WP 2.1: Germ-free birth in preterm pigs (Cancelled)

1. Related WPs, MG contact person: Synergies with WPs 1.1, 1.2b, 1.5, 2.1-4,3.1. MG contact: Thomas Thymann

2. Key involved personnel, their institution and mail address (project leader + main study site underlined):
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3. Main aim and sub-aims:
The main aim is to determine if delayed microbial colonization after preterm birth, has effects (beneficial or detrimental) on gut-, immunity and brain development.

4. Background and a central hypothesis:
Timing of the first exposure to gut microbes is believed to be important for maturation of gut, immunity and brain. The links between microbiota and gut, brain and immunity may be direct or indirect, but the recent finding that gut microbial colonization affects brain maturation and motoric control in mouse pups supports direct links. It is very important to document these fundamental effects in an animal model that more closely resemble human infants. This effect of early colonization could be particularly important after preterm birth when immunity, gut and brain are vulnerable to insults due to immaturity.
We hypothesize that immunity, gut and brain functions are dependent on timing of microbial colonization after birth (immediate versus delayed).

5. Key analyses and methods:
Preterm pigs are derived by cesarean section. Half of the newborn pigs are kept in conventional incubators while the other half is kept in germ-free isolators for up to 7 days before they are conventionalized. Key analyses include open field test as well as structural, functional, microbiological and immunological parameters of the developing gut and brain. Specific endpoints include immunological parameters (goblet/leucocyte cell counts, tissue gene expression and protein content of cytokines), gut parameters (digestive enzymes, cell cycle, histology, microbiota composition, short chain fatty acids) and brain parameters (morphology, blood brain barrier)

6. Expected results:
We expect to consolidate the notion that the timing of microbial colonization impact on gut and brain development. This project provides important mechanistic information for further clinically-relevant studies. Artificial rearing under germ-free conditions cannot be expected to be of direct translational value. However, germ-free rearing during the initial 7 days after birth enables us to determine whether late colonization is beneficial with regards to clinical and organ functional endpoints. Provided that we can substantiate the beneficial effects of delayed colonization, it creates the scientific rationale for studying how colonization can be delayed/inhibited under more clinically relevant conventional conditions.
Predicted publication: The effects of delayed microbial colonization and early life enteral nutrition on gut and brain development.

7. Estimated time frame

Task	2013				2014				2015				2016				2017			
Planning, protocol					x	x	x	x	x	x	x	x								
Sample collection																				
Behaviour																				
Microbiota comp. and SCFA																				
Goblet/leucocyte cell count																				
Mucus production																				
Cytokines																				
Histology																				
Blood brain barrier proteins																				
Publication(s)																				

8. Estimated budget from NEOMUNE: 1.8 mio DKK

9. Estimated budget from elsewhere: 0.3 mio DKK
Self-financing (i.e. university funded salaries) and collaboration with SPF Vejen, Denmark (intellectual and practical support from a commercial supplier of germ free pigs).

10. Additional comments:

- Germ free experiments are considered high-risk as contamination of the isolator can occur after even the smallest breach. We collaborate with experienced personnel to minimize this risk. The model may be refined after the first series of experiments, but is unlikely to become a routine experimental animal model. In the event that the experimental periods of earlier WP's within WP2 are extended, WP2.1 will be postponed.
- The protocol may be supplemented also with a diet-intervention, formula versus colostrum under both germ free and conventional conditions.
- Due to expanded contents of studies in WP 2.2-2.4, we have decided to cancel this WP. Funds allocated among other WPs in pigs to increase output from these WPs.