The Biotic Comforter®: *A low cost device for transfer of maternal vaginal flora to neonates*

Summary

In developing countries, chronic autoimmune diseases are continuously increasing. In recent decades, the rate of caesarean sections compared to vaginal delivery has also increased. Children born by the caesarean delivery have an increased risk for chronic autoimmune diseases later in life. A current hypothesis for the development of autoimmune diseases is that the immune system needs to be trained in order to mature and that disturbances in maturation might lead to autoimmune disorders later in life. The mode of delivery has a major role in the composition of the intestinal microbiome in infants. The intestinal microbiome of new-borns differs depending on the route by which they are delivered, skin flora is more prominent in children delivered by caesarean section and vaginal bacteria predominate in naturally born children. Thus, restoration of the microbiome of children delivered by caesarean section to that of vaginally delivered children is likely to have positive health effects.

The aim of this project is to develop a device, which consist of an applicator and a tampon like structure mounted to a comforter, to transfer the vaginal flora to neonates delivered by caesarean section.

We hypothesize that by the introduction of the maternal vaginal flora to neonates delivered by caesarean section will provide the neonate with the appropriate bacterial strains necessary for proper development of the immune system and thereby contribute to prevention of autoimmune diseases later in life.

Introduction

Developed countries are currently characterized by a drastic decline of infectious diseases compared to the past centuries, whereas chronic autoimmune diseases such as juvenile arthritis, inflammatory bowel diseases and asthma are continuously increasing. In less developed countries these autoimmune disorders are far less predominant and here infectious diseases contribute most to health impairment. Evidence suggest that early life risk factors may be underlying these chronic autoimmune diseases, since the disease prevalence changes in populations migrating from underdeveloped to developed countries(Bach 2002, Sevelsted, Stokholm et al. 2015)

In recent decades, the rate of caesarean sections compared to vaginal delivery has also increased in developed countries and children born by caesarean delivery have an increased risk for chronic autoimmune diseases later in life. A current hypothesis for the development of autoimmune diseases is that the immune system needs to be trained in order to mature and that a disturbance in this ''maturation'' may lead to adverse health effects later in life. In this respect it is thought that the composition of the gut microbiome plays a pivotal role.

Before birth, the gastrointestinal tract is almost sterile and upon birth one acquires a microbiome by a sequential colonisation of different microbial genera. The first gut colonists are vaginally derived, most likely acquired during the passage through the birth channel. Indeed, the mode of delivery has a major role in the composition of the intestinal microbiome in infants and affects the early stage of bacterial colonization (Biasucci, Rubini et al. 2010). It has been shown that the microbiome of infants born by caesarean sections and vaginal delivery can be separated in two different clusters on genus level. These differences persist for prolonged periods. Vaginal delivery was dominated by Bacteroidetes and caesarean by Firmicutes (Biasucci, Rubini et al. 2010)

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Of course food industries are aiming to contribute to human health by adding probiotics to formula milk to improve the quality of the microbiome and thereby the training of the infant immune system. However, to mimic the complex flora found in young humans might be too ambiguous yet. We are at infancy in understanding the complex composition and interactions between the gastrointestinal tract and the immune system(Hanage 2014). Also a persistent colonization of the intestine with probiotics at a stage when the gut is already colonized remains difficult (Bezkorovainy 2001). Moreover the existence of specific individual stains within the vaginal microbiome and also the existence of ethnic specific compositions of the vaginal microbiome will make the development of probiotics for newborns a complex affair. These new observations suggest that the appropriate combination of strains, derived from the mother, is necessary in for a full sequence of gut colonisation in a neonate.

Objective

The aim of this project is to develop a device to transfer, in a safe controllable and natural way, the vaginal flora to children delivered by caesarean section.

Hypothesis

The introduction of the maternal vaginal flora to a neonate delivered by caesarean section will provide the neonate with the appropriate ''first settler'' bacterial strains. This will help proper development of the immune system and contribute to prevention of autoimmune diseases later in life.

Method

Validation of the Biotic Comforter®

As it is unknown which species of the vaginal microbiome are most important for the development of a healthy intestinal microbiome in infants, our device must be able to transfer a representative set of microbes. Published vaginal microbiome compositions will be used as a standard for the design and optimisation of the mother to child microbiome transfer device: *the biotic comforter®*. We will first start with a prototype consisting of an applicator and a tampon like structure for inoculation with maternal vaginal flora. The tampon will be mounted (after the inoculation) to a comforter in order to transfer, in a safe mode, the vaginal flora to neonates delivered by caesarean section (fig. 1) . Inoculations will be done at different insertion depths and with different tampon materials. If necessary, gentle mechanical vibration to ensure absorption of a representative set of bacteria from the vaginal cavity will be used.

The amount of viable aerobic and anaerobic bacteria that can be transferred by the device will be determined by culture on selective agar plates. Species will be identified using Maldi-Tof.

After optimizing the device and addressing safety hazards such as the transfer of opportunistic pathogens such as *Candida albicans*, a pilot trial will be performed.

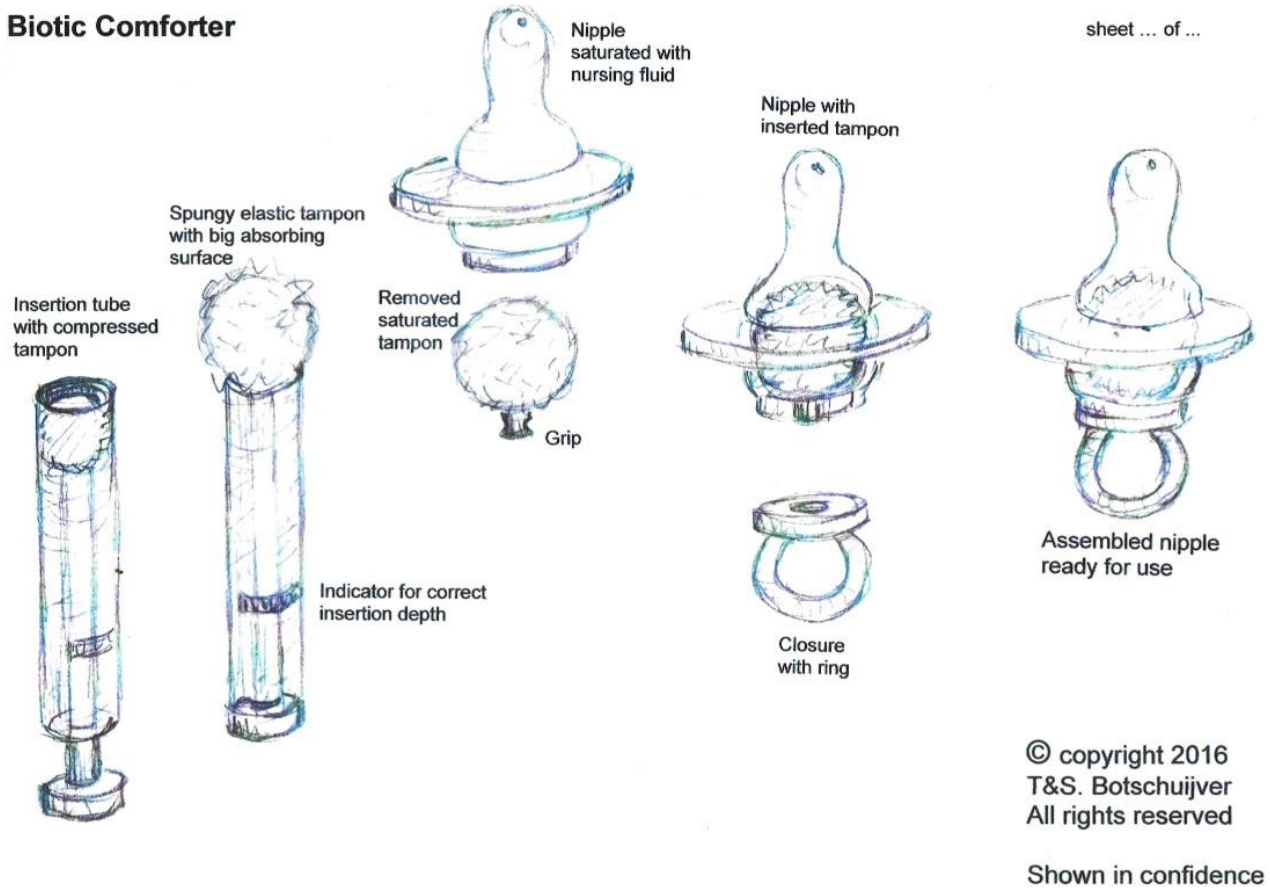

Figure 1 The Biotic Comforter® prototype.

Clinical trial

Healthy pregnant individuals undergoing an elective caesarean section will be addressed to participate in this trial under informed consent. Only woman free of STD and without bacterial vaginosis will be included in the study. The Biotic Comforter© will be applied by qualified medical personnel under hospitalized conditions within an experimentally determined limited time frame, post-partum. Faeces of the neonates will be collected daily for 14 days, followed by a weekly collection for up to three months. Babies that need antibiotic treatment within this period will be excluded from the study. The development of the microbiome of babies transferred with the maternal vaginal flora will be followed with 16 sequencing and compared with published data.

Expected results

A restored intestinal microbiome in infants delivered by caesarean section, , resembling vaginal delivery and that likely minimizes the early life risk factors for the development of chronic inflammatory diseases.

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