which transforms the taurine or the glycine conjugates of chenodeoxycholic acid (CDCA) and colic acid (CA) into the secondary bile acids, ursodeoxycholic acid (UDCA), deoxycholic acid (DCA) and lithocholic acid (LCA). However, little is known regarding how the development of the intestinal microbial community is associated with maturation of intestinal BA metabolism. To address this, we monitored the succession of gut bacterial community and its association with fecal BA profile in the first three years of ten healthy Japanese infants. The BA profiles were classified into four types, defined by high content of conjugated primary BA (Con), unconjugated primary BA (CDCA and CA) (Pri), UDCA (Urs), and DCA and LCA (Sec). Most subjects begun with Pri or Con profiles during lactation and eventually transited to Sec through Urs after the start of solid food intake. BA deconjugation was associated with Bifidobacterium-dominant microbiota corresponding to lactation microbiome. Urs subjects were strongly associated with Ruminococcus gnavus colonization, mostly occurring between Pri and Sec. Sec was associated with adult-type complex microbiota dominated by a variety of Clostridia and Bacteroidetes species. These results suggested that the gut microbiota is developed stepwise through the definite pattern in association with the maturation of BA metabolism.
Neonatal microbial colonisation drives postnatal gut maturation and supports the development of the immune system, thus possibly affecting (long-term) health. We investigated the microbiome composition of stool samples that were collected within the observational cohort of the INCA study. In total 436 term infants were recruited, of whom 151 received an antibiotic treatment during their first week of life for suspected neonatal infection (AB+). Clinical outcomes of the infants were registered continuously during the first year of life and faeces was sampled at nine time points from birth until two years of age. Maternal faeces was sampled after birth. A subset of 1677 faecal samples (496 AB+) were analysed using 16S Illumina sequencing. The results show a temporal pattern in the infant intestinal microbiota mainly driven by consecutive dominance of Proteobacteria during the first week of life, followed by Actinobacteria up to 6 months and finally Firmicutes at 2 years of age. During this development, the infant’s microbial community characteristics, such as alpha diversity, move towards that of the adult maternal faecal samples. Antibiotic administration during the first week of life seems to delay the normal intestinal microbiota development, especially delaying the peak of Actinobacteria in vaginally born babies that are being breastfed. Clinical interventions around birth like antibiotic treatment seem to affect the microbiota development in early life depending on the delivery and feeding mode of the infant. The possible health implications of these microbiota changes need to be further investigated and considered for cost-benefit analyses of medical interventions.
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Madre, Niños y Microbioma (MNM) Study: A pilot prospective birth cohort to understand establishment and development of the infant gut microbiome

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Maternal nutrition and feeding practice may influence the infant gut microbiota, potentially due to vertical transmission in utero, during delivery and/or via breastfeeding. These early-life microbiota alterations might influence child health in the long term. We aim to determine how pre- and postnatal nutrition influence infant gut microbiome development.

We enrolled 40 pregnant women in their 3rd trimester (Barcelona, Spain). Stool samples were collected from family triads pre- (fathers, mothers) and postnatally (mothers, infants) on days D0, 7, 14, 30, 60, 90, 120, 270, 365. Samples were sequenced for the 16S rRNA V3-4 region and quantitative taxonomic profiling was performed using QIIME2.

Mean infant species diversity remained stable through D120 and significantly increased thereafter until D365 (Shannon index 2.75 vs. 4.18, p=8.25 x 10^-5), but remained lower than that of adults (Shannon index 6.8). Infant microbial composition (mean distance, unifrac\text{unweighted}) was consistently more similar between infants than to adults through D120 (0.57±0.09 vs. 0.85±0.04, P\text{PERMANOVA}=0.001), but on D270 and D365 became more similar to adults (0.78±0.05 to 0.71±0.06; P\text{PERMANOVA}=0.001). Infants exhibited inter-individual variability in relative frequency of taxa. Mean infant \text{Gammaproteobacteria} and \text{Streptococcus} abundance declined from D7 to D365 (12-3% and 14-2%). \text{Bifidobacterium} abundance increased from D7 to D120 (33-64%) with a decline at D270 (42%) accompanied by an expansion of \text{Firmicutes} (13-28%).

Observed temporal changes in infant taxa suggest breastfeeding and complimentary feeding impact the microbiome and are consistent with development of the infant gut microbiota. Future analyses of breastmilk and dietary data will clarify these preliminary findings.
The Neonatal Period

Neonatal antibiotic exposure: the influence on the gut microbiota

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Exposure to antibiotics early in life is thought to affect various physiological aspects of neonatal development. Here, we investigated the long-term impact of neonatal antibiotic treatment on child growth in an unselected birth cohort of 12,443 children born at full term. We found significant attenuation of weight and height gain during the first 6 years of life after neonatal antibiotic exposure in boys, but not in girls, after adjusting for potential confounders. Neonatal antibiotic exposure was associated with significant differences in the gut microbiome, particularly in decreased abundance and diversity of fecal Bifidobacteria until 2 years of age. Finally, we demonstrate that fecal microbiota transplant from antibiotic-exposed children to germ-free mice resulted in significant growth impairment. Thus, we conclude that neonatal antibiotic exposure causes a long-term gut microbiome perturbation which results in reduced growth during the first six years of life.
Intrapartum antimicrobial prophylaxis (IAP) is an important protocol to reduce mortality by invasive Group B streptococcal (GBS) disease. In the 80s was demonstrated that administering IAP during labour to women at risk for transmitting GBS prevented invasive disease and infections of newborns decreased by 80%. However, we are still starting to understand the effect of IAP on the gut microbiome establishment. The correct gut microbiota colonization at the beginning of life is a key event for the foundation of early and future health and is affected by different perinatal factors. It is known that antibiotics highly affect the microbiota and that early postnatal exposure increases risk of later diseases. Moreover, the most frequent cause of contact with antibiotics during the perinatal period is the use of IAP, present in over 30% of deliveries.

We have analysed the impact of IAP on the gut microbial colonization in premature (n=69) and full-term babies (n=41) and, how IAP affects the levels of antibiotic resistance genes, enzymes and transporters involved on the antibiotic resistance process.

We have shown that IAP delays the colonization of beneficial bacteria. In addition, we have observed higher levels of antibiotic resistance genes, enzymes and transporters involved on the antibiotic resistances on babies whose mothers were subjected to IAP.

Our results confirm an impact of the IAP treatment on the correct establishment of the intestinal microbiota and highlight the need of new intervention strategies development based on microbial modulation, with aim to minimize the IAP effects in early life.
004 - Probiotics and Prebiotics in Pregnancy and Infancy

MODULATION OF THE IMMUNE SYSTEM IN FETO-MATERNAL TISSUES BY PREBIOTICS SUPPLEMENTATION DURING PREGNANCY: A FUTURE STRATEGY FOR ALLERGY PREVENTION.

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Allergies are multifactorial diseases related to the dysfunction of the microbiota, epithelial barriers and the immune system leading to a defect in the establishment of immune tolerance. Pregnancy represents an optimal window of intervention in the regulation of the allergic process through a modulation of the fetal immune and microbial systems. Prebiotics are able to improve the immune system, the microbiota and the intestinal barrier. A preclinical study carried out in our laboratory shows that prebiotic supplementation during pregnancy and lactation reduces the development of food allergy in offsprings. The aim of our study was to understand the immunological processes of prebiotics administered during pregnancy only. To do this, pregnant mice received a standard diet or a diet enriched in prebiotics (GOS/inulin). After 18 days of gestation, we analyzed the frequency of different lymphoid and myeloid populations in different maternal tissues (decidua, placenta, uterus, lymph nodes) as well as the hematopoietic stem cells in the femoral marrow of the mother and fetuses. Among the different populations of immune cells analyzed, the frequency of regulatory B lymphocytes (CD19⁺CD9⁺ and CD19⁺CD25⁺ B cells) increased in the placenta and the uterus of mice supplemented with prebiotics. The rate of CD4⁺CD25⁺FoxP3⁺ regulatory T cells was also increased in the placenta of supplemented mice. Prebiotics had no effect on dendritic cells nor hematopoietic stem cells homeostasis. In conclusion, prebiotic supplementation during pregnancy leads to the establishment of a tolerogenic environment which could protect the foetus against future allergies.
004 - Probiotics and Prebiotics in Pregnancy and Infancy

Can a perinatal educational dietary intervention alter the diversity of the infant gut microbiome? Preliminary results from a randomised controlled trial

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Background: The early life gut microbiota may regulate biological pathways underlying non-communicable disease risk. It is unclear whether the perinatal diet influences the diversity and composition of the infant gut microbiota.

Objective: To assess the efficacy of a perinatal dietary intervention in influencing microbial diversity in infant stool samples collected four weeks postpartum.

Methods: Participants were 45 infants born to mothers (n=44) participating in a prospectively registered dietary RCT (ACTRN12616000936426). In week 26 of pregnancy, mothers were randomised to receive dietary advice as part of standard pregnancy care, or to additionally receive a dietary intervention focussing on the Australian Dietary Guidelines and promoting intakes of prebiotic and probiotic foods. 16SrRNA data were generated from infant stool samples. Between-group differences in Shannon index were estimated using a parametric T-test and Cohen’s D effect size was estimated. Sensitivity analyses adjusted for infant age, mode of birth, feeding, antibiotics, and sample storage duration. Group allocation is still blinded for ongoing statistical analysis.

Results: There was a mean difference of 0.3 Shannon Index units between groups A (n=23) and B (n=22), (t=2.3, (95%CI: 0.03, 0.54), p=0.027). This difference represented a medium effect size of 0.69 (95%CI: 0.07, 1.31). Adjustments did not markedly change the effect.

Conclusions: A perinatal dietary intervention was associated with differences in alpha diversity of the infant gut microbiota. Given the modest sample size, these preliminary findings should be interpreted with caution, however we anticipate they will support larger studies aiming to guide the assembly of the early life gut microbiota.
In addition to providing nutrition and bioactive factors necessary for infant development, a number of studies have suggested that human breast milk also contains bacteria, referred to as the breast milk microbiome. It has been proposed that the bacteria present in the breast milk contribute to the establishment of commensal microbiota in the gut of the infant. The composition of this bacterial community however differs substantially between studies. One potential source of this variance is the different approaches employed to extract bacterial DNA. We hypothesised that, when applied to a mock breast milk sample containing defined bacterial communities, or to aliquots of individual human breast milk samples, different widely used approaches would give rise to substantially different 16S rRNA gene amplicon profiles.

Five commonly employed methodologies, including commercial kits, exhibited significant differences in DNA yield and purity (p<0.05), with the more stringent approaches that involved multiple methods of bacterial cell disruption, performing better. In addition, microbiota composition, as assessed by 16S rRNA gene sequencing, differ significantly, including in the contribution of artefactual bacterial signal. Concerningly, many of the bacterial taxa identified as contaminants have been reported as major components of the breast milk microbiota in previous studies.

These findings highlight the importance of using stringent, well-validated, DNA extraction methodologies for breast milk analysis. They also suggest the need for caution when interpreting sequencing derived microbiota data generated from low-biomass human samples.
The microbial colonization of infant gastrointestinal tract plays a key role in human health. Consequently, disruptions during this development of the infant gut microbiota have been reported to increase susceptibility to disease later in life. In early infancy, antibiotic use by the infant is one of the important external factors that influences the gut microbiota composition, associated with decreased numbers of bifidobacteria and Bacteroides. Therefore, promoting specific early feeding practices, such as breastfeeding and supplementation with prebiotics, could be seen as an opportunity to steer the disturbed microbial community towards a more beneficial and resilient state. In this study, TNO large-intestinal model (TIM-2) will be used to examine to what extent the intake of specific prebiotics, before and during antibiotic administration, can improve the resilience of infant gut microbiota. The type of prebiotic will be selected based on the combination of human milk oligosaccharides (HMOs) and prebiotics from KOALA study, as well as novel prebiotics. Samples will be analysed for HMO/prebiotic utilization, microbiota activity (lactate, succinate, short chain fatty acid, branch chain fatty acid, and ammonia), microbiota composition (16S rRNA gene), and function (metatranscriptomics).

Keywords: prebiotics, human milk oligosaccharides, antibiotics, infants, gut microbiome
Extremely low birth weight (ELBW; below 1000 g) infants often develop gut microbial dysbiosis which is related to severe clinical complications. Probiotic supplementation has resulted in clinical improvements in infants with a very low birth weight (VLBW; above 1500 g), but the effect in ELBW infants is still debated. Here, we investigated the effect of *L. reuteri* supplementation on the gut microbiota in ELBW infants. 134 ELBW infants born before post-menstrual week (PMW) 28 were enrolled in a prospective randomised, double-blind, placebo-controlled, multi-center trial, and supplemented with either *L. reuteri* DSM 17938 or placebo from birth to PMW36. Stool samples were collected weekly during the first month of life (1w, 2w, 3w and 4w), at PMW36, and at a follow-up at two years of age. The bacterial community composition of 558 stool samples was characterised by 16S amplicon sequencing. Probiotic supplementation significantly affected the 16S rDNA-based bacterial community composition (beta diversity) and structure (alpha diversity). The community composition (assessed using NMDS visualisation and ANOSIM test) significantly differed between the probiotic and *L. reuteri* group. Probiotic supplementation was associated with significantly higher richness (number of taxa) and alpha diversity (Shannon index), as well as significantly higher abundance of *Lactobacillus reuteri* during the first month of life, as determined by ANCOM analysis. In conclusion, probiotic supplementation modulates gut microbiota diversity and composition in ELBW infants during the neonatal period, but no effect is observed at PMW36 and at two years of age, when the microbiota has developed into a more mature community.
DYNAMICS OF INFANT’S GASTROINTESTINAL MICROBIOME DURING THE FIRST YEAR OF LIFE CAPTURED BY TWO HUNDRED AND FIFTY SAMPLING TIME POINTS
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Introduction: The gastrointestinal microbiome in children is a very dynamic system, susceptible to changes in response to different factors, providing the opportunity to study the compositional changes.

Methods: The dynamics of gastrointestinal colonization of one infant from birth to its first year of life was analyzed by the sequencing of 16S rRNA gene. Sets of infant's meconium/stool and buccal samples were collected with an information about nutrition type and other important factors, including mother’s samples as reference.

Results: Transition from breastfeeding to formula resulted in a decrease of *Bifidobacterium*, and increased of *Bacteroides* and overall microbial diversity. An effect of orally administered probiotic product Colifant Newborn containing *E. coli* A0 34/86 strain on the distribution of bacteria in the gut was confirmed.

Conclusion: Bifidobacteria being among the first microbial colonizers and represented an important commensal group during the first year of life. High levels of bifidobacteria in the infant's gut have been associated with the development and maturation of the immune system. The first *Escherichia* colonizer originated from the application of probiotic preparation Colifant newborn. Probiotic *E. coli* A0 34/86 successfully colonized infant's intestinal tract and became resident during the first year of life.
Commercially Processed Donor Human Milk-Derived Products Retain a Wide-Spectrum of Functionally Active Oligosaccharides

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Background: Human milk (HM) contains many bioactive substances, including a family of highly abundant and structurally unique glycans, called human milk oligosaccharides (HMOs). HMOs constitute the third most abundant component in HM, following lactose and fat, and are more abundant than protein. The best-characterized function of HMOs is their prebiotic activity, which supports the colonization of the gut by mutualist bacteria in the period after birth.

Methods: The spectrum and concentration of oligosaccharides were determined by HPAEC-PAD, using standards for the ten most abundant HMOs. Native donor HM, pasteurized donor HM, and an HM-derived, HM protein fortifier were compared. I-screen assays (TNO, Zeist) using fecal matter from infant, adult, or elderly donors were used to determine pre- and post-biotic activity of HMOs. Taxonomic classification was determined using 16S V4 sequencing and presented as OTU. Production of short-chain fatty acids was determined using gas chromatography and mass spectrometry.

Results: We identified similar spectrums of neutral and acidic HMO between pasteurized donor HM, human milk-derived HM protein fortifier, and native donor HM before processing. I-screen assays demonstrated differences in OTU in infant fecal pools when compared to adult or elderly fecal pools. The profile of short-chain fatty acids was also different between sample pools.

Conclusions: Commercial processing of HM preserves the content, spectrum, and activity of naturally occurring HMOs with ex vivo fidelity of pre-and post-biotic activity. Our data support that fortification of an individual mother’s milk with a wide spectrum of HMOs preserved in human milk-derived products may complement the bio-protective effects of HM.
004 - Probiotics and Prebiotics in Pregnancy and Infancy

METAGENOMIC CHARACTERIZATION OF THE GUT MICROBIOTA OF EXTREMELY LOW BIRTH WEIGHT PRETERM INFANTS SUPPLEMENTED WITH THE PROBIOTIC LACTOBACILLUS REUTERI DURING THE NEONATAL PERIOD

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Variations in gut microbiota maturation during childhood are likely to affect child growth and overall health status. Postnatal administration of probiotics has resulted in clinical improvements in moderately preterm children. However, the impact of probiotics on the colonization of gut bacteria in extremely low birth weight (ELBW; below 1000 g) infants is lacking. We employed metagenome shotgun sequencing to characterize the species-level taxonomic and functional profiles of gut bacteria of ELBW infants sampled from an original clinical study, finding that probiotic supplementation promoted the infants' head growth during the first month of life. The clinical trial is a multi-centred, double-blinded, placebo-controlled study with 134 ELBW infants born before post-menstrual week (PMW) 28, who received either L. reuteri DSM 17938 or placebo from birth to PMW36. In total, 48 DNA extracts of stool samples collected 3 weeks after birth were sequenced using the Novaseq 6000 platform. Bioinformatic analyses were performed using a publicly available metagenome workflow and the HUMAnN2 analysis tool. L. reuteri was present at a significantly higher relative abundance in the probiotic than the placebo supplemented group. Pathogenic bacteria such as Enterobacter cloacae and Haemophilus parainfluenzae were abundant in both groups. Species-resolved functional profiling identified 387 metabolic pathways, in total, and presence of L. reuteri was associated with 47 different metabolic pathways. In conclusion, this study indicates that probiotic supplementation in ELBW infants may affect the metabolic potential of the neonatal gut microbiota.
Prevention of Mastitis by Oral Administration of Lactobacillus salivarius PS2 during Late Pregnancy and Early Lactation

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Background: Mastitis is considered the main medical reason for unintended breastfeeding cessation. Therefore, prevention of mastitis is essential in reducing the likelihood of breastfeeding discontinuation. Previous studies showed efficacy of supplementation with the probiotic Lactobacillus salivarius PS2 in preventing mastitis in a susceptible population. This study aimed to investigate the effect of L. salivarius PS2 (LMG P-27027) on mastitis incidence in healthy lactating women.

Methods: In this randomized double-blinded controlled trial (Trial NL4243), 328 healthy pregnant women were randomly assigned to the probiotic (10⁹ CFU/day) or placebo supplement (maltodextrin). Intervention started at the 35th week of pregnancy and continued until 12 weeks after delivery. Occurrence of mastitis, defined as having at least 2 of the following symptoms: breast pain, breast erythema, breast engorgement and temperature over 38ºC, was evaluated until 12 weeks after delivery.

Results: 9 subjects (6%) in the probiotic group and 20 subjects (14%) in the placebo group reported mastitis (p-value=0.022, K-M (log rank) test). Using Cox’s PH, including subject’s age at delivery as explanatory variable, the p-value was 0.028. The exponentiated coefficient for treatment (0.41) showed that subjects in the probiotic group were 59% less likely to experience mastitis compared to those in the placebo group (95% CI: 9%-81%). Number of Adverse Events (AE) and number of subjects with at least one AE were comparable between groups.

Conclusion: Supplementation with L. salivarius PS2 during late pregnancy and early lactation is effective in preventing mastitis in healthy breastfeeding mothers and thus might offer a strategy to support breastfeeding.
Early life microbiome interventions impact later life metabolic health in mice
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We investigated the effect of early life microbiota modulation on adult metabolic health in male C57BL/6 mice.

Postnatal mouse diets were supplemented with synbiotics (scGOS/lcFOS with *Bifidobacterium breve* M-16V) until postnatal (PN) day 42. Subsequently mice were subjected to a Western-style Diet (WSD; 20% w/w fat) until PN day 98. Body weight and composition and markers for glucose homeostasis and lipid metabolism in adulthood were characterized. We assessed host transcription profiles of selected tissues in adulthood and determined the gut microbiota composition on PN21, PN42 and PN98.

We observed that WSD-induced excessive body weight gain and fat accumulation was mitigated by early life supplementation with synbiotics. Insulin resistance, liver characteristics and markers for dyslipidemia were improved in synbiotic-supplemented mice on PN98. Long-term effects on gene expression were most pronounced in the ileum and were mostly related to lipid metabolism. Furthermore, we detected subtle synbiotics-induced differences in the gut microbiota composition. Transplantation of the modified microbiota after 6 weeks of synbiotics supplementation to age-matched adolescent germ-free mice, did not transfer the beneficial phenotype with protection against diet-induced metabolic derangements. This indicates that timing of beneficial microbiota modulation is critical for achieving long-lasting beneficial and protective metabolic effects.

These results highlight the potential to use beneficial microbiome modulation in early life to reduce obesity risk and support lifelong metabolic health.
Allergies are multifactorial diseases related to the dysfunction of 3 biological actors: the microbiota, the epithelial barriers and the immune system. These alterations lead to a defect in the establishment of immune tolerance. Compelling evidence for the early role of gut microbiota dysbiosis and barrier integrity with allergies is emerging. In this context, pregnancy represents an optimal window of intervention in the regulation of the allergic process through a modulation of the immune and microbial systems, which makes it a promising avenue for the prevention of allergy. Prebiotics are able to act on the immune system, the microbiota and the intestinal barrier. The aim of our study is to assess the effect of prebiotic supplementation during pregnancy on the development of food allergy in offspring. To do this, mice received a diet enriched in prebiotics (GOS/inulin) during pregnancy. Then, food allergy to wheat was induced in their offspring by intraperitoneally sensitization and one oral challenge with the allergen (gliadins). Subsequently, we analysed clinical symptoms and immunological and physiological biomarkers of allergy. In the context of prebiotic supplementation, we observed in offspring: 1/ a tendency to decrease allergic symptoms; 2/ no decrease of allergic markers (T\text{H}2, \text{IgE}); 3/ a tendency to increase tolerance biomarkers. These results show that prebiotics could induce tolerogenic environment on the offspring when they are used during pregnancy, but this strategy is not enough to reduce the allergy.
Although the healthy status of maternal gut microbiota seems to be an important environmental factor, it has been unknown whether and how the maternal gut microbiota contributes to the brain development of offspring. To address this question, we examined if perturbing the maternal gut microbiota by administering the non-absorbable antibiotics (AB) to pregnant dams by voluntary drinking on embryonic day 9-16 influences the behaviors of their offspring at postnatal week four. In the open field test to measure spontaneous activity in a novel environment, the reduced voluntary activity in the mice born from AB-treated dams (AB offspring) compared with that in control offspring was observed. In the open field test, AB offspring spent a shorter amount of time exploring the center of the novel environment than control offspring. The behavioral phenotypes of AB offspring were rescued to a certain extent by fostering of these mice by normal dams from P1, whereas the offspring born from control dams and fostered by AB-treated dams exhibited the phenotypes resembling those of AB offspring. Further analyses showed that the maternal gut microbiota of AB-treated dams exhibited a long-lasting decrease in alpha diversity after the perturbation of gut microbiota by antibiotics. To be noted, the gut microbiota of AB offspring also exhibited the properties like those of AB-treated dams during neonatal periods. These results suggest that this mouse model can be useful in screening for gut microbiota-influencing materials which have potentials to promote brain development and prevent neurodevelopmental disorders of the offspring.
Characterisation of in vitro model to study MAmmary Gland microbial Colonisation (MAGIC model)

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Despite established concept of mammary gland (MG) as microbial habitat with its own microbiota, the exact mechanism of MG colonisation is still elusive. The objective of this study was to thoroughly characterise in vitro cell culture model for study of MG microbial colonisation (MAGIC model). For the MAGIC model, we used immortalized cell line MCF10A, which expresses strong polarized phenotype similar to MG ductal epithelium when cultured on permeable support (Transwell®). The study was conducted in two parts. We analysed 31 human milk samples from self-reported, healthy Slovenian mothers in 3rd to 8th week of lactation. We used 16S rRNA gene NGS and cultivation/MALDI-TOF mass spectrometry identification approaches. In the second part, the culture medium on the apical side of polarised MCFA10 cells grown on Transwell® membranes was replaced by: (1) a medium containing various strains of pathogenic and probiotic bacteria; (2) breast milk, with known microbial composition. We evaluated the ability of bacteria to attach to the epithelial layer, changes in transepithelial electrical resistance (TEER), and changes in the expression of genes encoding tight junctions, mucins and cytokines. We obtained information regarding the microbiota of breast milk, which bacteria were able to attach to the epithelium and the impact of bacteria on the epithelial cells of the MAGIC model. Well-studied MAGIC cell culture model provides new possibilities for the research in other areas of the MG physiology, such as the impact of bioactive milk components on the microbial colonisation of the MG.
EFFECT OF ORAL ADMINISTRATION OF A PROBIOTIC, CONTAINING LACTOBACILLUS CRISPATUS LMG9479, ON VAGINAL LACTOBACILLI COMPOSITION OF PREGNANT WOMEN

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The restoration of lactobacilli population after treatment of vaginal dysbiosis is advisable. Oral administration of a probiotic, containing Lactobacillus crispatus LMG9479, ("EcofeminFloravag", DarsCare, Denmark (EF)) seems promising. In order to assess the impact of EF on vaginal lactobacilli composition, samples from 81 pregnant (5-11 weeks of gestation) women with apparent dysbiosis were obtained before and 1 month after treatment. The probiotic was prescribed as a second stage of dysbiosis correction after the treatment of vagina with 0.05 % chlorhexidine solution cavitated with low-frequency ultrasound, the regimen — 1 capsule twice daily for 2 weeks. The quantity of seven lactobacilli species (Lactobacillus crispatus (LC), Lactobacillus iners (LI), Lactobacillus gasseri (LG), Lactobacillus jensenii (LJe), Lactobacillus johnsonii (LJo) Lactobacillus acidophilus (LA), Lactobacillus vaginalis (LV)) was determined via real-time PCR with the kit for scientific application (DNA-Technology, Russia). We detected from 1to 4 lactobacilli species in every vaginal sample, a species was considered predominant when its proportion was the highest among all. Before EF treatment, LC was predominant in 6 (7.4%) samples, LI in 46 (56.8%), LG in 22 (27.2%), LJe in 4 (4.9%) and LV in 3 (3.7%) samples. After the treatment, LC was predominant in 11 (13.6%) samples, LI in 44 (54.3%), LG in 20 (24.7%), LJe in 4 (4.9%) and LV in 2 (2.5%) samples. We detected the change of predominant species in 11 (13.6%) women. There were no samples where LC was detected after EF treatment if it was absent in vaginal microbiota at the start of the therapy.
Differential composition of vaginal microbiome is associated with successful intrauterine insemination (IUI) in couples with idiopathic infertility: a prospective observational study.

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Vaginal and seminal microbiome have gained increasing interest for their involvement in reproductive health and fertility. However, their role in reproductive outcome is not fully understood yet. In this study, we aimed to correlate the vaginal and the seminal microbiome of 23 couples with idiopathic infertility to the clinical pregnancy rate after intrauterine insemination (IUI).

Vaginal swabs and seminal fluids were analyzed through PCR amplification of variable regions 3 and 4 (V3–V4) of 16S rRNA genes and Illumina MiSeq sequencing. The obtained taxonomic data were then correlated to IUI success, together with a panel of clinical and laboratory variables.

The cohort of idiopathic infertile women showed an average different composition of vaginal microbiome compared to age-matched controls, while for seminal counterpart no relevant differences were observed. Furthermore, among idiopathic infertile women, different patterns of Lactobacillus species dominations were observed, with a predominance either of L. crispatus, marker of a healthy vaginal ecosystem, or of L. iners and L. gasseri, associated with a more dysbiosis-prone environment. Considering all investigated variables, IUI success resulted strongly associated only with vaginal L. crispatus domination (p-value = 0.0002).

To the best of our knowledge, this is the first study investigating vaginal and seminal microbiome in couples with idiopathic infertility. Our results support the hypothesis that some cases of idiopathic infertility may be associated with an alteration of the vaginal flora and that microbiome characterization could be useful, together with standard clinical and laboratory assessments, in the pre-IUI evaluation of couples.
Prenatal and Postpartum Antibiotic Exposure Shapes the Preterm Mother’s Milk Microbiota


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Background: It is unclear whether antibiotics alter the mother’s milk microbiota, particularly among preterm mothers who are frequently prescribed antibiotics.

Objective: To determine how antibiotic classes and timing of exposure are associated with the preterm mother’s milk microbiota across the first 8 weeks postpartum.

Methods: Weekly milk samples (n=508) were collected from mothers (n=86) of infants born <1250g enrolled in the OptiMoM Fortifier Study (NCT02137473). Maternal antibiotic use was collected daily for 2 weeks prior to delivery (prenatal) and during her infant’s hospitalization (postpartum). Microbiota was determined using V4-16S rRNA gene sequencing.

Results: Fifty-one (59%) mothers were exposed to antibiotics. Penicillins (n=29 [57%]) and macrolides (n=21 [41%]) were the most common antibiotic classes administered. Bacterial richness (Chao1) in mother’s milk was lower among mothers with postpartum exposure to first-generation cephalosporins (mean difference [SE]: -25.4 [9.7], p = 0.009) and prenatal exposure to macrolides (-24.5 [7.3], p = 0.0008). Similarly, bacterial evenness (Shannon index) was lower in mothers with prenatal (-0.30 [0.09], p = 0.0005) and postpartum (-0.20 [0.10], p = 0.04) macrolide exposure, and postpartum penicillin exposure (-0.20 [0.09], p = 0.04). Postpartum cephalosporins were associated with a lower incidence of Staphylococcus (incidence rate ratio [95%CI]: 0.59 [0.43-0.82], FDR-adjusted p = 0.007), but higher incidences of Acinetobacter (2.27 [1.34-3.86], p = 0.008) and Pseudomonas (1.64 [1.16-2.32], p = 0.01). Postpartum aminoglycosides were associated with a lower incidence of Corynebacterium (0.12 [0.03-0.48], p = 0.03) and Finegoldia (0.22 [0.07-0.68], p = 0.04), while postpartum penicillins were associated with a lower incidence of Lactobacillus (0.27 [0.11-0.65], p = 0.04).

Conclusion: Antibiotic class and timing of exposure are associated with altered microbiota in preterm mother’s milk. Antibiotic stewardship is often a concern for preterm infants; however, considering these findings, a focus on the mother may also be necessary. Funded by CIHR (#FHG129919; #FDN143233).
Intestinal Archaea Inversely Associated with Childhood Asthma

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Background: Methanogenic archaea are a key part of the gut microbiota alongside bacteria. However, there is comparatively little research on the role of archaea in health.

Objective: As in-vitro and animal experiments have demonstrated immunological effects of archaea, we hypothesised that intestinal exposure to archaeal species would influence the risk of asthma and other allergic diseases. We present the first human study connecting gut archaea with childhood asthma.

Methods: We performed a cross-sectional analysis nested within the Dutch KOALA Birth Cohort Study. DNA from two common intestinal archaeal species, Methanosphaera stadtmanae and Methanobrevibacter smithii, was quantified in faecal samples from 472 children at school age, using qPCR.

Our primary outcome was parent-reported asthma at 6-10 years. Secondary outcomes were questionnaire-reported eczema, total serum IgE levels, sensitisation to aero- and food-allergens and lung function (FEV1/FVC).

Associations between the presence/absence of each archaeal species and outcome were assessed with logistic or linear regression models, adjusted for potential confounders.

Results: Presence of M. stadtmanae was significantly associated with a lower risk of asthma, adjusted OR 0.32 (0.08 – 0.98). In addition, asthma risk decreased monotonically across three categories of increasing M. stadtmanae abundance (adjusted p-for-trend = 0.035). We also observed a non-significant tendency for less eczema and IgE sensitisation amongst children with M. stadtmanae. M. smithii was not associated with any outcome.

Conclusion: Further longitudinal and experimental research is needed to explore whether archaea could be directly linked to asthma risk, or if archaeal abundance is indicative of other health-relevant variation in microbiota composition.
AN INTERIM ANALYSIS ON THE FIRST HONG KONG CHINESE NEWBORN MICROBIOME COHORT – SMART BABY

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Background: In late 2017, we set up the first Hong Kong Chinese newborn cohort “Stool Microbiome and Allergic Reaction Study (SMART Baby) to understand natural course of microbiome development.

Methods: Mothers were enrolled at the third trimester with health and social information collected by a standardized self-administered questionnaire, antenatal and delivery history were acquired from medical records, and infants’ information were reported by mothers monthly. Allergic conditions to be ascertained at 6, 12, 24 and 36-month clinic visits.

Results: At this interim report, 104 infants (55 boys, 49 girls) had their 6-month data completed for analysis. 14 (13.5%) mothers had gestational diabetes, 5.8% pre-eclampsia, 32.7% positive for Group B Streptococcus, and 51.9% received intrapartum antibiotics. Frequent use of probiotic supplement during pregnancy was common (26%), but only a few (5.8%) with prebiotic supplement.

79 (76%) babies were delivered vaginally, 9.6% and 14.4% were by elective- and emergency-cesarean section, respectively. 26.9% infants were fed mostly with breast milk, 14.4% mostly formula, and majority (57.7%) received a mixture of both. 57.9% infants had furry pets and 26% exposed to smokers in their home. Illnesses were reported during the first 6 month from 15.4% (gastrointestinal), 14.4% (skin rash), 47.1% (respiratory), and 21.2% (fever). 16 (15.4%) infants received systemic antibiotics.

Conclusions: Stool samples collected at birth, and then monthly till 6 months were analyzed with 16s rRNA sequencing targeting the V3-V4 regions. Correlation of microbial community development with epidemiological and health variables are being analyzed. These data will be presented in the conference.
Characterization of human breast tissue microbiota from core needle biopsies through the analysis of multi hypervariable 16S-rRNA gene regions

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Breast microbiota compositions are not well understood, and few recent reports have begun to explore the correlation between breast tissue dysbiosis and cancer. Given that various methods for breast microbiota detection were used, the aim of the present work was to clarify which hypervariable region of the 16S-rRNA gene (V2, V3, V4, V6 + 7, V8, and V9) is the most informative for breast tissue microbiota. Healthy and tumour tissues from core needle biopsies (CNBs) and surgical excision biopsies (SEBs) were compared to find a less invasive form of recovery useful for the analysis of a larger statistical population and potentially for diagnostic use of breast tissue microbiota. Finally, this study was the first to analyse the breast microbiota of tumours and paired normal tissues of a Mediterranean population. Our findings showed that the V3 region is the most informative for breast tissue microbiota, accounting for 45% of all reads. No significant differences were found between CNB and SEB specimens in terms of total reads and numbers of Operational Taxonomic Units (OTUs). Moreover, we find that more similarities than differences exist between tumours and adjacent normal tissues. Finally, for the first time, the presence of the Ralstonia genus was associated with breast tissue. Given that previous studies have highlighted differences between healthy tissues of cancer and non-cancer patients and between nipple fluids in women with or without a history of breast cancer, the presence of similar microbiota in cancerous and paired healthy tissues could indicate a predisposition to carcinogenesis.
Prenatal and Peripartum Exposure to Antibiotics and Cesarean Section Delivery Alters the Infant Meconium Microbiome

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Introduction: Meconium microbiome can offer insight into intrauterine and peripartum exposures. Although there is little data, microbiome influenced by prenatal and peripartum antibiotics is theorized to be linked to childhood obesity.

Aims: To investigate the effect of prenatal and peripartum antibiotic exposure, in addition to delivery mode, on the meconium microbiome.

Methods: 16S rRNA gene sequencing was performed on meconium samples from 105 infants in a longitudinal cohort study. Clinical information (prenatal and peripartum antibiotic use, delivery mode, maternal pregnancy weight gain and infant weight for length percentiles at twelve months of age) was collected.

Results: Of 105 full term infants included, 43 (41%) were delivered by CS and 62 (59%) by vaginal delivery (VD). In addition to 19 (31%) delivered by VD, all mothers undergoing CS received peripartum antibiotics.

After multivariable adjustment, beta diversity of meconium was significantly different by delivery mode (p=0.044), with prenatal antibiotic use (p=0.005) and peripartum antibiotic use (p<0.001). Cesarean Section (CS) and peripartum antibiotics were associated with greater alpha diversity (Shannon and Simpson, p<0.05). CS versus vaginal delivery samples had lower Escherichia (p<0.001) and higher Methylobacterium (p=0.001). OTUs from the genus Streptococcus were increased in CS, peripartum antibiotic use with and without CS and in the meconium of infants with excess weight at 12 months.

Conclusion: Prenatal and peripartum antibiotic use and CS delivery, are associated with changes in the diversity and composition of the pioneering gut microbes. Resulting long term health effects warrant further exploration.
Microbiome Changes Associated with Total Parenteral Nutrition Induced cholestasis in Neonatal Intensive Care unit Patients.

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Introduction: Cholestasis has been linked to certain gut–associated bacterial infections, such as E. Coli urinary tract infection or Klebsiella septicemia. In bile salt homeostasis intestinal microbiome plays a role.

Aims: To investigate the role of the intestinal microbiome in the development of TPN cholestasis from serial stool samples in twins discordant for TPN cholestasis.

Methods: Serial stool samples were collected from four premature twin sets simultaneously receiving TPN but discordant for TPN cholestasis. DNA was extracted and 300 bp paired-end reads were sequenced and analyzed.

Results: From the twins discordant for TPN induced cholestasis (direct bilirubin ≥1), 84 serial stool specimens were collected. Twins ranged from 25 weeks to 31 weeks gestational age (mean=27 weeks). There was no significant difference found in antibiotic use between twins with or without cholestasis.

Random decision forests was utilized to determine the genera of microbiome samples from infants with and without cholestasis. In the infants with TPN cholestasis Klebsiella, Veillonella & Enterobacter (p<0.05) genera were found to be significantly increased while Escherichia (p<0.05) was found to be significantly decreased. While beta diversity significantly differed there was no difference in alpha diversity between twins with and without cholestasis.

Conclusions: In the twins who developed TPN cholestasis vs. controls significant differences in beta diversity and increases in relative abundance of Klebsiella, Veillonella and Enterobacter were identified. Further studies are required to determine if microbiome changes are predictive of TPN cholestasis.
Differences in the Oral Microbiome by Delivery Mode

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Background: Future health is influenced by the developing neonatal microbiota, especially in the gut by playing a critical role in diseases and immune responses. It is theorized that some of the gut microbiome development can be triggered by the oral microbiome, which may be affected by the birth mode.

Aims: To compare the oral microbiota of newborns delivered vaginally and by scheduled Cesarean Section (CS).

Methods: From the newborns enrolled in Inova Translational Medicine Institute Preterm birth study saliva samples were collected within the first two days of life. DNA was extracted, sequenced and mapped into Operational Taxonomic Units (OTUs).

Results: After rarefaction 18/20 newborn saliva samples were included. 50% (9/18) samples were from vaginally delivered and 50% were from CS delivered newborns. The mean gestational age was 38.94 weeks (sd=0.73). As compared with vaginal delivery, the CS samples were significantly higher in proteobacteria and lower in Firmicutes levels. Between the two groups, Achromobacter was found to have highest difference at the genus level. There were no significant differences in alpha as well as beta diversity measures between delivery modes.

Conclusion: Proteobacteria, a microbial indicator of dysbiosis and Achromobacter, associated with infection in impaired immune or respiratory systems were higher in CS saliva samples.
Very Low Birth Weight infants (VLBW<1500g) are at risk of gut dysbiosis and neurodevelopmental deficits, including cognitive delay and behavior problems. This study explores correlations among gut microbiome richness and diversity of VLBW infants and their mental development and behavior at 4 years of age. 78 VLBW infant and parent dyads admitted in the Neonatal Intensive Care Unit (NICU) at a hospital in Tampa, FL. Over the first six weeks of their NICU stay, stool microbiome was collected. From DADA2-generated ESVs, OTUs were used to calculate Shannon, Simpson, Inverse Simpson, and Chao indexes (alpha diversity). A total of 24 preschoolers were followed up from the NICU stay with home visits at 4 years of age, completing the Child Behavior Checklist (CBCL). The majority of participants were white non-Hispanic females, born between 25- and 32-weeks gestation with a mean weight of 1074g (±229g) and length of stay in the NICU of 82 ±44 days. Gut microbiome richness and diversity in the NICU was related to t-scores of multiple CBCL domains, lower richness and lower diversity were associated with higher CBCL scores, indicating greater behavioral and emotional issues. The results are shown in Table 1. Gut microbiome diversity and richness has been associated with various maladaptive behavioral and emotional problems, including symptoms of autism, anxiety, ADHD, attention problems, and aggressive behavior. Given the relationship between the gut microbiome and later behavior problems, further research needs to examine the specific underlying causes of this association.

Table 1:

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<th>Gut microbiome richness and diversity correlations with behavior at 4 years old</th>
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<td>p</td>
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<td>0.042</td>
</tr>
<tr>
<td>Simpson r</td>
<td>-0.193</td>
<td>-0.387</td>
</tr>
<tr>
<td>p</td>
<td>0.010</td>
<td>0.062</td>
</tr>
<tr>
<td>Inverse r</td>
<td>-0.193</td>
<td>-0.387</td>
</tr>
<tr>
<td>p</td>
<td>0.010</td>
<td>0.062</td>
</tr>
<tr>
<td>Chao r</td>
<td>0.161</td>
<td>0.173</td>
</tr>
</tbody>
</table>

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).
**Role of the Maternal Gut Microbiota in Immune Activation at the Maternal-Fetal Interface: Impact on Preeclampsia**

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**Background:** Preeclampsia is a leading cause of morbidity and mortality in pregnant women, affecting 5-8% of gestations worldwide. Abnormalities in maternal immunity and gestational immune tolerance play a critical role in the development of this disease. Gut microbiota is an ecological community of commensal, symbiotic and pathogenic microorganisms, with a fundamental role in the maintenance of the host immune homeostasis. Accordingly, changes in the bacterial variety and composition affect systemic immune responses and can disrupt the balance between pro- and anti-inflammatory activation.

**Objectives:** Scope of the study was to evaluate how maternal dysbiosis impact immune responses at the maternal-fetal interface and the consequences for pregnancy outcome.

**Methods:** Oral antibiotic administration of C57BL/6J mice was performed from mating plug detection till embryonic day 14. Immune cell activation was analyzed by flow cytometry. Placental development and vascularization were assessed by immunofluorescence and immunohistochemistry. Microbiome profiling was performed through 16S rRNA metagenomic sequencing. Metabolites were identified by untargeted metabolic analysis.

**Results:** Maternal dysbiosis was associated with increased fetal resorption and lower placental efficiency. Of note, maternal dysbiosis impaired placental NK cell angiogenic function and downregulated CD31 expression in the labyrinth area. Furthermore, maternal dysbiosis significantly impact glucose homeostasis and increased maternal serum level of short-chain fatty acids and branched-chain amino acids.

**Conclusions:** Maternal gut microbiota emerges as a key player of host immune and metabolic homeostasis during pregnancy. Translational relevance of these findings will be further evaluated to identify possible new molecular and cellular targets amenable to therapeutic intervention.
Proteobacteria to Firmicutes Ratio Correlated with Epithelial Inflammation and Adhesion Responses

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Background: Intestinal dysbiosis, increased intestinal inflammation and permeability are present in necrotizing enterocolitis and neonatal sepsis. Sterile fecal filtrates can improve symptoms in patients with inflammatory bowel diseases. We hypothesize that fecal filtrates from stool with higher presence of Proteobacteria induce greater IL-6 (tight junction regulator) and IL-8 (neutrophil chemokine) productions from premature gut epithelium.

Methods: Fecal microbiome and sterile fecal filtrates were derived from two stool samples, collected before 2 weeks and at 4 weeks, from each of 6 preterm infants. Stool samples were homogenized in water then filtered out large particles and bacteria. Human fetal intestinal epithelium (FHs 74int) cell cultures were exposed to fecal filtrates in duplicates and the culture media were collected at 4, 24, and 48 hours for IL-6 and IL-8 measurements. Total epithelial cell mRNA collected at 48 hours was sequenced and analyzed.

Results: Stool samples were collected at median postnatal ages of 7 (IQR = 6-8) and 28 (IQR = 26-31) days. Later stool samples had higher Proteobacteria to Firmicutes ratio and induced higher cytokine productions. Cytokine levels correlated positively with fecal Proteobacteria to Firmicutes ratio at all time points (Figure 1). The analysis of total mRNA sequences showed increased expressions in cell inflammation and adhesion with greater neutrophil chemotaxis induced by the later stool samples (Figure 2).

Conclusions: Sterile fecal filtrates induced higher inflammation and adhesion activities that correlated with Proteobacteria to Firmicutes ratio in fetal epithelial cells. This is a promising model to test microbiome and cell interactions.
Figure 1: The correlation between IL-6 production and ratio of Proteobacteria to Firmicutes

Figure 2: Up-regulated gene ontology in fetal intestinal epithelial cells exposed to late vs. early stool samples
Placental microbiome: myth or reality?
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²Institut Pasteur, Department of Computational Biology USR 3756 CNRS, Paris, France

The discovery of bacteria in the placenta questioned the in utero environment sterility dogma, and a specific but poor placental microbiome was characterized. Except similarities found with the oral microbiome, its origin is still unknown. Moreover, its physiological role is not understood but pregnancy outcome seems to be associated with specific bacterial community patterns. However, recent publications failed to prove the existence of such microbiome, describing bacterial communities undistinguishable from negative controls. In front of these conflicting data, new studies are necessary to assess the existence of such placental microbiome. Our project aimed at studying bacterial community presence in human placenta (n=34) using several complementary methods: bacterial culture and molecular biology (qPCR targeting the 16S rRNA encoding gene and shotgun metagenomics) with appropriate controls. Bacterial colonization was analyzed in several in utero areas: chorionic villi, umbilical cord and fetal membrane. Several sampling methods were used to assess the impact of delivery mode on bacterial composition identified. Bacterial colonization has been identified only in external areas of the placenta and predominantly after vaginal delivery. Our data suggest that bacteria found inside the in utero environment result mainly from contamination during delivery. The inner parts of the placenta remained sterile or scarcely colonized which make it difficult to distinguish these bacteria with potential contaminants. Therefore, bacterial community that could be present in the placenta should not be called a microbiome.
THE ROLE OF LACTATE ACIDIFICATION ON INFLAMMATORY PATHWAY ACTIVATION IN VAGINAL EPITHELIAL CELLS

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\textbf{Introduction:} \textit{Lactobacillus} species dominance of the vaginal microbiome is often associated with optimal health. This is partly attributable to D- and L-lactic acid (LA) production which exhibits antimicrobial and immune-modulatory properties via a poorly defined mechanism. Here we test the hypothesis that LA inhibition of microbial-induced inflammation in vaginal epithelial cells (VECs) occurs via inhibition of specific inflammatory transcription factor (TF) activation.

\textbf{Methods:} Using a multilayer VEC culture model the effect of L-LA (30mmol/L) on inflammatory TFs, NFkB, AP-1 and IRFs, was assessed across a time course (30 min, 1h and 2h; n=6) by examining p65 (NFkB), c-Jun (AP-1) and IRF3 (IRFs) phosphorylation following FSL-1 (TLR2/6) and poly I:C (TLR3) treatment. Upstream kinase (p38, ERK, JNK and IKK) activation and mRNA levels of IL-8, IL-6, IL-1Ra, IL-1β, TNFα, RANTES, MIP3α and IRF3 were also investigated.

\textbf{Results:} Specific agonism of TLR2/6 activates NFkB and AP1 transcription activity which is robustly inhibited by LA (p<0.05). TLR3 agonism drives IRF3 activity, which is suppressed by LA (p<0.01). LA inhibits basal activation status of upstream kinases JNK, p38 and IK, but strongly activates ERK (p<0.05).

\textbf{Conclusion:} Bacterial and viral agonists differentially stimulate TF activation in VECs. Early inflammatory response to TLR3 activation in VECs occurs independently of NFkB and AP-1 and is likely modulated through IRF3. These findings provide mechanistic insight into the anti-inflammatory action of LA on VECs and highlight a hitherto unknown role for LA-dependent activation of ERK.
IS AUTISM THE RESULT OF THE DISRUPTION OF THE GUT MICROBIOTA FROM PREGNANCY TO CHILDHOOD?
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³Metagenom Laboratory, Department of Biology, Malaga, Spain
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Background: Balance of the gut microbiota is necessary for good health. The first encounter of the microbiota occurs in utero and at birth when in contact with the mother's bacteria, then it evolves and adapts during growth and is contacted with diverse environmental factors. Healthy cognitive function depends on this balance. Autism spectrum disorder (ASD) is a complex developmental disability. Composition of gut microbiota is one of the factors that is related to the origin of this condition. We study the gut microbiota of a group of ASD children.

Method: we performed stool cultures for aerobic and anaerobic microbiota (comprehensive stool analysis) in 33 children with ASD.

Results: Of a total of 33 children 28 (84.85%) male and 5 (15.15%) female, within the beneficial microbiota, we found that patients with ASD have a lower proportion of Lactobacillus spp. if compared to Bifidobacteria spp., Enterococcus spp., Escherichia coli and Clostridium spp. Klebsiella pneumoniae ssp. pneumoniae is the bacteria that most often causes dysbiosis. 69.70% of these patients presented positive fungal culture, the most frequent being Geothricum spp. and Candida parapsilosis.

Discussion: We can appreciate a lack of balance in the microbiota of the children with ASD, with the prevalence of dysbiosis, which makes it a challenge in the management of these disorders.

Conclusion: Currently 1 in 50 children have ASD. Intervention at all stages of child development could stop a rapid increase in the prevalence of ASD. The study of microbiota is vital for the prevention and treatment of this condition.
The Initial Gut Microbiota Composition is Individually Heterogenous and not Explained by Perinatal Exposures or Gestational Age in Preterm Infants

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\textsuperscript{2}Turku University Hospital, Department of Pediatrics and Adolescent Medicine, Turku, Finland
\textsuperscript{3}Spanish National Research Council IATA-CSIC, Department of Biotechnology, Valencia, Spain
\textsuperscript{4}University of Turku, Functional Foods Forum, Turku, Finland

Background: Preterm infants exhibit aberrant gut colonization patterns from the first days of life, which may be linked to detrimental health outcomes.

Objective: To investigate whether the initial gut microbiota composition of the preterm infant is affected by perinatal or maternal exposures.

Design: A prospective study of 55 preterm neonates born before 35 gestational weeks was conducted. Initial gut microbiota composition was analyzed by 16S rRNA gene sequencing from the first passed stool. The sequencing data was analyzed using QIIME2 and Calypso. Clinical patient data were collected and linked with the microbiota data. Perinatal exposures including duration of pregnancy, delivery method (DM, vaginal or caesarean section), the reason of prematurity (ROP, spontaneous preterm labor, preterm premature rupture of membranes or iatrogenic), intrapartum antibiotic use and maternal pre-pregnancy BMI were selected for the analysis.

Results: The clinical characteristics are presented in Table 1. The initial gut microbiota composition displayed marked variation between individuals at phylum (Figure 1A) and class levels (Figure 1B). Perinatal exposures had no significant impact on the alpha diversity assessed by Faith’s Phylogenetic Diversity. No clustering in relation to perinatal exposures was detected in PCoA using the Bray Curtis test (for ROP and DM, see figure 1C and figure 1D). Moreover, PERMANOVA beta diversity testing showed no significant differences.

Conclusions: Preterm infants represent a heterogeneous population with no uniform early gut colonization pattern. The highly individual initial preterm gut microbiota composition is not directly modulated by gestational age or environmental exposures.
Table 1. Clinical characteristics of the preterm infants.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean with range or percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (boy)</td>
<td>22 (40%)</td>
</tr>
<tr>
<td>Gestational Age (weeks)</td>
<td>(32^{+2/7}) (27^{+5/7} - 34^{+5/7})</td>
</tr>
<tr>
<td>Birth weight (grams)</td>
<td>1885 (755 - 3050)</td>
</tr>
<tr>
<td>Birth weight Z-score</td>
<td>-0.16 (-3.5 - 3.5)</td>
</tr>
<tr>
<td>Delivery Method (vaginal)</td>
<td>34 (62%)</td>
</tr>
<tr>
<td>Enteral milk intake from the first day of life (n=54)</td>
<td>54 (100 %)</td>
</tr>
<tr>
<td>Antibiotic use within the first 48 hours of life</td>
<td>52 (96%)</td>
</tr>
<tr>
<td>Maternal intrapartum antibiotics (n=54)</td>
<td>32 (59%)</td>
</tr>
<tr>
<td>Mother’s pre-pregnancy BMI (n=49)</td>
<td>25.2 (17.1 - 40.0)</td>
</tr>
<tr>
<td>Chorioamnionitis (n=49)</td>
<td>2 (4%)</td>
</tr>
</tbody>
</table>
Figure 1. Meconium microbiota analyses. A Taxa plots sorted by gestational age, phylum level B Taxa plots sorted by gestational age, class level. C Beta-diversity PCoA assessed by Bray-Curtis test on reason of prematurity. PERMANOVA testing showed no statistically significant differences ($p=0.057$). D Beta-diversity PCoA assessed by Bray-Curtis test on delivery method. PERMANOVA testing showed no statistically significant differences ($p=0.989$).
How Group B Streptococci Colonization in Pregnant Women Alter Vaginal Microbiome?

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²National Taiwan University Hospital, Department of Internal Medicine, Taipei, Taiwan R.O.C.

Background: Group B Streptococcus (GBS) is an encapsulated gram-positive coccus that colonizes the gastrointestinal and genital tracts of 15 to 40 percent of pregnant women. Although GBS colonization is asymptomatic in these women, maternal colonization is the critical determinant of infection in neonates and young infants. However, the microbiome is an important determinant of vaginal pathogen colonization. We evaluated the potential relationship of vaginal microbiome composition of GBS colonization.

Methods: We analyzed vaginal swabs collected at 35-37 weeks gestation from 22 women participating in a study of breast milk probiotic supplementation. Vaginal swab DNA was extracted using PowerFecal® DNA Isolation Kit. Vaginal microbiome were profiled by 16S ribosomal ribonucleic acid sequencing.

Results: Of 22 pregnant women, five were GBS colonized. There is no difference of the maternal parity, gestational weeks of delivery, neonatal birth body weight, Apgar score at one minute and five minutes between GBS colonization group and GBS non-colonization group. However, neonatal observation admission rate was higher in GBS colonization group. There was no difference in α-diversity and principal coordinates analysis based on GBS status. After excluding S. agalactiae, there is no significant difference in the amount of other Streptococcus species between GBS(+) and GBS(-) group. However, there is still a trend that other Streptococcus species was less likely to be detected in GBS(+) group. We assume that S. agalactiae suppresses other Streptococcus species.

Conclusion: We assume that S. agalactiae suppresses other Streptococcus species. However, further studies should use these data to investigate the relationship between GBS and other streptococcus species.
The HELMi (Health and Early Life Microbiota) cohort is a longitudinal, prospective birth cohort designed to determine environmental, lifestyle and generic factors affecting the intestinal microbiota development in the first two years of life, and further the effects on health and well-being of the child. The cohort consist of 1055 families with healthy term infants born in 2016-2018 mainly from the capital region of Finland. The infant gut microbiota development is monitored with 9 fecal samples from the first two years of life, focusing on the first year. Extensive data on child exposures, development and health are collected with >50 online questionnaires that the parents fill at weekly to monthly intervals.

In addition, fecal samples from both parents, breast milk sample and DNA sample from the infant were collected. Child psychological and cognitive development was evaluated with online questionnaires and assessed by psychologist at 18 months for a subgroup.

The data collection and analysis are ongoing. The poster will present the basic facts about the cohort as well as our first 16S rRNA gene amplicon sequencing results from ca. 4000 infant samples that are used to identify the differences in the developmental microbiota trajectory between healthy and allergic as well as infection-prone infants, carefully controlling the analyses for birth mode, breastfeeding, antibiotics, probiotics and other factors known to affect the early microbiota development.
MATERNAL NUTRITION AND INFANT FEEDING CHARACTERISTICS ALTER THE HUMAN MILK MICROBIOTA AT 3-MONTHS POST-PARTUM


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⁷University of Toronto, Centre for the Analysis of Genome Evolution and Function, Toronto, Canada
⁸Mount Sinai Hospital, Lunenfeld-Tanenbaum Research Institute, Toronto, Canada

Background: The role of maternal nutrition and infant characteristics on the milk microbiota remains poorly understood. We sought to explore the associations between maternal diet from delivery to 3-months postpartum, infant feeding characteristics and the microbial composition of human milk.

Methods: This prospective cohort study (NCT01405547) collected milk samples, maternal diet (food frequency questionnaire) and infant feeding information from mothers at 3-months postpartum. Metagenomic DNA extraction and 16S ribosomal RNA gene sequencing of the V4 hypervariable region (Illumina MiSeq) was carried out on 113 milk samples.

Results: Human milk microbiota clustering was associated with mother’s milk exclusivity (weighted UniFrac $R^2=0.034$, $p=0.021$; Bray-Curtis $R^2=0.042$, $p=0.007$), times fed at the breast (Bray-Curtis $R^2=0.056$, $p=0.038$) and fibre from grains (Bray-Curtis $R^2=0.056$, $p=0.049$). Total fibre was associated with a reduced incidence of Streptococcus (incidence rate ratio 0.96 [95% CI 0.92-0.99] and an increased incidence of Corynebacterium (1.04 [1.02-1.07]). Every one-gram increase in trans-fat was associated with an increased incidence of Gemella (1.67 [1.43-4.99]). Mothers who fed their infants exclusively mother’s milk had an increased incidence of Acinetobacter (2.44 [1.40-4.24]) and every additional time an infant was fed at the breast resulted in a reduced incidence of Corynebacterium (0.81 [0.71-0.92]).

Conclusions: Maternal nutrition and infant feeding characteristics appear to be determinants of the composition and diversity of the human milk microbiota. Further research is warranted to determine how these predictors modulate the bacteria present and the potential implications this may have on infant health.

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Identification of Microbiome and Clinical Influences Related to Very Early Childhood Obesity

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Background: Childhood obesity is a threat to public health worldwide. Changes in the intestinal microbiome have been associated with obesity in adults, yet there is a paucity of data regarding microbiome changes in early childhood obesity, during the critical development of the intestinal microbiome.

Aim: To identify intestinal microbiome and clinical factors associated with early childhood obesity.

Methods: Children (N=309) enrolled in a longitudinal study provided a stool specimen with anthropometric data to classify obese children (weight for length ≥95th% <24 months, BMI ≥95th% ≥24months). DNA was extracted and 16S (r) RNA gene sequencing performed on the Illumina Miseq platform. Downstream analyses were generated using R package phyloseq.

Results: No differences in alpha or beta diversity were found in age or weight cohorts. Previous associations from adult studies of relative abundance with obesity were not found. Increased levels of Proteobacteria trended towards association with obesity in children ≥24 months (p=0.09). In children ≥24 months without antibiotic use in the last 3 months (N=109) the following were associated with obesity: increased genus Klebsiella, p=0.022, from family Enterobacteriaceae p=0.048, order Enterobacteriales, p=0.026, phylum Proteobacteria, p=0.046. Clinically significant factors were birth weight, breast milk consumption and birth mode.

Conclusions: Clinical factors associated with early childhood obesity, such as a cesarean delivery, antibiotics or breast feeding, are associated with microbiome changes. However, in this analysis, significant differences in relative abundance were revealed only in those ≥24 months. This warrants further exploration with longitudinal specimen collection to examine when microbiome changes associated with obesity occur.
The Novel Identification of the Pediatric Urinary Microbiome

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³Johns Hopkins University, Department of Surgery, Baltimore, USA

Background: The urinary tract was once thought to be sterile and is starting to be explored in adult populations; however, the urinary microbiome in pediatric populations is largely unknown.

Aims: To examine the urinary microbiome of children ≤48 months.

Methods: Children ≤48 months undergoing a clinical urinary catheterization in the Pediatric Emergency Room were recruited (N=85) and urine samples along with demographic and clinical information were collected. DNA extraction and 16S ribosomal RNA gene sequencing on the Illumina MiSeq platform occurred; standard urinalysis and urine culture were performed. Alpha diversity, beta diversity and the relative abundance were compared across demographic and clinical factors.

Results: A urinary microbiome was identified in every child. Those with Escherichia coli urinary tract infections (UTIs) identified (N=9) had significantly decreased alpha diversity (t-test, p<0.001), the composition of the microbiome clustered separately, and there was a large increase in the relative abundance of the genus Escherichia. Antibiotic use within 2 weeks of urine collection resulted in decreased alpha diversity (t-test, p=0.017); no differences were detected if antibiotics were taken more than 2 weeks prior to sample collection. Some differences in microbiome metrics were seen between genders, but not between delivery mode, maternal ethnicity or probiotic use.

Conclusions: A urinary microbiome was observed in all children, including neonates. Changes in microbiome diversity and composition were observed in patients with a positive UTI. The urinary microbiome has just begun to be explored, especially in pediatric populations, and the implications on long term disease processes warrant further investigation.
The impact of gastric acid suppression on the developing intestinal microbiome of a child
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**Background:** Medications for gastric acid suppression are often used in children despite increased risk of Clostridium difficile infection. The underlying mechanism is unknown, but it is theorized that increased pH alters the gut microbiome, allowing C. difficile to proliferate.

**Aim:** To identify pediatric gut microbiome changes from gastric acid suppression.

**Methods:** Children under age 3 (N=5) gave stool samples before medication initiation and 2 months after. DNA was extracted and sequenced using a modified Illumina 16S Metagenomics Sequencing Library Preparation protocol. QIIME 1.9 software and Greengenes 16S rRNA database (version gg 13) were used for data preparation. Downstream analyses were generated using the R package phyloseq. Quantitative-PCR was used to detect C. difficile toxin A (TcdA) and B (TcdB) genes.

**Results:** 3 children took histamine receptor 2 antagonists (“H2RAs”), and 2 took proton pump inhibitors (“PPIs”). All exhibited an increase in Clostridiales (p=0.04). 4/5 children exhibited a decrease in Enterobacteriales approaching zero, with males starting at higher abundance and decreasing more. There were no significant changes in alpha-diversity before and after treatment. Greatest variation in beta-diversity was clustered by gender. Samples from the same individual before and after treatment clustered together. Nontoxicogenic C. difficile was present in only one patient, before and after PPI treatment.

**Conclusions:** This pilot study reveals that changes in relative abundance of taxa occur over time with H2RA and PPI use. While an increase in C. difficile carriage was not seen, additional exploration is needed to study the effects of these changes over a longer period.
LARGE ANIMALS - OPTIMIZED SAMPLING: 16S rRNA AND CULTURE BASED ANALYSIS OF MICROBIOTA IN AMNIOTIC FLUID AND MECONIUM OF CALVES DELIVERED BY ELECTIVE CAESA REAN SECTION

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¹Ghent University, Faculty of Veterinary Medicine, Merelbeke, Belgium
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Sterility of the fetal environment is still debated, due to technical challenges in reliable analysis of low-abundance microbiotas. Several studies have reported the existence of microbial DNA or even culturable bacteria in the mammalian fetus, while others interpret such observations as contamination. In this cross-sectional study, we have analysed the bovine fetal microbiota from amnion and meconium samples. Sampling methods, laboratory analyses and data processing were all designed to maximize the reliability of the study, and negative controls were included for all sample types. We collected samples of amniotic fluid and meconium from Belgian Blue beef cows and their calves during elective caesarean sections. The cows were at term with no rupture of the fetal membranes prior to surgery and no evidence of intra-uterine infections. After making an abdominal incision and exteriorizing the uterus, an incision through the uterine wall and the allantoic membrane was made to expose the intact amniotic membrane. Amniotic fluid was aspirated with a sterile syringe by passing the sterile needle through the amniotic membrane. Meconium samples were acquired from the calves immediately after birth, directly from within the rectum using double-guarded swabs. All samples were taken in duplicate, one stored in 30% glycerol for culturing, the other unprocessed for DNA extraction. We are currently performing the bacterial cultures and 16S rRNA gene qPCR and amplicon sequencing. We will present the results from our study at the conference, showing how large mammalian species can be used as models, considering the sterile womb dogma.
THE ROLE OF THE GUT MICROBIOME IN THE ADAPTATIONS OF THE MATERNAL IMMUNE RESPONSE DURING PREGNANCY IN MICE

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¹University of Groningen and University Medical Center Groningen, Pathology and Medical Biology, Groningen, The Netherlands

Pregnancy is associated with adaptations of the maternal immune response to tolerate the developing fetus. The mechanisms inducing these changes are not clear yet and we hypothesize that the maternal gut microbiome, which also changes during pregnancy, may be involved.

C57BL/6J OlaHsd conventional (n=24) and germfree (n=18) pregnant (day 18) and non-pregnant (dioestrus) mice were sacrificed and blood, spleen and Peyer’s patches (PP) were collected. Numbers of subpopulations of T cells and monocytes were measured by flow cytometry. The data were analyzed with ANOVA followed by Sidak’s multiple comparisons test and expressed as mean±SEM%.

In conventional mice, in the CD4+ cells, pregnancy decreased Th1 cells in the spleen (3.0±0.2% versus 4.8±0.3%, p<0.05), increased Th2 cells in the spleen (1.3±0.1% versus 1.0±0.1%, p<0.05) and in PP (2.9±0.2% versus 2.5±0.2%, p<0.05) and increased Treg cells in the spleen (27.9±2.0% versus 21.8±1.3%, p<0.05). In germfree mice, pregnancy decreased Th1 cells (3.3±0.3% versus 4.6±1.6%, p<0.05) in the spleen. In blood, pregnancy increased classical monocytes and decreased non-classical monocytes in both conventional and germfree mice. In conventional mice, not in germfree mice, pregnancy decreased MHCII positive classical (7.4±0.6% versus 15.5±2.0%, p<0.05) and intermediate (17.8±0.7% versus 26.3±2.3%, p<0.05) monocytes.

This study shows different immunological adaptations in pregnant germfree mice vs pregnant conventional mice. This may suggest a role for the gut microbiome in the adaptations in pregnant conventional mice, which is analyzed in more detail by applying 16S rRNA sequencing.
Recent evidence seems to indicate that there is a relationship between the vaginal microbiome and fertility, however, it is unknown whether this effect occurs when couples undergo ART. The aim of this study is to investigate if vaginal microbiome of the day of the embryo transfer could affect the pregnancy rate. A prospective study was performed. Thirty-one patients attended to our clinic for PGT-A with single frozen embryo transfer were recruited from May 2017 to April 2018. Vaginal samples were collected at the moment of the transfer from the posterior sac of the vagina (patients with positive pregnancy test n=17, patients not pregnant n=14). DNA was extracted using the PureLink-Microbiome-DNA-Purification-kit. Sequencing the rRNA16S V3V4 region were performed according to Illumina-Metagenomics-protocol. The bioinformatic tools QIIME2 and MicrobiomeAnalyst have been used. A total of 7,089,699 sequences were analyzed and 116 OTUS were identified. Regarding diversity analysis, alpha diversity is higher in patients who did not achieve pregnancy (p<0.05), just as for, the beta index, although without reaching statistical significance (p=0.08). Moreover, we showed a dominance of *Lactobacillus* with predominance of *L. crispatus* (47.05%), *L. helveticus* (22.85%), *L. iners* (21.95%) and *L. jenseii* (3.97%). There is a correlation between the vaginal microbiomes dominated by *Lactobacillus* and greater reproductive success against another profile not dominated by *Lactobacillus* and with the presence of *Gadnerella*. As a conclusion, these results suggest that the presence of a low diverse vaginal microbiome predisposes to the pregnancy. Also, *Lactobacillus* in the vaginal microbiome seems to be key to embryo implantation.
Unlocking the Human Microbiome with Strain-Level Metagenomics

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Next-Generation Sequencing has revolutionized microbiological sciences by revealing that virtually all environments, including the human body, are teeming with diverse microbial communities. Due to inefficiencies in common NGS workflows as well as limitations in open-source tools, generating accurate strain-level data remains one of the biggest challenges in the field today. We present an overview of the CosmosID-optimized metagenomics workflow, designed to deliver high-quality sequencing data and easy-to-access strain-level bioinformatics. During early-stage R&D, statistical power considerations, as well as controlled and standardized workflows, are key components in determining statistically significant observations in the microbiome between different cohorts. In order to establish robust correlative data and move towards causation studies, high-quality taxonomic and functional microbiome profiling using shotgun metagenomics, with strain-level resolution, should be generated to enable effect size prediction of differentially abundant taxa or features. This poster introduces common challenges specific to microbiome studies and surveys solutions across the microbiome R&D workflow using real-world examples.
The Relationship Between Preterm Microbiome Diversity and Linear Catch-Up Growth at 2 and 4 Years of Age Among Very Low Birth Weight Infants

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Preterm infants experience severe growth delays in neonatal intensive care units (NICUs), and recent findings have implicated the Proteobacteria-dominated NICU microbiome in growth. This study goal was to assess the relationship between NICU microbiomes and growth at 2 and 4 years of age. We followed 78 very low birth weight (VLBW) infants (<1500 g) through the first 6 weeks of their NICU stay and then followed up at 2 and 4 years of age (n = 20). Stool samples were sequenced via the V4 region of the 16S rRNA gene, exact sequence variants (ESVs) calculated using DADA2 pipeline, and subsequent OTUs used to calculate diversity indices. Height-for-age z-scores (HAZ) at 2 and 4 years were determined using the WHO Child Growth Standards and used to calculate catch-up HAZ from discharge to 2 and 4 years. Spearman’s rank correlation coefficient was calculated between diversity indices (Shannon, Simpson) and HAZ and catch-up HAZ, with partial correlation for gestational age. Shannon’s index was significantly associated with HAZ (rho = -0.57, p = 0.04) and catch-up HAZ (rho = -0.56, p = 0.04) at 2 years of age. There were no significant results at 4 years, but the nature of the associations changed (rho = -0.12, p= 0.63 and rho = 0.41, p = 0.11, respectively). Simpson’s index was not significant with any measure, indicating that species richness is more essential than evenness for growth outcomes. Future work will use more complex modeling to detail the longitudinal relationship between microbiome composition and growth.
Asymptomatic Vaginal Colonization and Adverse Pregnancy Outcomes Including Preterm Birth: a Systematic Review and Meta-analysis.

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Background: Genital tract infection is an important risk factor for adverse pregnancy outcomes, including preterm birth (PTB). One common vaginal infection during pregnancy is candidiasis. Although some studies suggest an association between asymptomatic vaginal Candida colonization and adverse pregnancy outcomes, the evidence is inconsistent.

Objectives: To systematically review the association between asymptomatic vaginal Candida colonization and adverse pregnancy outcomes, including PTB.

Search strategy: We searched OVID Medline, OVID EMBASE and the Cochrane Controlled Register of Trials from inception to April 7th, 2019 for published studies on vaginal Candida/yeast and pregnancy outcomes.

Selection criteria: Studies that included pregnant women who were tested for asymptomatic vaginal Candida colonization and reported on adverse pregnancy outcomes were eligible.

Data collection and analysis: Two independent reviewers selected and appraised the data.

Main results: We found no difference in PTB rate between Candida-positive and -negative women (OR 1.09 [95%CI 0.98-1.22] in 15 studies among 33089 women), neither in treated (OR 1.09 [95%CI 0.80-1.49]) or in untreated (OR 1.11 [95%CI 0.86-1.43]) Candida-positive women. Asymptomatic vaginal Candida colonization was not associated with small for gestational age, perinatal mortality or any other adverse pregnancy outcome.

Conclusion: Asymptomatic vaginal Candida colonization is not associated with adverse pregnancy outcomes, including PTB. Previous studies reported that treatment of asymptomatic vaginal Candida colonization reduces PTB rate. Our results suggest that this effect is unlikely to rely on treatment of asymptomatic vaginal Candida colonization, but might be caused by antibacterial, antiprotozoal or anti-inflammatory properties of clotrimazole.
Evaluating Different Bacterial DNA Isolation Methods For Breast Milk Shotgun Metagenomics

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Microbes in breast milk are important for the development of the infant gut microbiome and maturation of the immune system. However, metagenomic sequencing of breast milk samples is challenging due to the presence of large numbers of host cells and small numbers of microbial cells. In this study we tested five different DNA isolation strategies for their suitability for shotgun metagenomics sequencing of five human breast milk samples. We further used conventional cultivation to validate the results in two of the five samples.

Shotgun sequencing revealed a large variation in bacterial composition between samples and low bacterial diversity. Detected bacteria corresponded with those previously found in human breast milk samples using 16S rRNA sequencing. No significant differences in species richness and Shannon diversity indices were observed between the different DNA isolation methods. In a principal component analysis, samples clustered by donor and not by isolation method, indicating that the results are independent of the DNA extraction method. Although one DNA isolation method had a significantly higher percentage of microbial reads compared to the other methods, only 4–8% of all high-quality reads were of microbial origin. The concordance of results from shotgun sequencing and from cultures was low and did not differ between DNA extraction strategies.

In line with literature, our study finds high inter-individual variability and low microbial content in breast milk. One DNA isolation method performed slightly better than the other tested methods. Nevertheless, further endeavors are needed to improve the quality of sequencing results from milk samples.
**MUMS: Microbiome Understanding in Maternity Study - The study protocol**

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**Introduction:** Alterations in the maternal microbiome may facilitate the normal adaptive changes throughout pregnancy for biological advantage. However, increasing rates of pre-pregnancy obesity, metabolic abnormalities, reduced physical activity and the proinflammatory environment related to the modern-day lifestyles may be leading to preconception dysbiosis and maladaptation which may lead to pathological pregnancy outcomes.

**Objective/hypothesis:** This mother-infant cohort is aimed to characterise maternal microbial signatures and their associations with pathological pregnancy phenotypes ie; Gestational Diabetes Mellitus, gestational hypertension and excessive gestational weight gain. The MUMS aim is to explore longitudinal differences in the faecal, oral and vaginal microbiome over the course of a pregnancy and one-year post-partum in women who have complicated and uncomplicated pregnancies.

**Methods:** MUMS is an Australian prospective longitudinal cohort of 100 mother-infant pairs. Recruitment will occur in the first trimester. Visits will occur in trimester one, two and three, birth then six weeks, six months and 12 months post-partum. Maternal and infant biological samples will be collected at seven timepoints. Simultaneous clinical, medication, anthropometric, body impedance analysis, dietary, physical activity and psychological data will also be collected. Faecal shotgun metagenomic analysis will be conducted and 16S rRNA gene sequencing will be conducted on the oral and vaginal specimens.

**Results:** In-depth microbiome analysis will occur for all samples with clinical correlation controlling for con-founders.

**Discussion:** Pregnancy and postpartum microbiome analysis may provide additional insight into role of the microbiome during pregnancy and the implications of pathological pregnancy phenotypes on women and their offspring.

**Key words:** microbiome, pregnancy, preeclampsia, hypertension, gestational diabetes.
Objective. The aim of this study was to understand how the maternal microbiota contributes to the initial neonatal oral microbiota.

Materials and methods. The microbiota analysis was performed in 12 mother-neonate pairs from the Finnish Family HPV Study cohort. Samples from the maternal oral mucosa, uterine cervix, placenta and the neonate’s oral cavity immediately after birth were used. Six neonates were delivered vaginally and six by caesarean section. The gender distribution was equal.

The microbiota composition and diversity were characterized by 16S rRNA gene sequencing (V3-V4 region). The microbiota analyses and comparisons were carried out with Calypso software version 8.1 and with SourceTracker 1.0.1.

Results. The most abundant phyla in all of the neonatal oral cavity samples were *Firmicutes* (42.2 %), followed by *Proteobacteria* (20.5 %), *Actinobacteria* (18.0 %) and *Bacteroidetes* (14.6 %). More variation occurred on the family level, but overall *Streptococcaceae* (9.3 %), *Lactobacillaceae* (8.0 %) and *Propionibacteriaceae* (6.5 %) were the predominant families.

Samples from the neonatal oral cavity showed moderately high bacterial diversity and low richness. The neonatal oral cavity microbiota seems to share features mainly with the placenta microbiota, followed by the cervical microbiota and the maternal oral microbiota.

No statistically significant differences in diversity (Shannon index, p=0.14), richness (Chao1, p=0.53) or in microbial composition were observed according to delivery mode.

Conclusions. The neonatal oral cavity microbiota is not significantly modulated by the birth canal or maternal oral microbiota but displays clear associations with placental microbiota. These results suggest that the neonatal oral microbiota may have a prenatal origin.
Prevalence of lactobacilli in vaginal microbiota is considered a positive factor for normal course of pregnancy.

In order to evaluate vaginal microbiota at the first trimester of pregnancy, real-time PCR (RT-PCR) test was performed with “Femoflor” kit (DNA-Technology, Russia) for 238 pregnant women (aged 20-43, 5-12 weeks of gestation). The kit allows detecting the quantity (expressed in genome equivalents per 1 ml (GE/ml)) of lactobacilli and 15 groups of opportunistic microorganisms (OM). The special software was used to automatically calculate the total bacterial load (TBL) and the proportion of OM and lactobacilli in relation to the TBL.

Depending on the proportion of lactobacilli and OM in the TBL, three types of vaginal microbiota were identified: normocenosis — the proportion of lactobacilli > 80% of the TBL; apparent dysbiosis (AD) — the proportion of lactobacilli < 20% and OM > 80% of the TBL; moderate dysbiosis — the proportion of lactobacilli and OM 20% < 80% of the TBL. Depending on the quantity of the associated bacteria (Mycoplasma hominis, Ureaplasma spp.) and yeast-like fungi (Candida spp.), «normocenosis» is divided into two groups: absolute normocenosis (AN) — when their quantity is < 10^4 GE/ml, and conditional normocenosis (CN) when it is >10^4 GE/ml. Depending on the prevalence of obligate or facultative anaerobes, three variants of AD or MD can be identified: aerobic, anaerobic or mixed.

Vaginal microbiota of pregnant women met the criteria of normocenosis: AN was detected in 112 cases (47.5%), CN — in 82 (34.45%); AD in 24 (10.1%) and MD in 20 (8.4%) cases.
ASSESSMENT OF VAGINAL MICROBIOTA DYNAMICS THROUGHOUT THE PREGNANCY VIA REAL-TIME PCR
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In order to assess the changes in vaginal microbiota during pregnancy, real-time PCR (RT-PCR) test was performed with “Femoflor” kit (DNA-Technology, Russia) for 112 pregnant women (aged 20-43) in the first, second, and third trimesters. The kit allows detecting the quantity (expressed in genome equivalents per 1 ml (GE/ml)) of lactobacilli and 15 groups of opportunistic microorganisms (OM). The special software (DNA-Technology, Russia) was used to automatically calculate the total bacterial load (TBL) and the proportion of OM and lactobacilli in relation to the TBL.

In the first trimester vaginal microbiota of all pregnant women met the criteria of absolute normocenosis (AN): the proportion of lactobacilli > 80 % of the TBL, the quantity of the associated bacteria (Mycoplasma hominis, Ureaplasma spp.) and yeast-like fungi (Candida spp.) < 10\(^4\) GE/ml.

In the second trimester AN was detected in 98 (88.5%) of 112 cases. In 14 (12.5%) cases vaginal microbiota met the criteria of conditional normocenosis (CN): the proportion of lactobacilli > 80 % of the TBL, the quantity of the associated microorganisms > 10\(^4\) GE/ml.

In the third trimester AN was detected in 96 (85.7%) of 112 women. In 14 (12.5%) cases CN remained. In 2 (1.8%) cases vaginal microbiota met the criteria moderate dysbiosis (MD) — the proportion of lactobacilli and OM 20% < 80%of the TBL.

So, women with AN of vaginal microbiota in the first trimester have a tendency to retain it throughout the pregnancy. Changes in vaginal microbiota were detected only in 16 patients.