Development of the intestinal microbiome in healthy children in the first years of life

This study is organized by:
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Dear madam,

We would kindly ask you and your unborn child to participate in our research project. Please find details of our research project below, first as a brief summary followed by another description in more detail.

Summary

1	**Aim of the project** The intestinal microbiota comprises all microorganisms residing in the intestine, including bacteria and viruses. At birth, the intestine is almost germ-free; however, within the first two years of life the intestine will gradually be colonised by bacteria. During this time, the microbiota undergoes a maturation process. During this process, the number of different bacterial species increases and their metabolic properties become more diverse. After approximately two years, the maturation of the microbiota is complete. The aim of the research project is to understand the normal development of the intestinal microbiota. We will also test whether the maturation of the microbiota is related to children's health issues (e.g. infections, overweight or abdominal pain). We hope that this study will provide a basis for further research and the development of future therapies.
2	**General inclusion criteria** We are aiming to include healthy pregnant women between 18 and 45 years of age from week 20 of pregnancy.
3	**General information about the project** Overall study duration for each participant is more than 10 years (from inclusion before birth to the child's 10th birthday). However, most contacts are planned within the first two years of life. We are aiming to analyse microbiota characteristics in stool samples and correlate this information to parameters of children's health. The study is conducted at the Inselspital Bern.
4	**Procedure** Inclusion in the study takes place after the 20th of week of pregnancy. Further controls are carried out after birth (day 0-3), on day 10 and in weeks 6, 10, 14, 24, 36, 48 and 96 after birth. In addition, we plan a study visit at 5 years and 10 years after enrolment. During study visits the investigator will ask for a stool sample from your child. Within the first two years, the stool sample can usually be taken directly out of the diaper or, if the diaper is empty, through a sampling kit for stool collection at home. We will also ask you for stool samples and, if you are breastfeeding at that time, for a few millilitres of breast milk. In addition, a skin swab will be taken from you and your child (using a cotton swab in the groin area). A voluntary vaginal swab will be taken, only with your consent. Shortly after birth of your child, a single placenta sample will be acquired. We will measure upper arm, hip and

	waist circumference of your child. Furthermore, the investigator will ask you general questions about the medical situation and nutrition of you and your child. At two visit we will ask for more detailed nutritional habits. After 2, 5 and 10 years, we will assess behavior of the child using a questionnaire.The investigator will also use the results of regular paediatric examinations for this study. Each visit should last no longer than 60 minutes.
5	**Benefits** No direct benefit for your health or the health of your child is expected from this study. However, by participating in this study you will help us to understand why other children experience for example developmental problems or more frequent stomach pain.
6	**Rights** You decide voluntarily whether you and your child want to participate in this project or not. Your decision has no influence on your medical treatment/care and you do not have to justify this decision.
7	**Duties** If you participate, the investigator asks you to meet certain needs. As a participant it is necessary that you support us in obtaining stool samples, breast milk samples and skin swabs. The investigator will also ask you to provide information about your health and the health of your child.
8	**Risks** By participating in the project, neither you nor your child are exposed to relevant medical risks. The risks related to acquisition of stool samples and skin swabs is negligible.
9	**Results** You will be informed of new results relevant for your decision to participate in this study during the project. You will be informed in the event of random findings that may contribute to the prevention, diagnosis or treatment of existing or future diseases in you or your child.
10	**Confidentiality of data and sampling** The investigator collects your personal and medical data and collects biological material from you or your child (stool, breast milk, skin swabs, placenta sample, vaginal swab if applicable). Data and samples can also be used for other projects if you provide separate consent. The investigator will comply with all legal regulations regarding data protection. All parties involved are subject to the duty of medical confidentiality.
11	**Withdrawal** You can withdraw from the project at any time and no longer participate. The data and samples collected until then will still be analysed.
12	**Compensation** You will not receive any expense allowance. The investigator will reimburse you expenses such as travel expenses directly related to your participation in this study. There will be no costs for you or your health insurance related to your participation in this study.
13	**Liability** The Inselspital Bern, which initiated this study and is responsible for conducting it, is liable for any damage that you may incur in connection with the research activities (e.g. examinations).
14	**Funding** The study is financed by the Clinic for Visceral Surgery and Medicine, Inselspital Bern. The sponsor will apply for funding from public third-party funds (e.g. Swiss National Science Foundation, SNF).

15	**Contact person(s):** You can get information on all your questions. Prof. Dr. med. Benjamin Misselwitz Clinic for Visceral Surgery and Medicine, Inselspital Bern. Freiburgstrasse 18, 3010 Bern benjamin.misselwitz@insel.ch Phone 031 632 5719 PD Dr. med. Christiane Sokollik University Clinic for Pediatrics, Inselspital Bern Freiburgstrasse 18, 3010 Bern christiane.sokollik@insel.ch Phone 031 632 9837 Prof. Dr. Stephanie Ganal-Vonarburg Department for BioMedical Research Clinic for Visceral Surgery and Medicine Murtenstrasse 35, 3008 Bern stephanie.ganal@dbmr.unibe.ch

1. Aim of the study

The human body hosts a large amount of microorganisms. They populate almost every surface of our body and form the so-called "microbiota". The microbiota plays a central role in many diseases.

The child's intestine is almost germ-free before birth. At birth and also afterwards, the intestine is colonised by bacteria from the birth canal and the environment. In the following years, the complexity of the intestinal flora then increases further until a stable and mature intestinal microbiota is reached at the age of about two years. The maturation of the intestinal flora can be influenced by alimentation, diseases and medication. Certain diseases such as allergies, inflammatory bowel diseases, obesity, infectious diseases and environmental enteropathy in children with malnutrition in developing countries are associated with changes in the intestinal flora.

The intestinal microbiota and its maturation is a field of active research. In recent years, the techniques for the analysis of the intestinal microbiota have improved considerably. With modern analysis techniques, the genetic information of the microbiota can be recorded almost entirely. From the resulting data, information about the metabolic activity of the intestinal bacteria can be derived. In addition, investigators can determine the chemical composition of stool samples. The combination of these methods will enable a deep understanding of the intestinal microbiota and the diseases associated with it.

The aim of this study is to collect and analyse stool samples from healthy children in the first years of life at several points in time in order to understand the normal development of the intestinal microbiota. The investigator will also test whether the maturation of the microbiota is influenced by environmental factors (nutrition, breastfeeding, life style) and related to the health of the children (e.g. infections, overweight, abdominal pain, allergies, behaviour). Both could provide a basis for further research and the development of future therapies.

2. Inclusion and exclusion criteria

The investigators are looking for healthy pregnant women from week 20 of pregnancy, aged between 18 and 45 years, to be included into this study. The study inclusion concerns you as well as your newborn child.

3. General information

This study is conducted locally in Switzerland. The study is explorative and aims to promote the basic understanding of the intestinal microbiota (basic research). Recruitment will take place over a period of three years. The study duration for each particular participants is slightly more than ten years (from inclusion before birth until the child's 10th birthday). However, most contacts are planned within the first two years. The study will be conducted at the Inselspital Bern and 250 pregnant women will be recruited.
The investigator is conducting this study in accordance with the laws in Switzerland. In addition, the investigator is following all internationally recognised guidelines. The responsible cantonal ethics committee has examined and approved the study.

4. Procedure

Inclusion in the study takes place after the 20th week of pregnancy. Further controls are carried out after birth (day 0-3), on day 10 and in weeks 6, 10, 14, 24, 36, 48 and 96 after birth. In addition, there will be a study visit 5 years and 10 years after birth.

During study visits, the investigator will ask you for a stool sample from your child. Within the first two years, the stool sample can usually be taken directly out of the diaper or, if the diaper is empty, through a sampling kit for stool collection at home. We will also ask you for a stool sample from and, if you are breastfeeding at that time, for a few millilitres of breast milk. In addition, a skin swab will be taken from you and your child (using a cotton swab in the groin area). In addition, a skin swab will be taken from you and your child (using a cotton swab in the groin area). A voluntary vaginal swab will be taken, only with your consent. Shortly after birth of your child, a single placenta sample will be acquired. Furthermore, the investigator will ask you general questions about the medical situation (e.g. allergies, abdominal pain) and nutrition of you and your child. At two visit we will ask for more detailed nutritional habits. After 2, 5 and 10 years, we will assess behavior of the child using a questionnaire. We will also measure the mid upper arm circumference of you and your child as well as hip and waist circumference of your child. The investigator will also use the results of regular paediatric examinations for this study. Each examination should last no longer than 60 minutes.

During the first 10 years of your child's life, your child will already be comprehensively medically examined during regular paediatric examinations. We strongly encourage you to attend each of these regular paediatric examinations. The investigators will also use the results of these examinations for this study. Should you or your child develop a disease during the course of this study, the investigator will ask your paediatrician or treating physician for further medical information. From biological material such as stool, skin swab, vaginal swab, placenta sample and breast milk, genetic analyses of the bacteria present in the probes will be made. We will also determine the chemical composition of the breast milk.
For technical reasons, human genetic information will also be acquired in these investigations. This human genetic information is not the subject of the main analyses in this study. However, it is possible that analyses of this genetic information will become important in future projects. The investigator asks you to give your consent for these possible additional analyses as well. If you do not agree to this, please tick the corresponding box in the declaration of consent. A refusal to additional analyses does *not* exclude you from this study. The cultivation of intestinal bacteria (reproduction under laboratory conditions) and their further examination is also possible. Similarly, antibodies and molecules of the immune system in breast milk and stool and their relationship to the intestinal bacteria can be examined.

5. Benefit

No direct benefit for your health or the health of your child is expected from this study. However, by participating in this study you will help us to understand why other children experience for example developmental problems or more frequent stomach pain. This is

particularly important because a parallel study with a similar study design is being conducted in a country with few resources (Zimbabwe, Africa), and children participating in this study will be directly compared with children from Zimbabwe. Results from this study will help to better understand the development of the intestinal flora and this could be important for the future development of new therapies.

6. Rights

You and your child participate in the study voluntarily. If you do not wish to participate or later withdraw your participation, you do not need to justify your decision. Your medical treatment/care is guaranteed regardless of your decision. You may ask questions about participating in the study at any time. For questions, please contact the person named at the end of this information. Your child is 10 years old at the time of the last examination. The investigator will then briefly explain the study to your child in an appropriate and understandable manner. If your child refuses to participate the investigator will respect this.

7. Duties

If you participate, the investigator asks you to meet certain needs. As a participant it is necessary that you support us in obtaining stool samples, breast milk samples and skin swabs. The investigator also asks you to provide information about your health and the health of your child.

8. Risks and burdens for the participants

By participating in the project, neither you nor your child are exposed to relevant medical risks. The risks related to acquisition of stool samples and skin swabs is negligible.

9. Results from the study

The investigator or project leader will inform you during the project of any new findings that may affect the study benefit or your safety and thus your consent to participate. You will be informed in the event of random findings that may contribute to the prevention, diagnosis or treatment of existing or future diseases in you or your child. Such random findings could also occur with very low probability in the context of a possible analysis of human genetic information (if approved by you). In such a case, the investigator will inform you in cooperation with your paediatrician and, if appropriate, offer you appropriate advice. If you do not wish this, please contact the study team.

If you do not wish to be informed, please speak to your investigator/project manager.

10. Confidentiality of data and samples

For this study, personal and medical data from you and your child will be collected. Very few professionals will see your unencrypted data, and this only to perform tasks in the context of the study. When data is collected for study purposes, the data will be encrypted. Encryption means that all reference data that could identify you or your child (e.g. name, date of birth) is deleted and replaced by a key. The list of keys always remains in the Clinic for Visceral Surgery and Medicine at the Inselspital Bern. Those people who do not know the key cannot draw any conclusions about your identity. In a publication, the summarised data cannot be traced back to you or your child as individuals either. Your name or the name of your child never appears on the Internet or in any publication. Sometimes there is a requirement for individual data (so-called raw data) to be submitted for publication in a journal. If individual data has to be transmitted, the data is always encrypted and therefore as well not traceable to you or your child as an individual. All persons who have access to your data in context with the study are subject to medical confidentiality. The data protection regulations are observed and you as a participating person have the right to view your data at any time.

If data/ samples are stored on site, it is a database/ biobank for research purposes. These data and samples can be encrypted and sent to another database/ biobank in Switzerland (for mass spectrometry Prof. U. Sauer, Institute for Molecular Systems Biology, ETH Zurich. Otto-Stern-Weg 3, 8093 Zurich, storage there is planned for up to 6 months).

It is possible that your data and samples, or those of your child, may be used for other investigations at a later date or may be sent and used later on to another database/biobank in Switzerland or abroad for investigations that have not yet been further defined (further use). This other database/biobank must comply with the same standards as the database/biobank for this study. For this re-use, the investigator asks you to sign a further declaration of consent at the very end of this document.

This study may be audited by the responsible ethics committee or another institution. The investigator may need to disclose the personal and medical records of this study for such review. All individuals involved in this process must maintain absolute confidentiality.

11. Withdrawal

You can terminate your participation in the study prematurely at any time if you wish. In this case the investigator would cancel your further study appointments. However, the data and samples collected up to that point will still be evaluated in encrypted form, otherwise the entire project will lose its value.
It is not possible to make your data and samples anonymous when you withdraw, as the investigator is legally obliged to keep the study documents with your name (such as for example the informed consent of this project). Thus, data and samples remain encrypted. Please check if you agree to this before you participate in this project.

12. Compensation for participants

You will not receive any expense allowance. The investigator will reimburse you for expenses such as travel expenses which are only caused due to your participation in the study. There are no costs for you or your health insurance company due to your participation. The results of this study may help to develop commercial products. However, by participating in the study, you are not entitled to claim commercial developments (e.g. patents).

13. Liability

The Inselspital Bern, which initiated this study and is responsible for conducting it, is liable for any damage that you may incur in connection with the research activities (e.g. examinations). The requirements and the procedure for this are regulated by law. If you have suffered damage, please contact the investigator.

14. Financing of the study

The study is financed by the Clinic for Visceral Surgery and Medicine, Inselspital Bern. The sponsor will apply for funding from public third-party funds (e.g. Swiss National Science Foundation, SNF).

15. Contact person(s)

If you have any questions, uncertainties or emergencies that arise during or after the study, you can always contact the contact persons listed below.

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Written declaration of consent to participate in a study project

Please read this form carefully. Please ask if you do not understand or want to get information on something. Your written consent is required for participation.

BASEC-Number (after submission):	2019-00510
Title of the study (scientific and layman language):	„Trajectory of microbiota maturation in healthy Bern infants – a network approach" Maturation of the healthy infant intestinal microbiota
Responsible institution **(Sponsor with address):**	Prof. Dr. med. Benjamin Misselwitz University Hospital for Visceral Surgery and Medicine, Inselspital Bern Freiburgstrasse 18, 3010 Bern benjamin.misselwitz@insel.ch, 031 632 5719
Place of conduct:	University Hospital for Visceral Surgery and Medicine, Inselspital Bern Women's Clinic (Frauenklinik), Inselspital Bern
Responsible investigator at the study site: First and last name in block letters:	
Participant: Name and first name of mother in block letters: Mother's date of birth: Name and first name of the child in block letters: Child's Date of birth:	Child: ☐ female ☐ male

- I have been informed verbally and in writing by the undersigned investigator about the purpose, the course of action of the study and about possible advantages and disadvantages as well as risks.
- I voluntarily participate in this study and accept the content of the written information provided. I had sufficient time to make my decision.
- The consent to participate in the study applies to me as well as to my unborn child. If my child does not wish to continue to participate in the study at the age of 10 years (after having been informed verbally in a child-friendly manner) The investigator will respect this.
- My questions in conjunction with my participation in this study have been answered. I will keep the written information and receive a copy of my written consent form.
- I agree that my family doctor will be informed about my participation in the study.
- I agree that the responsible experts of the responsible ethics committee may inspect my unencrypted data for review and control purposes, but in strict compliance with confidentiality.

- I will be informed in case of study results or accidental findings that directly affect my health. If I do not wish to be informed, I will inform my investigator.
- I understand that my health-related and personal data and samples from this study can be shared for research purposes in encrypted form only.
- I can withdraw from the study participation at any time and without giving reasons. My further medical treatment is always guaranteed, regardless of my participation in the study. The data and samples collected up to the point of withdrawal will be used for the analysis of the study.
- The liability insurance of the hospital/institution will cover any damages.
- I am aware that the obligations mentioned in the participant information must be followed. In the interest of my health, the investigator may exclude me from the study at any time.
- I allow ☐ / do not allow ☐ a vaginal swab to be taken

Location, Date	Signature of the participant (mother)

Investigator's confirmation: I hereby confirm that I have explained the nature, significance and scope of the study to this participant. It was explained that the consent applies to both the mother and the child. I confirm that I will comply with all obligations in connection with this study in accordance with applicable law. If at any time during the conduct of the study I learn of any aspects that might influence the participant's willingness to participate in the study, I will inform him/her immediately.

Location, Date	Name and first name of the investigator in block letters Signature of the investigator

Declaration of consent for further use of (genetic) data and biological material in encrypted form

Participant:	
Name and first name in block letters:	
Date of birth:	

I allow that the genetic data and samples of me and my child from this study may be used for medical research. This means that the samples may be stored in a biobank and used indefinitely for future, as yet undefined research projects. This consent is valid for an unlimited period of time.

I decide voluntarily and can revoke this decision at any time. I only have to inform my investigator and do not have to justify this decision. If I resign, however, the data collected so far will continue to be used for analysis, otherwise the entire project would lose value. This also applies to data and samples from my child.

I understand that the data and samples are encrypted and the key is kept safe. The data and samples can be sent to other databanks and biobanks in Switzerland and abroad for analysis, provided they comply with the same standards as in Switzerland. All legal requirements regarding data protection are complied with.

Normally, all data and samples are evaluated as a whole and the results are published in summary form. If a result that is important for my health should arise, it is possible that I will be contacted via my investigator. If I do not wish to be contacted, I will inform my investigator.

If results from the data and samples are commercialized, I have no claim to a share of the commercial use.

Location, Date	Signature of the participant

Confirmation by the investigator: I hereby confirm that I have explained to this participant the nature, significance and extent of the further use of samples and/or genetic data. It has been explained that consent applies to the mother and child samples.

Location, Date	Name and first name in block letters of the informing investigator Signature of the investigator

Declaration of consent for the analysis of human genetic information (DNA)

Participant: Name and first name in block letters: Date of birth:	

☐ I permit ☐ I do **not** permit

that the genetic information about me and my child obtained in the course of the study may be analysed and evaluated for medical research.

Location, Date	Signature of the participant

Confirmation by the investigator: I hereby confirm that I have explained to this participant the nature, significance and scope of the analysis of human genetic information (DNA). It has been explained that the consent applies to the mother and child samples.

Location, Date	Name and first name in block letters of the informing investigator Signature of the investigator