Jahresversammlung der niedersächsischen Perinatalerhebung 2017

# Das Mikrobiom, die menschliche Entwicklung und Reproduktion – überraschende Einsichten


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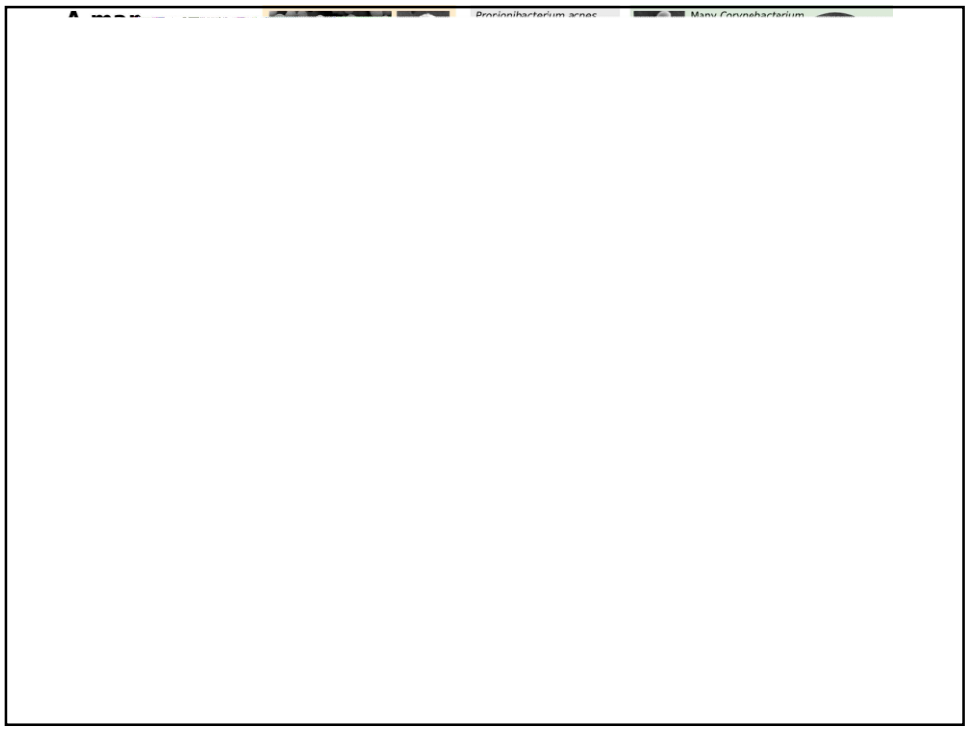
**and Developmental Biology**


# Inhalt

- **Klinisches Szenario**
- **Darm-Gehirn Entwicklung**
- **Implantation**
- **Klinische Perspektive**

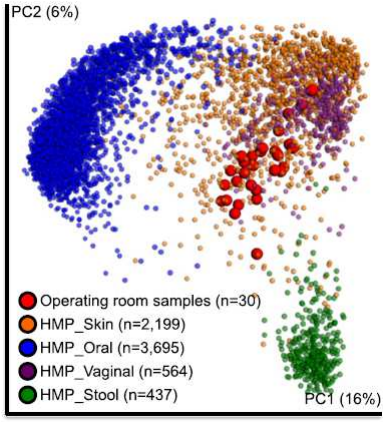


# Mikrobiom des Darms

- Risikofaktor für Adipositas
- Abhängig von Geburtsmodus
- Abhängig von Ernährung – Muttermilch
- Kann durch Stuhltransplantation bei Fehlbesiedlung verändert werden

Hill *et al. Microbiome* (2017) 5:4

# The first microbial environment of infants born by C-section: the operating room microbes




Shin *et al. Microbiome* (2015) 3:59

## Partial restoration of the microbiota of cesarean-born infants via vaginal microbial transfer

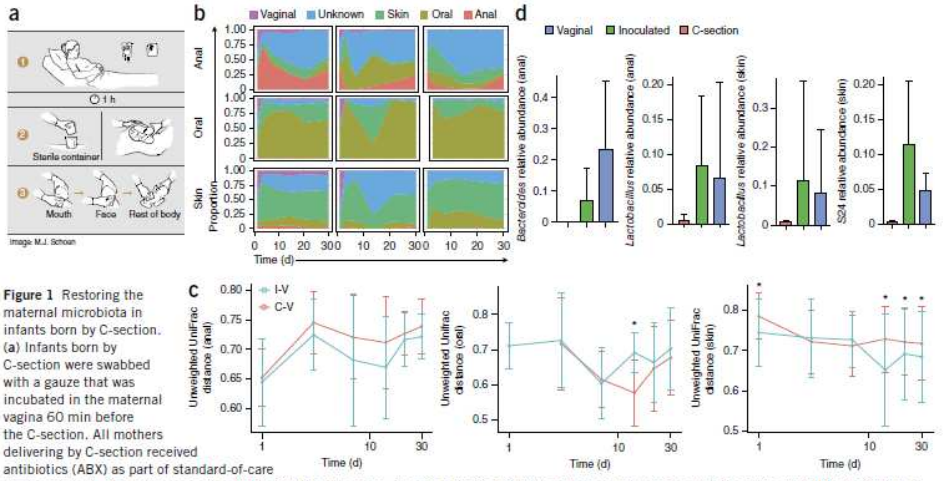



**Figure 1** Restoring the maternal microbiota in infants born by C-section. (**a**) Infants born by C-section were swabbed with a gauze that was incubated in the maternal vagina 60 min before the C-section. All mothers delivering by C-section received antibiotics (ABX) as part of standard-of-care treatment (top). The gauze was extracted before the procedure, kept in a sterile (middle) container and used to swab the newborn within the first 1–3 min after birth, starting with the mouth, then the face and the rest of the body (bottom). (**b**) Proportion of microbiota from anal (top), oral (middle) and skin (bottom) samples of infants delivered either vaginally (left; $n = 7$ subjects sampled at six time points), by C-section (unexposed) (right; $n = 7$ subjects sampled at six time points) or by C-section and exposed to vaginal fluids (middle; $n = 4$ subjects sampled at six time points) that are estimated to originate from different maternal sources (colored regions), using bacterial source-tracking. (**c**) Bacterial community distances (unweighted UniFrac distances) in anal (left), oral (middle) and skin (right) samples between vaginally delivered babies and C-section–delivered babies that were either exposed (I-V) or not exposed (C-V) to the vaginal gauze during the first month of life. Because the babies were sampled more frequently during the first week of life, the *x* axis is represented as a log scale. Error bars indicate mean ± s.d. $^*P < 0.01$; analysis of variance (ANOVA) and Tukey's honest significant difference test. (**d**) Representative bacterial taxa enriched in infants with perinatal exposure to vaginal fluids during the first month of life. S24, Bacteroidales family S24-7 members. Error bars indicate mean ± s.d.

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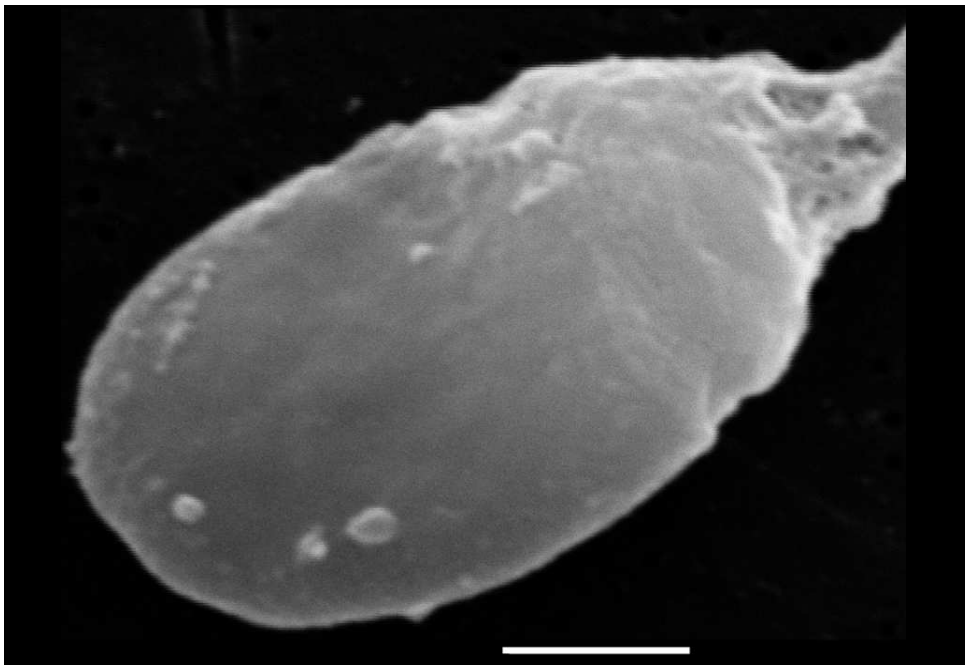
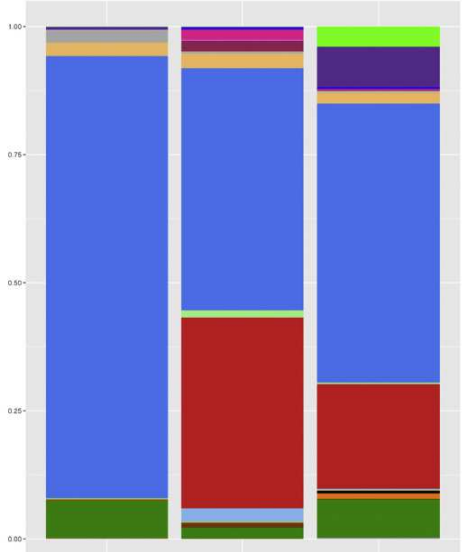


Courtesy of Dr. C. Knox, Queensland University of Technology, Australia

## Mikrobiom des Endometriums ist assoziiert mit Implantationserfolg

**FIGURE 11**
**Low abundance of endometrial Lactobacillus is associated with poor reproductive outcome**




Bar charts showing mean values of 20 most abundant operational taxonomic units (OTUs) in receptive subjects grouped by their reproductive outcomes: live births (LB) correspond to patients who became pregnant and successfully delivered; nonpregnant (NP) are patients who did not conceive; finally, miscarriage (MISC) applied for those patients who became pregnant but experienced either biochemical or clinical pregnancy. Twenty most abundant taxa are represented, while sum of remaining OTUs are included as "others" that comprise: *Cloacibacterium*, *Ethanoligenens*, *Akkermansia*, *Acidimicrobiales*, Cytophagaceae, Haliangiaceae, *Halomonas*, *Hydrogenophaga*, *Brochothrix*, *Ureaplasma*, *Bilophila*, *Actinobaculum*, *Diaphorobacter*, *Phascolarctobacterium*, SMB53, *Oligella*, WCHB1-84, Ellin6075, *Cellvibrio*, *Balneimonas*, *Ochrobactrum*, *Petrobacter*,

**Lebendgeburten Fehlgeburten Keine Schwangerschaft**

American Journal of Obstetrics & Gynecology DECEMBER 2016

## Mikrobiom des Endometriums ist assoziiert mit Implantationserfolg

- Mikrobiom des Endometirums ist zyklusunabhängig

- Non-Lactobacillus Bakterien im Endometrium sind mit niedrigen Reproduktionserfolgen assoziiert

- Risikofaktor für fehlende Implantation oder Schwangerschaftverlust

- Bakterien sind Teil der Reproduktion – und ggf. Voraussetzung

American Journal of Obstetrics & Gynecology DECEMBER 2016

# Mikrobiom der Plazenta

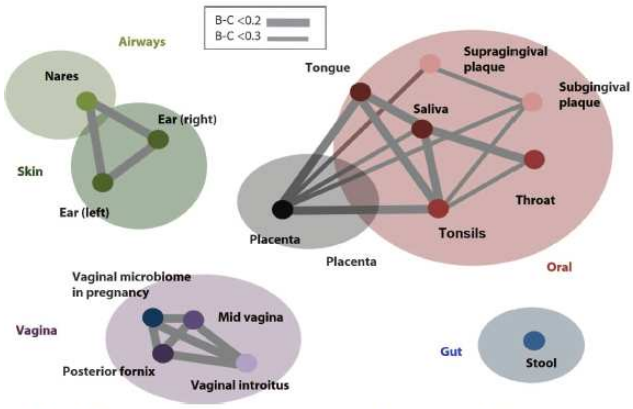



**Fig. 1. The placental microbiome has a taxonomic profile that is similar to the oral microbiome.** Bray-Curtis (B-C) dissimilarity was calculated using WGS-generated phylum-level abundance of bacteria from each body site, including placental data from this study; gut, vagina, posterior auricular skin, and nasal airways data from the HMP; and vaginal data from previously published gravidae (1–4). The thicker the connecting line, the greater the similarity of the taxonomic profile (Bray-Curtis <0.2). Strong phylum-level similarity was observed between the placenta and tongue, tonsils, saliva, and subgingival plaque taxonomic profiles. The colors of dots reflect the vicinity of the body sites.

Aagard et al., Sci.Transl.Med., 6: 237, 2014

# Mikrobiom der Plazenta

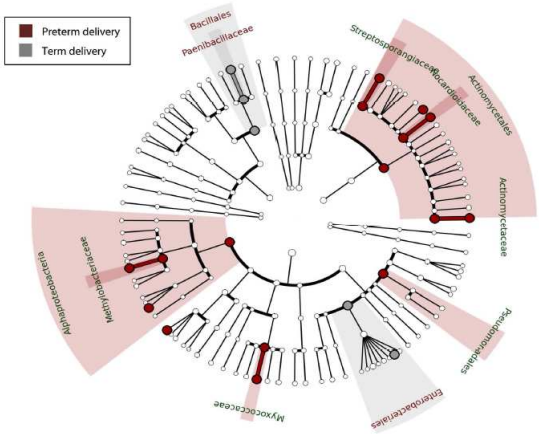



**Figure S3. LEfSe-generated cladogram indicates the enrichment of placental microbiome taxa in gravidae who experience a spontaneous preterm birth.** Red indicates enrichment in preterm delivered samples, gray indicates enrichment in term delivered samples.

## Transfer genetisch markierter Bakterien von der Mutter zum Feten in utero

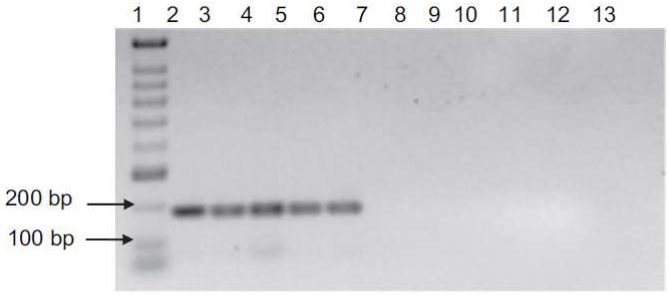



Fig. 2. PCR detection of *E. fecium* JLM3 among colonies isolated from meconium. Lane 1, 100 bp ladder (Bioline, London, UK); lane 2, PCR positive control (genomic DNA obtained from transgenic soy); lanes 3–7, colonies obtained from group A mice that grew on MRS-Cm agar plates; lanes 8–12, colonies obtained from group B mice; lane 13, PCR-negative control (*E. fecium* HA1).

*E. Jiménez et al. / Research in Microbiology 159 (2008) 187–193*

## Effect of probiotics on vaginal health in pregnancy. EFFPRO, a randomized controlled trial

**OBJECTIVE:** The purpose of this study was to evaluate whether an oral probiotic food supplement supports the maintenance or restoration of a normal vaginal microbiota during pregnancy.

**608.e1** **American Journal of Obstetrics & Gynecology** NOVEMBER 2016

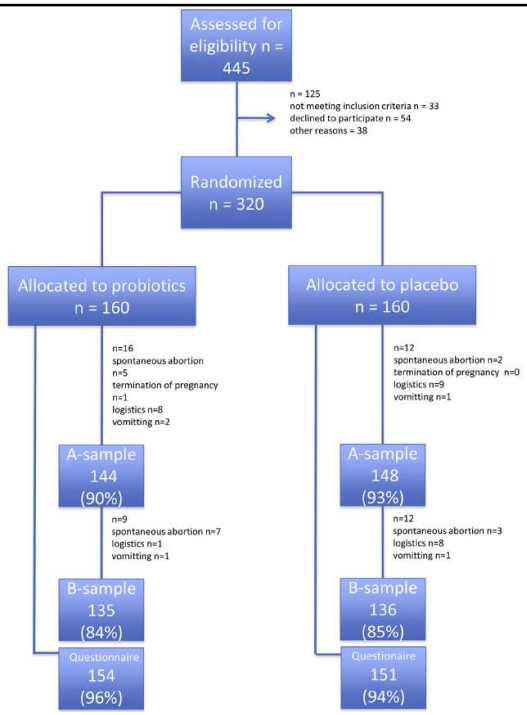


**TABLE 2**
**Nugent score**

| Nugent score | Treatment, n (%) | Placebo, n (%) | *P* value 1-Tailed | *P* value 2-Tailed |
|---|---|---|---|---|
| Normal microbiota sample | | | | |
| A | 119 (82.6) | 117 (79.1) | .265 | .460 |
| B | 105 (77.8) | 101 (74.3) | .297 | .560 |
| Bacterial vaginosis sample | | | | |
| A | 4 (2.8) | 8 (5.4) | .202 | .376 |
| B | 3 (2.2) | 2 (1.5) | .497 | 1.000 |

Frequency of normal vaginal microbiota (Nugent score, <4), and bacterial vaginosis (Nugent score, 7-10) in treatment and placebo group before (A-sample) and after (B-sample) intervention. For statistical analysis, values were compared with the use of Fisher's exact test.

*Gille et al. EFFPRO: Effect of probiotics on vaginal health. Am J Obstet Gynecol 2016.*

**TABLE 3**
**Secondary outcomes**

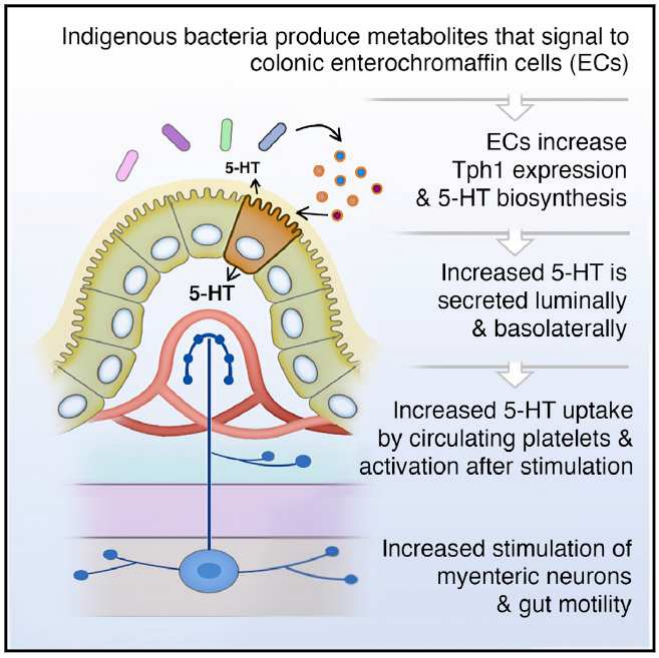
| Outcome | Treatment | Placebo | *P* value: 2-tailed |
|---|---|---|---|
| Intervention-associated discomfort, n (%) | 13 (8.3) | 5 (3.1) | .06 |
| Preterm delivery <37+0 weeks of pregnancy, n (%) | 6 (3.8) | 8 (5.0) | .59 |
| Median duration of pregnancy delivered <37+0 weeks of pregnancy, wk (range) | 35.1 (29.3–36.9) | 31.6 (23–36.6) | .22 |
| Miscarriages (delivery <22+0 weeks of pregnancy), n (%) | 12 (7.7) | 5 (3.1) | .08 |
| Congenital malformations, n (%) | 9 (6.3) | 12 (8.2) | .65 |

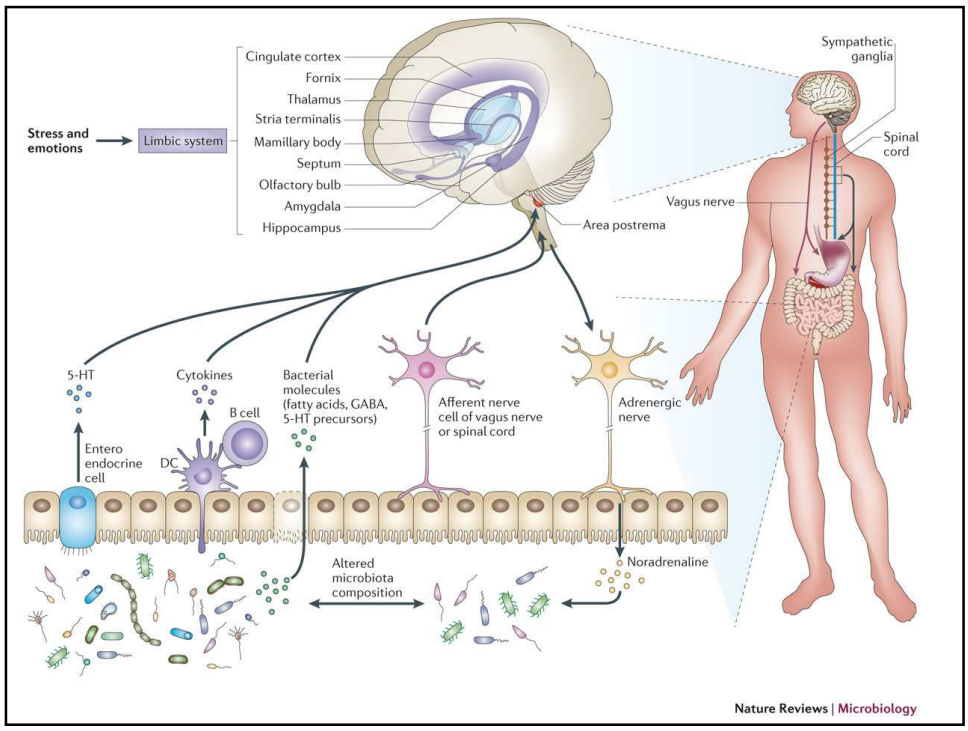
Secondary outcomes are presented in treatment and placebo group; for statistical analysis, values were compared with the use of Fisher's exact test.

*Gille et al. EFFPRO: Effect of probiotics on vaginal health. Am J Obstet Gynecol 2016.*

## Stuhlpassage vermindert…

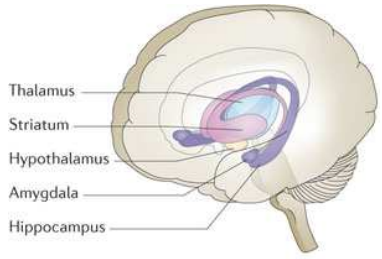
In 1967 zeigten Abrams und Bishop, dass gnotobiotische Tiere eine verminderte Stuhlpassage hatten als Tiere mit einem Mikrobiom (Abrams and Bishop, 1967).






## Gehirn Entwicklung in gnotobiotischen Mäusen

- Reduzierte Gedächtnisfunktion
- Erhöhte Risikobereitschaft und Explorationsverhalten
- Neurobiochemie verändert:
  - Umsatz von Dopamin und Noradrenalin und Serotonin erhöht
  - Weniger Synapsen (Synaptophysin)

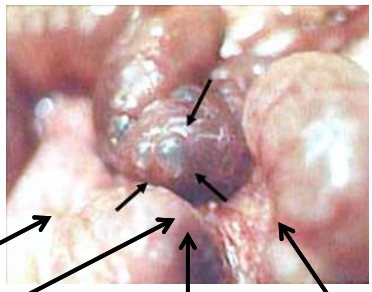



Nature Reviews | Microbiology

Pathogenese von NEC beinhaltet enterale Ernährung, Ischämie & Bakterien

Santulli et al. *Paediatrics* 1975;55:376-87

***Neurologische Entwicklung beeinträchtigt***



>90% der Babies mit NEC wurden gefüttert

Genetik

Lumeninhalt

Immaturiät Immunsystem

Perfusion

**Methods**: A retrospective study of 83 preterm infants born before gestational week 28+0 and treated at the NICU at the Queen Silvia Children's Hospital in Gothenburg, Sweden. The NEC group (n=39) was diagnosed according to modified Bell's staging criteria ≥ 2A, and the controls (n=44) were preterm infants not diagnosed with NEC. GAS score is calculated based on feeding volume, change of feeding volume since previous day, gastric residuals and frequency of stools. Data was recorded from the day of NEC diagnosis and 6 days prior.
Average age at NEC diagnosis was 13 days; data from the controls were recorded from day 13 of life and 6 days prior. The GAS system would score each parameter resulting in a score from 0-8 per day where a higher score would imply a tendency towards gastrointestinal distress.

**Results**: Average stools per day during the studied period were 1,8 ± 0,5 in the NEC group and 3,5 ± 0,4 in the control group. Days to passing of first stool was 4 ± 2,3 days in the NEC group and 2,8 ±1,8 days in the control group. Mean total gastric residual volume in the NEC group was 4,3 ± 1,3 mL and 5,8 ± 0,6 mL in the control group. The NEC group had a higher GAS score in total.

# NEC & eNose

- Microbiome ist metabolisch aktiv

- Metabolite als flüchtige organische Substanzen (volatile organic substances (VOCs))

- VOCs können durch Gas oder Massenspektroscopie

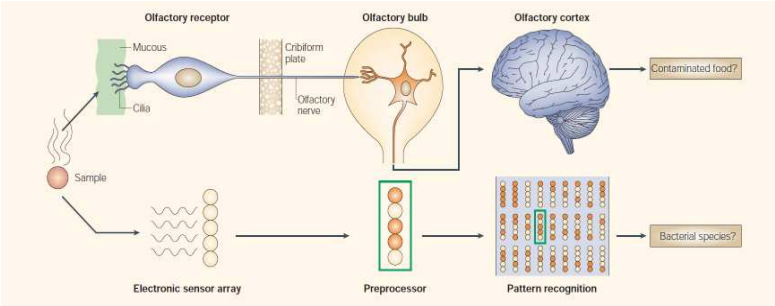



**Table II.** Subject characteristics of the 3 subgroups NEC, sepsis, and controls

| | NEC 13 | Sepsis 31 | Control 14 |
|---|---|---|---|
| Sex | | | |
| Male (n [%]) | 4* [31] | 18 [58] | 11* [79] |
| Birth weight (median [IQR]), g | 740 [155] | 880 [300] | 937 [346] |
| Gestational age (median [IQR]), wk + d [d] | 26 + 6 [6] | 26 + 3 [17.5] | 26 + 5 [15.3] |
| Feeding pattern (n [%]) | | | |
| Breast milk ± formula | 12 [92] | 29 [94] | 13 [93] |
| Exclusive formula | 1 [8] | 1 [3] | 0 [0] |
| Missing value | 0 [0] | 1 [3] | 1 [7] |
| Postnatal age at $T_0$ (median, [IQR]), d | 18.5† [8] | 10†,‡ [7] | 17‡ [0] |
| AB use before $T_0$ (%) | n = 13 (100) | n = 29 (94) | n = 13 (93) |
| Days AB use before $T_0$ (median [IQR]), d | 7 [5] | 5 [4] | 7 [4] |
| Deceased (n [%]) | 7 [54] | 3 [10] | 0 [0] |
| Age deceased (median [d]) | 20 | 18 | NA |

*AB*, antibiotics; *NA*, not applicable.
*$P$ = .013.
†$P$ = .005.
‡$P$ = .008.

De Meijs et al., J. Pediatr., 2015

**Table I.** Number of fecal samples (n) in each group per selected time windows used for VOC analysis

| | (n) | | |
|---|---|---|---|
| Window | NEC | Control | Sepsis |
| $T_{-1,0}$ | 9 | 9 | 23 |
| $T_{-3,-2}$ | 12 | 12 | 20 |
| $T_{-5,-4}$ | 10 | 10 | 18 |

**Table IV.** Isolated pathogens n (%) from blood cultures in 31 sepsis patients

| | |
|---|---|
| *Staphylococcus aureus* | 2 [6] |
| CNS | 21 [68] |
| *Staphylococcus epidermidis* | 10 [32] |
| *Staphylococcus capitis* | 5 [16] |
| *Staphylococcus haemolyticus* | 1 [3] |
| *Staphylococcus warneri* | 2 [6] |
| Combination of 2 different CNS | 3 [10]* |
| *Escherichia coli* | 3 [10] |
| *Enterococcus faecalis* | 1 [3] |
| *Serratia marcescens* | 1 [3] |
| *Candida albicans* | 1 [3] |
| More than 1 pathogen cultured | 2 [6]† |

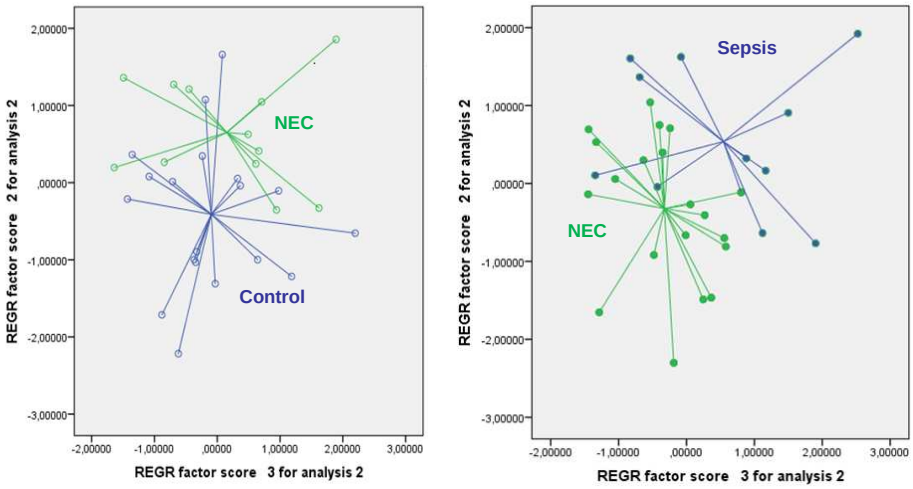
*CNS*, Coagulase negative *Staphylococcus*.
*2 × *Staphylococcus capitis* and *Staphylococcus epidermidis*; 1 × Staphylococcus capitis and Staphylococcus haemolyticus.
†1 × *Enterobacter cloacae* and *Staphylococcus wameri*; 1 × Acinetobacter baumanii and Staphylococcus capitis

Bakterien aus der Blutkultur stammen aus dem Darm.

De Meijs et al., J. Pediatr., 2015

Berkhout et al., JPGN , 2016

# Electronic Nose (eNose) Sepsis versus NEC




De Meijs et al., J. Pediatr., 2015

# eNose Stuhlproben

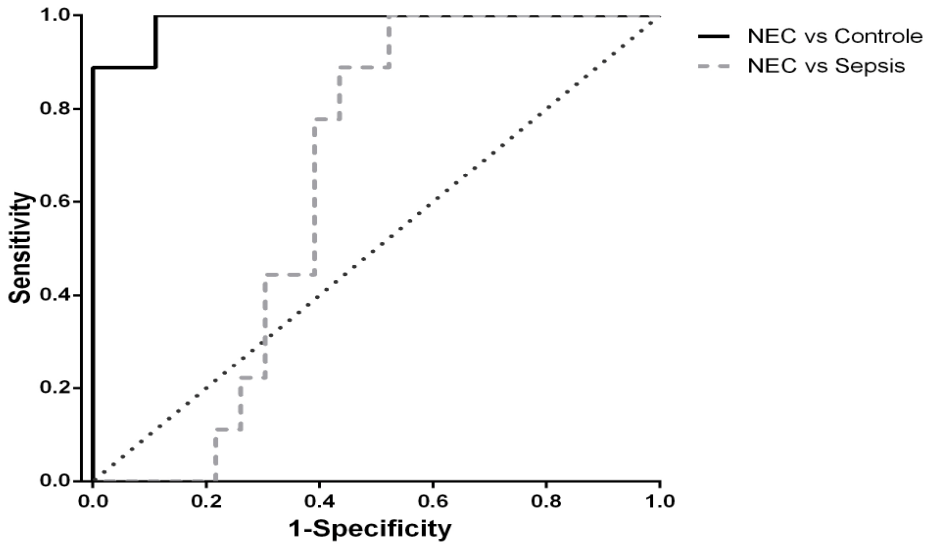



**Table III.** Performance characteristics of fecal VOC analysis for the discrimination of NEC, sepsis, and controls

| Time window | AUC ± 95% CI | *P* value | Sensitivity | Specificity | +LR | −LR |
|---|---|---|---|---|---|---|
| NEC vs control | | | | | | |
| $T_{0,-1}$ | 0.99 ± 0.04 | >.001 | 88.9 | 88.9 | 8,1 | 0,1 |
| $T_{-2,-3}$ | 0.77 ± 0.21 | .024 | 83.3 | 75.0 | 3,3 | 0,2 |
| $T_{-4,-5}$ | 0.65 ± 0.25 | .257 | 60.0 | 60.0 | 1,5 | 0,7 |
| NEC vs sepsis | | | | | | |
| $T_{0,-1}$ | 0.64 ± 0.18 | .216 | 88.9 | 56.5 | 2,1 | 0,20 |
| $T_{-2,-3}$ | 0.80 ± 0.17 | .004 | 83.3 | 75.0 | 3,3 | 0,2 |
| $T_{-4,-5}$ | 0.52 ± 0.23 | .886 | 50.0 | 40.0 | 0,8 | 1,3 |

+*LR*, positive likelihood ratio; −*LR*, negative likelihood ratio.
Sensitivities, specificities, and positive and negative likelihood ratios are reported for the optimum cut-points.

De Meijs et al., J. Pediatr., 2015

# Zusammenfassung:

- Mikroben sind Bestandteil des menschlichen Körpers
- Mikroben haben Anteil an Funktionen wie Stuhlentleerungsfrequenz und Implantation
- Beginn des Verständnis – Interventionen werden entwickelt

# Vielen Dank !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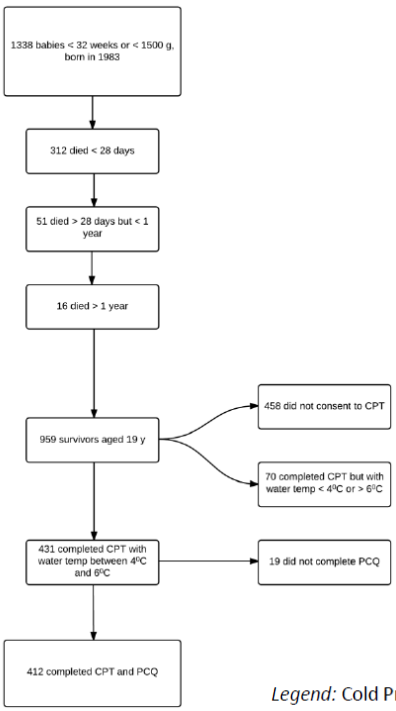
Van Ganzewinkel, submitted



*Legend:* Cold Pressor Test (CPT), Pain Coping Questionnaire (PCQ)

**Table 2: Proportion of participants 'surviving' in ice water to the set limit of 180sec.**

| | | Pain threshold | | Pain tolerance | |
|---|---|---|---|---|---|
| | | T1 | | T2 | |
| | | n/N (%) | *p* value | n/N (%) | *p* value |
| SGA/AGA | AGA | 16/244 (7) | 0.62 | 67/244 (27) | 0.03 |
| | SGA | 9/168 (5) | | 31/168 (18) | |
| Gender | female | 10/228 (4) | 0.11 | 44/228 (19) | 0.02 |
| | male | 15/184 (8) | | 54/184 (29) | |
| NEC | no | 25/382 (7) | 0.15 | 96/382 (25) | 0.02 |
| | yes | 0/30 (0) | | 2/30 (7) | |
| Sepsis | no | 19/278 (7) | 0.35 | 67/278 (24) | 0.83 |
| | yes | 6/134 (5) | | 31/134 (23) | |
| IVH | no | 23/349 (7) | 0.29 | 87/349 (25) | 0.20 |
| | yes | 2/63 (3) | | 11/63 (17) | |
| Respiratory support | no | 12/204 (6) | 0.88 | 46/204 (23) | 0.56 |
| | yes | 13/208 (6) | | 52/208 (25) | |