WP 2.0: Development of a preterm pig brain model

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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 3-4 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 piglets that are initially raised in our neonatal intensive care unit (NICU) and subsequently (after ~10 d) transferred to our pediatric unit for longer-term rearing (~25 d). We assess brain function, structure and development at various levels and

at all stages of postnatal development that are accessible to us. The following brain related endpoints are assessed for their suitability for further use in the final preterm pig model. Functional: Basic motor functions (eye lid opening, first walk/stand, development of suckling reflexes), total activity in home cage, general movement analysis (balance and coordination), locomotion, basic electro-cortical activity by electroencephalography (EEG), and cognitive performance (learning and memory in a novel visual delayed non-match to sample behavioral assay). Structural: neuronal proliferation, blood brain barrier maturation, microglia phenotyping and cytokine expressions, concentration and/or localization of neurotransmitters, receptors and trophic factors (e.g. serotonin (5-HT), N-methyl-D-aspartate (NMDA) receptor, brain derived neurotrophic factor (BDNF), and others) in different brain regions, brain weight and structural magnetic resonance imaging (volumetric analyses and myelination) and stereology (volume and surface area of cerebral cortex) in preterm *vs.* term delivered piglets. Standard gut analyses, including gut microbiota.

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 this field by providing a novel diet-sensitive model of brain development and maturation in weak newborns.

Predicted publications:

1) Feasibility of using preterm pigs as model for early brain development.
2) A visual delayed non-match to sample task for cognitive assessments in preterm and term pigs.
3) Brain prematurity in preterm and term pigs. Cognitive, functional and structural neurodevelopment and associations with early nutrition.
4) Longitudinal EEG and MRI in preterm and term piglets.
5) The gut microbiota in preterm and term pigs and associations with brain maturation.

7. Estimated time frame

Task	2013				2014				2015				2016				2017			
Planning, protocols	x	x	x																	
Study execution and sample collection		x	x	x																
Activity, locomotion, balance			x	x	x															
General movement analyses		x	x	x	x															
EEG brain analyses		x	x	x																
Cognition tests		x	x	x	x	x														
Neuronal proliferation/apoptosis			x	x	x															
Blood brain barrier		x	x																	
Cytokines, neurotransmitters (5-HT)					x	x	x													
NMDA receptor, BDNF growth factors					x	x	x													
MRI, volumetric, myelination	x		x		x															
Publication 1				x	x	x														
Publication 2					x	x	x	x												
Publication 3					x	x														
Publication 4+5					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.

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.