WP 2.0: Development of a preterm pig brain model

1. Related WPs, MG contact person: Synergies with WPs 1.4a, 2.1-2.4, 3.1. MG contact: Thomas Thymann

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3. Main aim and sub-aims:

The primary aim is to develop a clinically-relevant preterm pig brain model of preterm human infants. We will identify and describe functional and structural neurodevelopmental differences between preterm- and term-delivered piglets e.g. by using a series of *in vivo* methodologies and approaches traditionally used by neonatologists, chosen to maximize the translational value to preterm human infants. We also aim to relate any neurobehavioral and –functional deficits and/or delays with various relevant brain and gut structural, cellular and molecular endpoints within the first 4 weeks of life. Of these, the most sensitive endpoints will serve as key outcome parameters in subsequent interventions.

4. Background and a central hypothesis:

The pig is emerging as a valuable animal model of humans in biomedical research related to both neuroscience and to gastrointestinal diseases. Our group has >10 yrs experience with preterm pig models of gastrointestinal disorders such as necrotizing enterocolitis (NEC), a prevalent disease in preterm delivered infants which is associated with an increased risk of suboptimal neurodevelopment. Humans and pigs are thought to share similar brain growth and development patterns. A growing awareness of the important interplay between the early milk diet, the gut and the brain, and their possible interdependence during postnatal development, has prompted us to expand our research to investigations of brain maturation in the preterm pig model. This preterm pig model may be used to develop diet and feeding regimens that optimize brain development in the most vulnerable newborn infants. *We hypothesize that prematurity in pigs is associated with functional and structural neurodevelopment deficits and delays that reflect those observed in preterm infants.*

5. Key analyses and methods:

A comparison of newborn caesarean-delivered preterm (~90% gestation) and term pigs initially reared in our neonatal intensive care unit (NICU) and subsequently (after 12 d) transferred to our pediatric unit for longer-term rearing (26 d). We assess brain function, structure and development at various levels and at all stages of

postnatal development. The following brain related endpoints are assessed for their suitability for further use in the preterm pig model. Functional: Basic motor functions (eye lid opening, first walk/stand), total activity in home cage, balance and coordination assessments, general and specific object exploratory interest and locomotion in an open field arena, longitudinal basic electro-cortical activity by electroencephalography (EEG) and cognition assessed by learning performance in a novel poke-reward test and short term-memory in a novel object recognition test. Gut microbiota analysis using MiSeq-based tag-encoded 16S rRNA gene targeted high throughput amplicon sequencing. Structural: blood brain barrier maturation, concentration and/or localization of neurotransmitters, receptors and trophic factors (e.g. brain derived neurotrophic factor (BDNF) and Sonic Hedgehog. We also apply a qPCR array of 84 neurogenesis-pathway related genes (targeting aspects related to neuronal migration, cell differentiation, synaptic functions, growth factors & cytokines, apoptosis, signal transduction and transcription factors) in selected brain regions in a subset of the piglets, followed by a more targeted approach using a high through put microfluidic q-PCR system on samples from all piglets. Brain weight and structural magnetic resonance imaging (volumetric analyses and diffusivity of large white matter tracts) and stereology for an ontogenetic description of brain development in preterm *vs.* term delivered piglets.

6. Expected results:

We will develop a preterm pig model with a high translational value that will be used in subsequent intervention studies. We expect this work to facilitate advancements in pediatric research within the field by providing a novel diet-sensitive model of brain development and maturation in weak newborns. An estimated 5 publications will result from WP 2.0.

7. Estimated time frame

Task	2013				2014				2015				2016				2017			
Planning, protocols	x	x	x																	
Study execution and sample collection		x	x	x		x														
Activity, locomotion, balance			x	x	x	x														
General movement analyses		x	x	x	x	x														
EEG brain analyses		x	x	x	x															
Cognition tests			x	x	x	x	x													
Blood brain barrier		x	x	x	x	x	x	x	x											
qPCR assay					x	x	x	x												
Sonic Hedgehog , BDNF					x	x														
MRI, volumetric, myelination	x		x		x	x		x												
Publication 1						x	x	x												
Publication 2+3						x	x	x	x	x	x	x								
Publication 4								x	x	x	x	x	x							
Publication 5+6										x	x	x	x	x	x					

8. Estimated budget from NEOMUNE: 2.6 mio DKK

Mainly derived from contributions from ARLA Food Ingredients and Danone allocated for 2013 and 2014.
Some co-financing from NEOMUNE base funds are expected (max 1.0 mio DKK)

9. Estimated budget from elsewhere:

2.0 mio DKK (co-financing from numerous collaborating institutions and partners, sample analyses etc.)

10. Additional comments:

- Development of the preterm pig brain model is a high-risk, high-priority NEOMUNE study part. Maximal effort is put into this project during the first 1½-2 years because it forms a foundation for later parts of NEOMUNE (e.g. WPs 2.3, 2.4 and some human studies).
- Refinement of the model continues beyond the initial test phase.