NEOMUNE News
April 2018

Milk:

Microbiota:

Immunity
Gut
Brain

NEOMUNE subgroup steering

Yanqi, Gorm
Infant feeding
WP 1.2c/1.3b/1.4/1.6a/1.7

Malene, Stine, Per
Steering Administration Communication

Yanqi
Piglet/mouse immunity and anti/probiotics
WP 2.1/2.2/3.1/3.2

Ninh, Hanne

Dennis, Gorm
Infant delivery, immunity and anti/probiotics
WP 1.1/1.2a/1.2b/1.3a/1.5/1.6b

Thomas, Per
Piglet feeding, gut & brain
WP 2.0/2.3/2.4
General information

This newsletter provides a brief description of work package (WP) status, publications, and publications in progress. For in depth descriptions of the 20 work packages, see the WP synopses at www.neomune.ku.dk.

The NEOMUNE project progresses according to plan. The practical work of most WPs is now complete and we are in the results publication phase. The project runs until end 2019 and until then the aim is to stimulate as many spin-off projects and collaborations as possible. This has already been successful in new projects on milk and microbiota interventions in early life (e.g. the NEOCOL, STIMMUNE, InfantBrain, Infant-I projects).

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NEOMUNE main results and status

WP 1: Infant studies

WP 1.1: Mode of delivery and gut microbiota in term infants
**Results:** Resemblance between the infant’s and the mother’s microbiota is influence by delivery mode with less resemblance after C-section compared to vaginal birth. Cessation of breast-feeding, rather than introduction of solid food is required for maturation into an adult-like microbiota and has a major impact on early microbiota composition and function (Bäckhed et al. 2015). **Status:** The WP was finalized in 2016. **Publications in progress:** Several publications based on follow-up on the cohort in preparation.

WP 1.2a: Breast-feeding and infections in term infants
**Results:** Increased breast feeding does not decrease the prevalence of infections in term infants within the first year of life. Differences between full breastfeeding and any breastfeeding remain to be analyzed. The project is near completion. A Ph.D. project will end March 1, 2017. The use of SMS-tracking has been successful with total data sets on of 900 families, data analysis and preparation of a manuscript is currently taking place. **Status:** Last data are being compiled from the Odense cohort, data collection and publications are submitted. **Publications in progress:** Review in J. Ped. Inf. Dis. (Infections and vitamin D); one manuscript submitted (infections and birth mode; one manuscript in preparation (Christensen et al. infections, breast-feeding, SMS track data)

WP 1.2b: Maternal antibiotics and term infant gut colonization
**Results:** 1. Antibiotics (cefuroxime) treatment of mothers prior to or after cesarean section has no effects on infant gut microbiota (publication in press). 2. The cefuroxime half-life is 3 times longer in newborn infants than in adults (Zachariassen et al. 2016). **Status:** Experiments and analyses are completed. **Publications in progress:** Very early life exposure to cefuroxime does not influence gut microbiota colonization and antibiotic resistance profiles (Zachariassen et al. in press)

WP 1.2c: Milk and immunity in children during chemotherapy
**Results:** 1) Toxic responses to chemotherapy have opposing effects on immune regulation via regulation of miRNAs. A pro-inflammatory miRNA profile is sustained during the first three weeks after allogeneic hematopoietic stem cell transplantation (Pontoppidan et al. 2015). 2) Chemotherapy increases the expression of genes related to innate immune functions involved in surveillance, protection, and homeostasis of mucosal surfaces in the developing intestine of pigs (Rathe et al. 2016B). 3) Bovine colostrum may reduce gut toxicity during chemotherapy in piglets by preserving intestinal function and reducing inflammation (Pontoppidan et al. 2015). 4) Chemotherapy induces diarrhea, growth deficits, and leukopenia in piglets. In these pigs, colostrum reduce intestinal permeability, tissue IL-8 levels and C-reactive protein while it increase intestinal villus height and activities of brush border enzymes (Shen et al. 2016A). 5) Chemotherapy to piglets induces diarrhea and weight loss and reduces white blood cells, hexose absorptive, plasma citrulline and organ weights and increases gut permeability and plasma C-reactive protein levels. Increasing bovine colostrum
supplementation to chemotherapy-treated piglets has no effects on the measured parameters. Only-BC pigs, however, have lower diarrhea severity toward the end of the experiment when intestinal toxicity was reduced (Shen et al. 2016B). 6) Chemotherapy reduces circulating levels of surfactant protein D and is associated more systemic inflammation on d 8-15 and elevated intestinal mucositis scores on d 15 (Rathe et al. 2016A). A review paper on pig models of chemotherapy-induced mucositis has been published (Sangild et al. 2017).

Status: All pig studies complete. Enrolment of children with leukaemia in Copenhagen and Odense was delayed but now complete, data analyses ongoing. PhD students Mathias Rathe and Rene Shen defended their theses. 

Publications in progress: Final publication will await completion of child recruitment (2018)

WP 1.3a/b: Probiotics/bioactive milk formula for term infants

Status: All 800 infants have been recruited in China (Nanjing, Shanghai, and Beijing). Follow-up will end in Aug 2017. Sample analysis has begun 2017 (when last blood samples were collected) and expected time for first publications is medio/end 2018.

WP 1.4a: Donor human milk to preterm infants

Results: Pasteurized donor milk and preterm formula, as supplemental feeding during the first 10 days of life, yield similar outcomes (sepsis or meningitis, necrotizing enterocolitis, or mortality) during the first 60 days in very low-birth-weight infants when own mother’s milk was insufficient (Corpeleijn et al. 2016). Status: The WP was finalized in 2016. Publications in progress: A follow-up publication on microbiota and neurodevelopmental outcomes is planned. Publication is expected to be submitted in 2018.

WP 1.4b: Database of feeding preterm infants

Results: Nutritional practices and associated clinical outcomes in very low-birth-weight infants vary markedly in hospitals around the world. This may be related to differences in clinical practice, traditions, and national recommendations. Status: The WP work was finalized in 2016. Some delay in publication phase, in part due to some central actors on maternity leave or in new job positions. Publication in progress: NeoNutriNet-NEOMUNE: Global comparison of feeding strategies for preterm infants, an observational cohort study (Waard et al., submitted).

WP 1.5: Probiotics, feeding and NEC in preterm infants

Results: A historically controlled study does not indicate significant effects of probiotics on NEC in preterm infants (Nielsen et al. 2016). Nasogastric feeding tubes from a neonatal department yield high concentrations of potentially pathogenic bacteria- even 1 d after insertion (Petersen et al. 2016a). NEC diagnosis and surgery are highly variable (Petersen et al., 2017). Status: The WP is complete. PhD student Sandra Meininch has defended PhD thesis in 2017. Publications in progress: Planned follow-up publication combined with WP1.4b.

WP 1.6a: Minimal enteral colostrum for preterm infants

Results: Bovine colostrum supplementation is well tolerated and raises enteral protein intake markedly in very premature infants during the first week of life. Status: Phase A and B completed. Laboratory analysis for phase C continues (microbiomics and metabolomics on fecal samples) and the WP terminates in mid 2018. Patent

WP 1.6b: Probiotics for preterm infants. WP cancelled (due to new published results on probiotics for infants and lack of funding for the re-prioritized NEOMUNE WP)

WP 1.7: Ethical, social and cultural processes of translational research
Results: Social, ethical and cultural aspects of translating animal based research into the neonatal intensive care units have been explored in relation to cultural understandings and interdisciplinary collaboration. Through ethnographic methods and collaborations with basic and clinical scientists in NEOMUNE this work has resulted in submission of three publications. In the first publication, “Treating pigs: Balancing standardization and individual treatments in translational neonatology research”, we develop the notion of ‘patientising’ to capture the way researchers strive to create a clinically relevant pig model balancing traditional scientific norms of standardization against requests for a more individual approach that mimics the way premature infants are cared for (Dam et al., 2016). The second publication, “Feeding premature neonates: Kinship and species in translational neonatology” (Dam et al., 2017) offers important new insight into the ‘species flexibility’ of premature beings and the way perceptions of feeding and kinship profoundly shape the translational processes in the field of neonatology research. The third publication, “Translational Neonatology Research: Transformative encounters across species and disciplines” (Dam et al., 2018), unfolds every day collaborations in the preterm pig laboratory and argues that transformative cross-fertilizations across species and disciplines in translational research facilitate the day-to-day survival of piglets, the academic survival of scientists, and (the promise of) survival of preterm infants.

Status: Finalized. Publications: Se above.

WP 2: Pig studies

WP 2.0: Development of a preterm pig brain model
Results: 1) Preterm pigs show delayed neonatal arousal and impaired physical activity, coordination, exploration, and learning, relative to term pigs. Supplementation of parenteral nutrition in the first 5 days with an enteral milk diet does not affect later outcomes. In preterm pigs, many physiological characteristics of immaturity disappear by week 4, while some neurodevelopmental deficits remains (Andersen et al. 2016, Hansen et al. 2016). 2) Preterm pigs show decreased physical activity compared to term pigs, and the first enteral diet stimulate both gut growth and physical activity, where bovine colostrum and human milk is superior to formula (Cao et al. 2015). 3) Limited effects of preterm birth and the first enteral nutrition on cerebellum morphology and gene expression in piglets (Bergström et al. 2016). 4) Decreased physical activity precedes the clinical symptoms of NEC in the small intestine of preterm pigs, and increased gastric residuals predict NEC lesions in the colon (Cao et al., 2016). 5) Relative to near-term and term pigs, newborn preterm pigs have low blood leukocyte counts, poor neutrophil phagocytic rate, and limited cytokine responses to TLR1/2/5/7/9 and NOD1/2 agonists. After a week blood leukocyte numbers, NK cell proportion, neutrophil phagocytic rate and TLR2-mediated IL-6 and TNF-α production increase. However immune parameters remained different between preterm and near-term pigs for 2-3 weeks (Nguyen et al. 2016B).
**Status:** The WP practical and analytical work has been finalized in 2016. Several manuscripts are still in the publication process. We have had great out from several experiments that allowed us better to see how gut, immunity and brain development occur in preterm pigs, independently and together. This work is considered crucial for the future work of the preterm pig model.

**Publications in progress/submitted:** Cerebrospinal fluid samples from preterm pigs with NEC induce higher neurotrophic effects on primary hippocampal neurons (in vitro) compared to non-NEC samples ([Pankratova et al. manuscript in prep.](#)). Motor function and cerebellar gene expression in preterm pigs ([Bergström et al. manuscript in prep.](#)). Characterization of the gut microbiota in early life. ([Kamal et al. manus in prep.](#)). Epigenetic profiling of samples from the middle intestine in preterm and term pigs show different adaptive and innate immune regulation ([Pan et al. manuscript in prep.](#)) Neocortical development in a pig model of preterm birth ([Palner et al. submitted](#)).

**WP 2.1: Germ-free birth in preterm pigs** (The WP was cancelled early in NEOMUNE. This allowed expansion of WP 2.0, 2.3 and 2.4 with additional studies. Germ-free studies was considered of reduced importance, partly due to the cancelled probiotics studies in infants, WP 1.6b)

**WP 2.2: Anti- and probiotics in preterm and term pigs**

**Results:** 1) Enteral, but not parenteral, administration of antibiotics reduces gut colonization, inflammation, and prevents NEC lesions in formula-fed preterm pigs. Enteral administration of antibiotics further enhances blood neutrophil maturation and reduces gut permeability and Gram-positive bacteria ([Birck et al. 2016](#)). 2) Oral antibiotics may benefit mucosal and systemic immunity via delayed gut colonization and enhanced blood neutrophil maturation just after preterm birth ([Nguyen et al. 2016A](#)). 3) Feeding bovine colostrum rather than formula to 23 d old pigs reduces gut colonization by pathogenic E. coli and modulates the intestinal immune system, whereas no difference is observed in piglets fed bovine colostrum and conventionally reared by the sows ([Sugiharto et al. 2015](#)). 4) A challenge with pathogen E. coli F18 to newborn term pigs increased the occurrence of diarrhea. Co-inoculation with either of two probiotic strains further increases the diarrhea score and show either no or adverse effects on body growth, intestinal structure, function and permeability ([Andersen et al. in press](#)).

**Status:** The WP will be finalized in 2017. **Publications in progress:** Transplantation of fecal microbiota from healthy 3d old term pigs to newborn preterm pigs protects against NEC, but increases mortality ([Brunse et al. Manuscript in prep.](#)). Fecal microbiota transplantation (FMT) to prevent bacterial dyscolonization and necrotizing enterocolitis in preterm neonates ([Brunse et al. expected submission in spring 2017](#)). Antibiotics and probiotics to term pigs do not show lasting effects on gut parameters ([Poulsen et al. Manuscript in prep.](#)). Anti/probiotics to term newborn pigs. Probiotics administration to the sow enriches the neonatal pigs, but does not influence the measured endpoints related to GI function. Likewise, a 3-day enteral antibiotics administration during the first postnatal week, did not show lasting effects on the gut microbiota up to day 42 after birth. Ann-Sofie Riis Poulsen has defended her PhD and a **manuscript is in preparation**

**WP 2.3 Enteral and parenteral feeding for preterm pigs**
Results: 1) Early gradual formula feeding induces intestinal dysfunction and NEC lesions, whereas bovine colostrum supports gut maturation during the first week after preterm birth (Shen et al. 2015). 2) Intestinal structures show growth adaptation the first week after preterm birth but some digestive functions remain immature until at least week 3-4, especially without enteral nutrition during first 5 days. (Hansen et al. 2016A). 3) Enteral feeding, particular with formula, induces subclinical inflammation in the premature intestine and a more open chromatin structure in key inflammatory genes (Willems et al. 2015). 4) Formula-feeding causes epigenetic down-regulation of genes associated with intestinal metabolism. Pre- and postnatal changes in intestinal DNA methylation may contribute to high NEC sensitivity in preterm neonates which may be changed via environmental stimuli (e.g. diet, nutrition, microbiota) (Gao et al. 2014). 5) Preterm birth predisposes to NEC and reduces nutrient absorption but does not induce up-regulation of immune-related genes or cause bacterial dyscolonization in pigs (Østergaard et al. 2015). 6) Provision of amniotic fluid during parenteral nutrition increases weight gain but has limited effects on gut structure, function, immunity, and microbiology in newborn preterm pigs (Østergaard et al. 2016).

Status: The WP terminates ultimo 2017. The in vivo part of the TPN-lipid study (CNRC, Fresenius Kabi, Copenhagen) has been conducted and laboratory analysis are ongoing. Publications in progress:

Preterm birth, leading to immature GIT structure and function, influences the gut microbiota (GM) in pigs at least until 26 days of life. The feeding regimes (parenteral nutrition alone or followed by enteral nutrition) affect the GM short-term, but has no lasting GM effects (Kamal et al. manuscript in prep.). Early enteral feeding promotes bacterial clearance and protects against meningitis in preterm pigs with Staphylococcus epidermidis induced neonatal sepsis (Brunse et al. Manuscript in prep.). Early enteral feeding also ameliorates sepsis-induced blood-CSF barrier disruption and alters the brain innate immune response to SE infection (Brunse et al. Manuscript in prep.).

WP 2.4: Bioactive formula diets for preterm pigs

Results: 1) Following activation of growth factors, low-heat-treated whey protein concentrate (WPC) induces higher IL-8 levels in intestinal epithelial cells (IEC). Higher levels of bioactive proteins in low-heat-treated WPC may enhance proliferation and cytokine responses of IEC (Nguyen et al. 2016D). 2) Spray drying and pasteurization affects bovine colostrum proteins, but do not reduce the trophic and anti-inflammatory effects on the immature pig intestine (Støy et al. 2016). 3) Bovine colostrum, and to some degree donor human milk, are superior to infant formula in stimulating gut maturation and body growth, using a gradual advancement of enteral feeding volume over the first 11 days after preterm birth in piglets (Rasmussen et al 2016). 4) The maturational and protective effects on the immature pig intestine decreases in the order bovine colostrum > bovine milk > whole milk powder, but all three intact bovine milk diets are markedly better than formula (Li et al. 2014). 5) Bovine colostrum restores intestinal function after initial formula-induced inflammation in preterm pigs (Støy et al. 2014). 6) TGF-β2 of dietary or endogenous origin may regulate the intestinal epithelial cell responses against LPS stimuli, thereby supporting cellular homeostasis and innate immunity in response to bacterial colonization and the first enteral feeding in early life (Nguyen et al. 2015). 7) Low doses of bovine lactoferrin (bLF) to porcine intestinal epithelial cells up-regulate proteins associated with glycolysis, energy metabolism and protein synthesis, indicating support of cell survival. In contrast, high doses bLF up-regulate apoptosis-inducing proteins, down-regulate anti-apoptotic and proliferation-inducing proteins and proteins
related to energy and amino acid metabolism (Nguyen et al. 2016C). 8) Higher levels of bioactive proteins in low-heat-treated WPC, especially from acid whey, may enhance proliferation and cytokine responses of porcine intestinal epithelial cells (Nguyen et al. 2016D, Li et al., 2017)

**Status:** The WP was finalized in 2016. Funding for additional studies was obtained from ARLA Foods Ingredients and Danone/Nutricia. One patent application was approved in 2016. **Publications in progress:** Two full long-term studies including bioactive components from AFI and Danone, and with focus ion brain endpoints, have been conducted. They are currently in the laboratory/manuscript phase (AFI study) and manuscript submission phase (Danone study). We expect to publish three manuscripts based on these data in 2018.

**WP 3: Mice studies**

**WP 3.1: Antibiotics in newborn mice**

**Results:** Oral antibiotics during pregnancy impair diversity of colonizing bacteria and decrease hematopoiesis in the mice pups. **Status:** WP is finalized in 2016. **Publications in progress:** Antibiotic treatment during pregnancy affects myelopoiesis and T cell development in newborn mice (Fuglsang et al.). Antibiotic treatment of mice influences their defense towards influenza infection but only during antibiotic treatment (Fuglsang et al.). Influenza affects microbiota of mice treated with antibiotics differently from non-treated mice (Fuglsang et al).

**WP 3.2: Probiotics in newborn mice**

**Status:** The study has been performed and data is being processed.

**WP 4: Administration, meetings and education**

**Administration and Steering:**
The Administration group meets daily/weekly in Copenhagen. Management group (MG) meetings have been held 4-7 times per year during the project period (4 meetings in 2017). Governing board (GB) meetings have been held once annually during the project period and twice in 2017 (February, October)

**NEOMUNE research meetings:**
Scientific research meetings have been held several times annually during the project period, both in Denmark and internationally. In 2017 two larger meetings were held in Copenhagen (February, August) with large international attendance. Two larger meetings were also held in China (April, Nov, Shenzhen, Guangzhou).

**Education:**
The annual NEOMUNE PhD course “Food, health and philosophy in East and West” was completed April 2017. The next course will be 5-14 April in Hong Kong, Shenzhen and Guangzhou.

**NEOMUNE has been fully or partly responsible for completion of the following PhD studies:**
Afrouz Abbaspour (WP 2.0, Karolinska Institute, SE, finishes 2018)
Anders Brunse (WP2.0; 2.2; 2.3, Copenhagen University, DK, finished 2017
Anne Mette Plomgaard (WP 2.0, Rigshospitalet, DK, finished 2016)
Ann-Sofie Riis Poulsen (WP2.2, Aarhus University, DK, finished 2016)
Eva Fuglsang (WP 3.1; 3.2, Copenhagen University, DK) finalized 2017)
Publications fully or partly sponsored by NEOMUNE


