Abstract Preview - Step 3/4

- print version -

Topic: Basic science

Title: Preterm birth, early nutrition and gut microbiota affect intestinal immunity via DNA methylation

Author(s): Xiaoyu Pan[1], Fei Gao[1], Thomas Thymann[1], Per Sangild[1]

Organisation(s): [1]University of Copenhagen, Department of Veterinary and Animal Sciences, Copenhagen, Denmark

Text:

Objectives and Study:
DNA methylation plays an important role for normal organ development and for adaptation to adverse conditions, resulting in altered gene transcription and cellular functions. Consequently, preterm birth and postnatal exposure to milk and microbes may affect intestinal development via DNA methylation. Using pigs as models for infants, we investigated the intestinal DNA methylome in response to shortened gestational age at birth, the first feeding and bacterial colonization, to understand how the immature intestine adapts to birth, diet and bacteria just after preterm delivery.

Method:
The intestinal DNA methylome was characterized by reduced representation bisulfite sequencing of the middle small intestine from caesarean-delivered preterm or term pigs. In study 1, preterm or term pigs were euthanized shortly after birth or fed either total parenteral nutrition (TPN) or minimal enteral nutrition (MEN with bovine colostrum, COL) for five days. In study 2, preterm pigs were fed COL or infant formula (FOR) for five days. In study 3, preterm pigs were fed FOR for five days, with or without oral antibiotics to delay bacterial colonization.

Results:
In study 1, the newborn preterm intestines showed genome-scale hypermethylation (relative to term), affecting genes related to innate immunity (LBP, lipopolysaccharide binding protein) and glucocorticoid signaling (NR3C1, glucocorticoid receptor). Promoter hypermethylation of LBP in preterm intestines down-regulated gene expression at birth and day 5, and it was associated with impaired LPS tolerance, regardless of feeding regimen (TPN or MEN). In study 2, FOR feeding increased intestinal LBP expression, relative to COL feeding, and increased bacterial epithelial adherence, complement protein (C3) and hypoxia-inducible factor 1-alpha (HIF1A). This was associated with LBP gene promoter hypomethylation. In study 3, antibiotics treatment reduced intestinal bacterial density, incidence of necrotizing enterocolitis (NEC) and the expression of HIF1A and C3. These changes were associated with promoter hypomethylation of a C3 inhibitor.

Conclusion:
Gestational age at birth (preterm or term), the first feeding and gut bacterial colonization induce intestinal DNA methylation changes. These changes may be associated with impaired or altered responsiveness to critical factors just after preterm birth (e.g. glucocorticoids, gram-negative bacteria), potentially explaining poor intestinal adaptation in preterm neonates. Even slow introduction of infant formula may induce bacterial dyscolonization and molecular changes that predispose preterm neonates to intestinal inflammation. Delaying bacterial colonization with antibiotics ameliorated intestinal inflammation and hypoxic stress, which may partly be mediated by DNA methylation changes. It remains to be investigated if the neonatal changes in intestinal DNA methylation have long term effects for intestinal development and health in preterm neonates.

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Conference: 51st ESPGHAN Annual Meeting · Abstract: A-968-0002-00321 · **Status: Submitted**

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Abstract Preview - Step 3/4

- print version -

Topic: Neonatal and infant nutrition

Title: Nutrient fortification improves postnatal growth without effects on neurodevelopment in preterm pigs

Author(s): Agnethe May Ahnfeldt[1], Stine Brandt Bering[1], Line Møller Willumsen[1], Ester Arévalo Sureda[2], Tilla Busk-Anderson[1], Anne Ruge[1], Susanne Søndergaard Kappel[1,3], Ole Bæk[1], Karina Obelitz-Ryom[1], Charlotte Holme Nielsen[1], Thomas Thymann[1], Per Torp Sangild[1]

Organisation(s): *[1]University of Copenhagen, Department of Comparative Pediatrics and Nutrition, Frederiksberg, Denmark, [2]Lund University, Department of Biology, Lund, Sweden, [3]Rigshospitalet, Department of Neonatology, Copenhagen, Denmark*

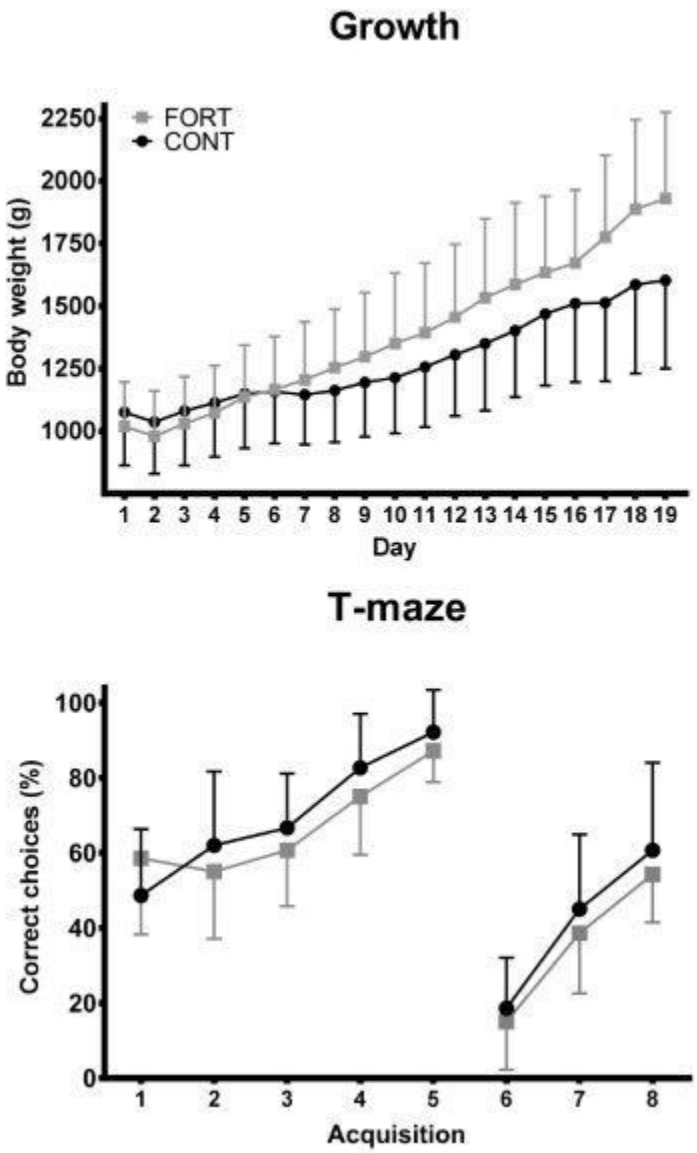
Text:

Objectives and study:
Relatively high nutrient and energy intakes are recommended in preterm infants to achieve a postnatal growth rate similar to corresponding intrauterine growth rates. These recommendations are based on studies associating poor postnatal growth with negative long-term outcomes, including poor neurodevelopment. Recommended amounts of nutrients, especially protein, cannot be met alone by feeding mother's own milk or donor human milk. Nutrient fortification of human milk is therefore required to support growth and development in preterm infants. Conversely, excessive enteral nutrient intake may induce gut dysfunction and feeding intolerance. Using preterm pigs as a model, we investigated if nutrient fortification with bovine colostrum, which contains high levels of proteins and bioactive components, could improve growth, organ development and brain function within the first three weeks after preterm birth.

Method:
Preterm pigs (90% gestation, n = 31) from two sows were block randomized into two groups. In a blinded setting, they were fed increasing enteral volumes (32-180 mL/kg/d) of bovine milk (CONT, n = 16) or bovine milk fortified with colostrum (FORT, n = 15) for 19 d. Protein intakes were 3-5 and 3-10 g/kg/d in CONT and FORT groups respectively. Cognitive function was assessed in a spatial T-maze system, testing memory and learning at 13-18 d. At 19 d, blood biochemistry values and weights of all internal organs were recorded to assess how nutrient fortification affected body and organ development.

Results:
Preterm pigs receiving the FORT diet grew faster than CONT pigs (32 vs. 19 g/kg/d, $P < 0.01$, Figure), thus preventing extra-uterine growth restriction. Improved growth was accompanied with increased levels of creatinine kinase, phosphate and aspartate aminotransferase and decreased level of iron (all $P < 0.05$). Among organs, the FORT diet increased stomach, intestine, colon, liver, spleen, kidney and heart weights (20-60%, all $P < 0.05$) at 19 d. Expressed relative to body weight (g/kg), intestinal length and weight, liver and spleen were affected (10-50% gain, $P < 0.05$). The FORT diet tended to increase absolute weights of some brain regions (cerebellum, cerebrum, brain stem, $P < 0.15$), but relative brain weight was lower in FORT vs. CONT pigs (19 vs. 23 g/kg, $P < 0.01$). Memory and learning were identical between the FORT and CONT preterm pigs, as tested in a spatial T-maze test showing identical group performances in two different settings, a basic and reverse test phase (see Figure).

Conclusion:
Growth restriction during the first 3 weeks after preterm birth was prevented by nutrient fortification with a tendency to improved brain growth but without changes in functional neurodevelopmental outcomes in pigs. This indicates pronounced brain sparing effects in nutrient-restricted preterm neonates, at least short-term. Conversely, fortification had marked effects on liver and spleen growth, potentially stimulating metabolic and immune development. Long-term effects of nutrient fortification in early life remain to be better investigated to balance the risk and benefits of high nutrient intake and rapid growth in preterm infants.



[Growth and cognition performance in control and nutrient-fortified preterm pigs]

Conference: 51st ESPGHAN Annual Meeting · Abstract: A-968-0020-00749 · **Status: Draft**

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Abstract Preview - Step 3/4

- print version -

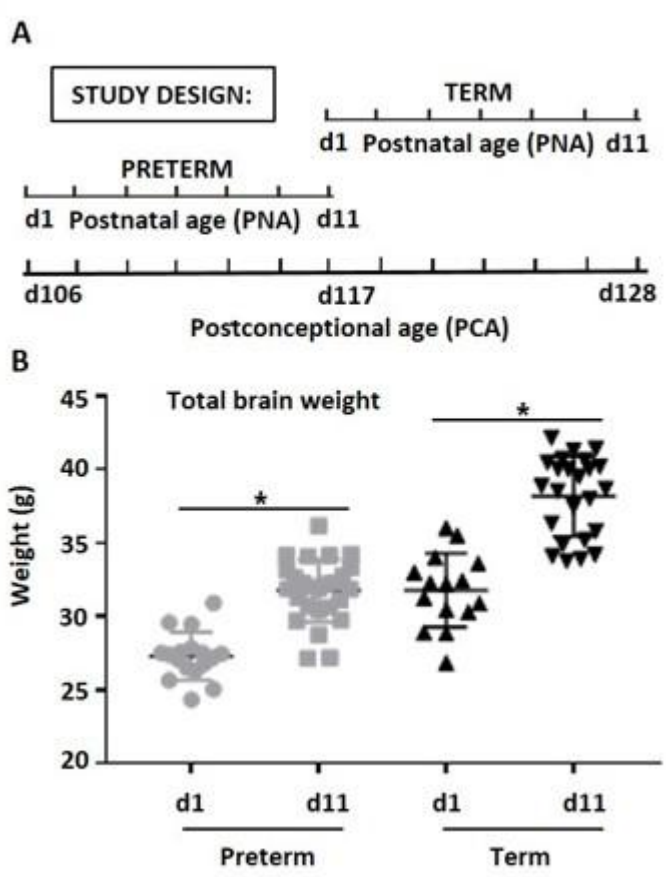
Topic: Basic science

Title: Brain development in preterm and term pigs in response to identical nutrition after birth

Author(s): Charlotte Holme Nielsen[1], Anne Bladt Brandt[1], Karina Obelitz-Ryom[1], Stanislava Pankratova[1], Charlotte Vanden Hole[2], Chris Van Ginneken[2], Per Torp Sangild[1], Stine Brandt Bering[1], Thomas Thymann[1]

Organisation(s): *[1]University of Copenhagen, Comparative Paediatrics and Nutrition, Frederiksberg, Denmark, [2]University of Antwerp, Department of Veterinary Sciences, Wilrijk, Belgium*

Text: **Objectives and Study:** Preterm birth interrupts the intrauterine maturation of organs and may impair the normal brain development in the postnatal period. The brain develops rapidly in the perinatal period and may or may not be highly susceptible to environmental changes such as birth, feeding and microbial colonization. Prematurity in pigs is associated with neurodevelopmental deficits, but it is unclear how these deficits may be determined by postnatal age (PNA), postconceptional age (PCA) and environmental factors. Using preterm pigs as a model for preterm infants, we hypothesized that preterm birth impairs structural and functional brain development after birth, although mainly influenced by PCA.
Method: Pigs were derived by cesarean section at 90% gestation (PRETERM, n=43) or full term (TERM, n=41) and randomized to groups euthanized either at birth (d1) or after 11d (n=17-24 per group) (Fig. 1A). Clinical, para-clinical and brain outcomes were measured at d1 and d11. Functional brain outcomes included basic motor skills (first eyelid opening, first stand and first walk), independent drinking, balance and walking coordination (gait analysis). Structural brain outcomes included wet weight of the brain and selected brain regions, permeability of the blood-brain barrier and biochemistry of blood and cerebrospinal fluid (CSF).



*[Fig. 1. A: Study design, postconceptional vs. postnatal ages. B: Total brain weight. *P<0.05.]*

Results: Term pigs showed higher weight gain than preterm pigs, despite identical nutrient intakes (+30%, $p < 0.05$). Comparison of preterm and term pigs at the same PCA showed no difference in absolute or relative weights of the entire brain (Fig. 1B) and brain regions between d11 preterm and d1 term pigs. Only striatum weight was higher in d11 preterm versus d1 term pigs ($p < 0.05$). The brain hydration level was higher in preterm versus term pigs at both ages ($p < 0.05$). At PNA d11, weights of the entire brain and cerebrum, cerebellum and brain stem were higher in term pigs relative to preterm pigs. For hippocampus, preterm pigs had lower weights than term pigs at d1 but not at d11 ($p < 0.05$), indicating a postnatal catch-up growth of the preterm hippocampus. Lactate concentration in plasma and CSF of preterm pigs mimicked that in term pigs, whereas glucose levels were lower at both ages. The blood-brain barrier permeability (measured by raffinose levels in CSF after an i.v. bolus) was highest in preterm pigs ($p < 0.01$). Finally, preterm pigs showed a delay in time to learn to drink and required longer time for first eyelid opening, first stand and first walk. In the gait analysis, term pigs had longer normalized stride and step length, while preterm pigs had higher normalized maximum stride height.
Conclusion: The lack of difference in brain weights between d11 preterm and d1 term pigs indicates that PCA, rather than environmental factors and PNA, is the main determinant of brain growth. The functional tests confirmed that preterm pigs showed impaired motor coordination, indicating reduced neuro-motoric maturation. Despite the delays in brain development in preterm pigs, postnatal maturation occurred rapidly with PNA, with some parameters reaching that of term newborns at the corresponding PNA (d11). The developing brain of both preterm and term newborns may be relatively resilient to environmental stimuli such as birth, nutrition and microbial colonization.

Conference: 51st ESPGHAN Annual Meeting · Abstract: A-968-0017-00356 · **Status: Draft**

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Abstract Preview - Step 3/4

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Topic: Basic science

Title: Prenatal endotoxin exposure induces postnatal systemic inflammation but does not increase diet-induced necrotizing enterocolitis in preterm pigs

Author(s): Duc Ninh Nguyen[1], Shuqiang Ren[1], Thomas Thymann[1], Sandra Goericke-Pesch[2], Wei Wei[1], Anders Brunse[1], Boris Kramer[3], Tim Wolfs[3], Per Sangild[1]

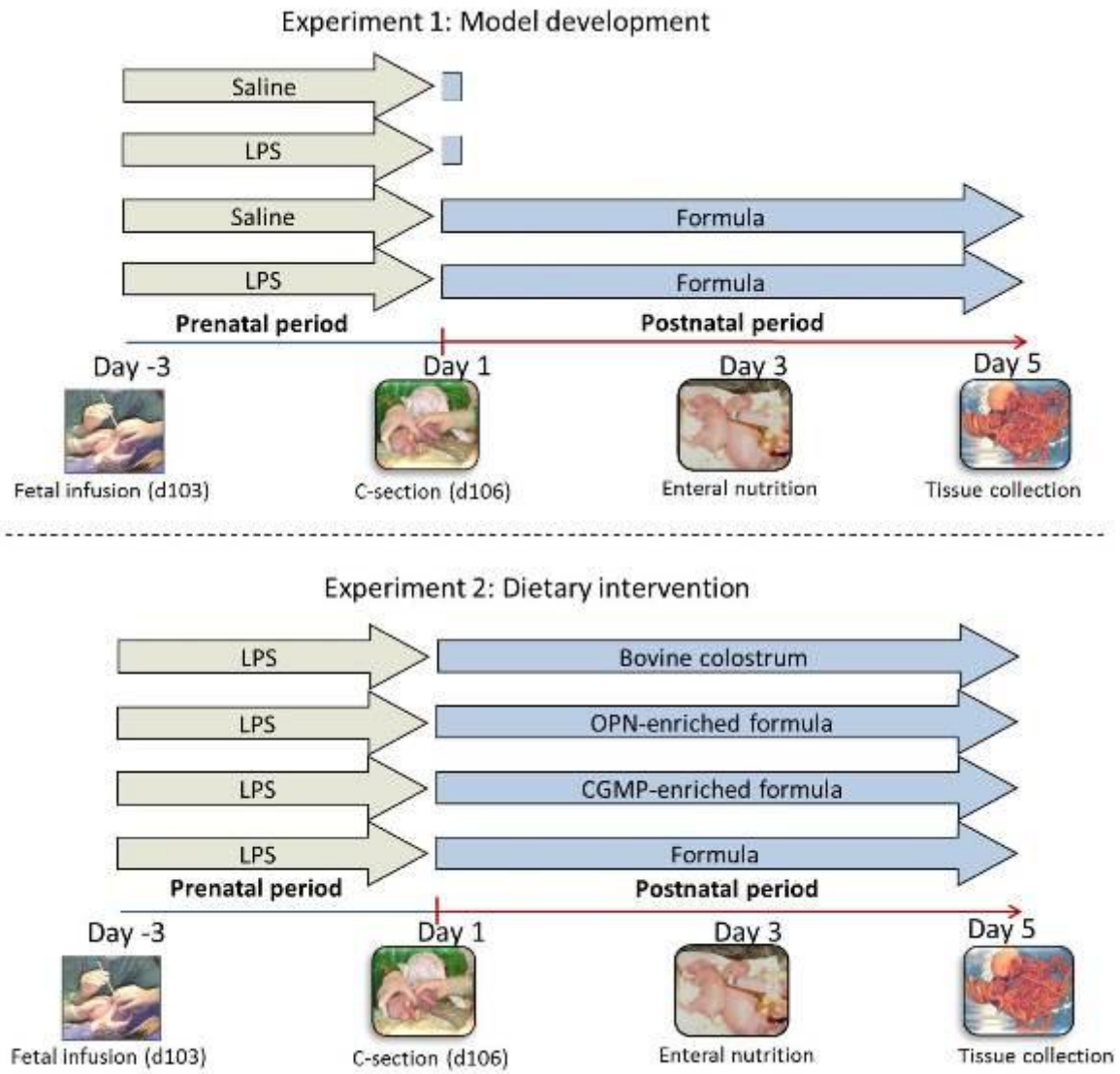
Organisation(s): [1]University of Copenhagen, Veterinary and Animal Sciences, Frederiksberg, Denmark, [2]University of Copenhagen, Veterinary Clinical Sciences, Frederiksberg, Denmark, [3]Maastricht University, Department of Pediatrics, Maastricht, Netherlands

Text: **Objectives and Study:** Prenatal inflammation is a major risk factor for preterm birth but it remains unclear how it affects the postnatal immune system and diet-induced gut inflammation. Using newborn preterm pigs as a model for preterm infants, we investigated the systemic immune status and the gut sensitivity to necrotizing enterocolitis (NEC) following fetal exposure to lipopolysaccharide (LPS) and after feeding different enteral milk diets just after preterm birth.

Method: At 103 d (88%) of gestation, fetal pigs received an intra-amniotic dose of LPS (1 mg/fetus, n=81) or control saline/no injection (CON, n=32). Preterm pigs were delivered by caesarean section three days later, euthanized immediately or fed for five days with formula (FORM, NEC-sensitive diet), bovine colostrum (COL, NEC-protective diet), or formula enriched with bovine milk caseinoglycomacropeptide (CGMP, CGMP-10, Arla Foods Ingredient, AFI) or ostepontin (OPN, OPN-10, AFI, n=10-15/group). Fetal membranes, amniotic fluids, blood and gut tissues were collected for analysis at birth and on day 5.

Results: At birth, intra-amniotic LPS induced histological chorioamnionitis, intra-amniotic inflammation (increased leukocyte counts and inflammatory cytokines) and a fetal systemic inflammatory response (increased cord blood neutrophils with impaired phagocytic function). On day 5, LPS pigs had systemic inflammation with elevated blood leukocytes and cytokines, and reduced serum albumin and cholesterol levels. At birth, the fetal intestine showed increased inflammatory cytokine levels and decreased villus height following LPS exposure, while LPS effects on gut parameters (cytokines, digestive functions, NEC sensitivity) were largely absent on day 5. COL feeding to LPS pigs reduced diarrhea incidence (40 vs. 91%), colon NEC (0 vs. 36%), gut inflammatory cytokines, and increased gut lactase activity, distal intestinal and colon goblet cell density and serum cholesterol and ion levels, relative to FORM. COL feeding to LPS pigs also reduced $CD4^+$ expression in helper-T cells of mesenteric lymph nodes, and tended to increase blood T-helper cell frequencies and to decrease blood neutrophil phagocytic capacity, relative to FORM. CGMP and OPN supplementation to LPS pigs increased gut lactase activity and tended to reduce colon NEC incidence, relative to FORM. Neither CGMP nor OPN supplementation affected systemic immune parameters.

Conclusion: Fetal exposure to endotoxin predisposes preterm pigs to postnatal systemic inflammation but does not increase NEC sensitivity. Immunomodulatory milk diets may reduce gut inflammation in LPS-exposed preterm pigs but have limited short-term effects on systemic immunity. The postnatal milk diet is the most critical factor protecting preterm neonates from gut inflammation, regardless of exposure to endotoxin before birth.



[Figure 1: Experimental design]

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Abstract Preview - Step 3/4

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Topic: GI motility, GERD and functional GI disorders

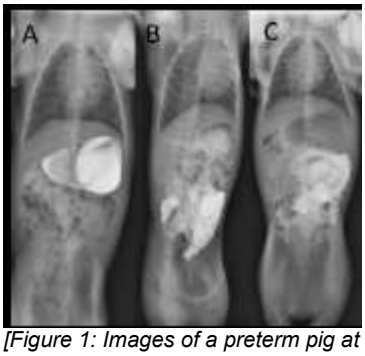
Title: Nutrient fortification of milk prolongs intestinal transit time without effects on gastric emptying rate in preterm piglets

Author(s): Susanne Søndergaard Kappel[1,2], Christel Renée Friborg[2], Agnethe May Ahnfeldt[1], Magdalena Gormsen[3], Per Torp Sangild[1,2], Lise Aunsholt[2]

Organisation(s): *[1]University of Copenhagen, Comparative Pediatrics and Nutrition, Frederiksberg, Denmark, [2]University hospital of Copenhagen, Rigshospitalet, Department of Neonatology, Copenhagen, Denmark, [3]University hospital of Copenhagen, Rigshospitalet, Department of Radiology, Copenhagen, Denmark*

Text: **Objectives and Study:** Human milk fortifiers (HMFs) are commonly added to mother's own milk (MM) and donor human milk (DM) to secure optimal growth of preterm infants. When to initiate fortification is unclear and there are concerns of feeding intolerance defined by eg. delayed stomach emptying as well as constipation, abdominal distension and necrotizing enterocolitis (NEC) after adding HMF. We used preterm piglets as a model for preterm infants to investigate how nutrient fortification of bovine milk with protein-rich bovine colostrum, would affect gut motility the first weeks after birth.
Method: Piglets were delivered by cesarean section at 90% gestation and block randomized into two groups fed either increasing volumes of dilute bovine milk (CONT, n=22), or dilute bovine milk fortified with bovine colostrum powder (FORT, n=16) for 19 days. Total protein contents were 27g/L (CONT) and 55 g/L (FORT). Food passage time was evaluated by stomach-intestine-colon contrast examination using serial x-ray imaging (***Figure 1**: The stomach filled with contrast (A). The contrast has reached caecum (B). Contrast in colon and rectum and a nearly empty small intestine (C))* on days 4 and 18 (10-360 min after a meal containing contrast solution: 4 ml/kg Iodixnol, Visipaque, GE Healthcare, Brøndby Denmark).

Results: On day 4, at enteral feeding volumes of 96 mL/kg/d, the stomach emptying time, intestinal transit time, and time to reach from caecum to rectum, were similar between CONT and FORT. However, on day 18, with enteral feeding volumes of 180 ml/kg/d, intestinal transit time were longer in FORT (2.69 ± 0.31 hours) piglets relative to CONT (1.57 ± 0.16 hours)($p < 0.05$), while stomach emptying time, and colon transit time, were similar between groups.
Conclusion: Results indicate that fortification of milk with bovine colostrum prolonged intestinal transit time on day 18 without effects on gastric emptying or colon transit time. Fortification with bovine colostrum will unlikely increase feeding intolerance defined by eg. stomach emptying in preterm neonates, and prolonged intestinal transit time may allow more time for nutrient absorption. Examination of food transit time by stomach-intestine-colon x-ray contrast examinations is a feasible method to investigate factors influencing gut motility and feeding intolerance in preterm neonates.



[Figure 1: Images of a preterm pig at day 18]

Conference: 51st ESPGHAN Annual Meeting · Abstract: A-968-0009-00765 · **Status: Draft**

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Abstract Preview - Step 3/4

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Topic: Basic science

Title: Preterm birth induces persistent delay in gut and immune maturation in the postnatal period of pigs

Author(s): Shuqiang Ren[1], Duc Ninh Nguyen[1], Anne Bladt Brandt[1], Thomas Thymann[1], Per Torp Sangild[1,2]

Organisation(s): *[1]Comparative Pediatrics and Nutrition, University of Copenhagen, Department of Veterinary and Animal Science, Frederiksberg C, Denmark, [2]Copenhagen University Hospital, Department of Pediatrics and Adolescent Medicine, Copenhagen, Denmark*

Text: **Objectives and Study:** The adaptation and maturation of immature organs following preterm birth may be associated with increased risks of infection and organ dysfunctions. Using preterm pigs as a model for preterm infants, we compared the maturational trajectory of gut functions and systemic immunity between preterm and term neonates. We hypothesized that these maturations depend more on changes in the external environment (e.g. birth, nutrition, microbes, postnatal age, PNA) than on age after conception (post-conceptional age, PCA).
Method: Pigs were delivered by Caesarean section at d 106 (preterm, n=43) and d 116 of gestation (term, n=41) and euthanized for tissue collection at birth (d 1) or d 11. Postnatally, pigs were fed the same amount (per kg) of parenteral nutrition and enteral nutrition (gradual transition from bovine colostrum to mature milk). Values were compared between preterm and term pigs both at the same PNA (d 1 and 11) and PCA (preterm d 11 vs. term d 1).
Results: At the same PCA, all investigated organs, except the brain, showed different levels of maturation between preterm and term pigs. For instance, preterm pigs on d 11 showed greater relative gut weight, gut cytokine IL-1β levels, sucrose and maltase activities but shorter villus height, lower goblet cell density and lactase activities than term pigs on d 1. Similarly, preterm pigs on d 11 showed greater levels of blood leukocytes, neutrophils and neutrophil phagocytic function but lower levels of blood cytotoxic T cells and NK cells than term pigs on d 1. In contrast, brain weight was closely related to PCA, with weights being identical between d 11 preterm and term newborn pigs. When comparing preterm and term pigs at the same PNA, preterm pigs showed delayed maturation shown by multiple parameters including lower growth rate, cord plasma cortisol and cortisol response to ACTH. Relative gut and brain weight, villus height, gut I-FABP levels and disaccharidase activities increased with advancing PNA but remained at lower levels in preterm than term pigs on d 1 and/or d 11. Gut permeability decreased over time but remained higher in preterm than term pigs at both PNA. Blood leukocyte and neutrophil numbers were lower in newborn preterm vs. term pigs, but increased to similar levels between groups by d 11. In contrast, the proportion of blood NK cells and cytotoxic T cells were lower in preterm than term pigs on both d 1 and 11.
Conclusion: Gut and immune parameters were immature in preterm vs. term pigs at birth and did not develop according to PCA. Some parameters showed a persistent delay in structural and functional maturation in preterm animals, relative to term animals at the same PNA. Our data suggest a highly distinct trajectory of gut and immune development during the first weeks of life in preterm neonates. Factors related to the birth transition and environment (e.g. nutrition, microbes) may determine gut and immune development in preterm neonates, rather than advancing PCA before or after birth. Conversely, PCA may mainly determine development of organs such as the brain that is relatively protected from the external environment. Relating postnatal maturation of preterm infants to their PCA is highly organ-specific and a poor measure of overall physiological maturation.

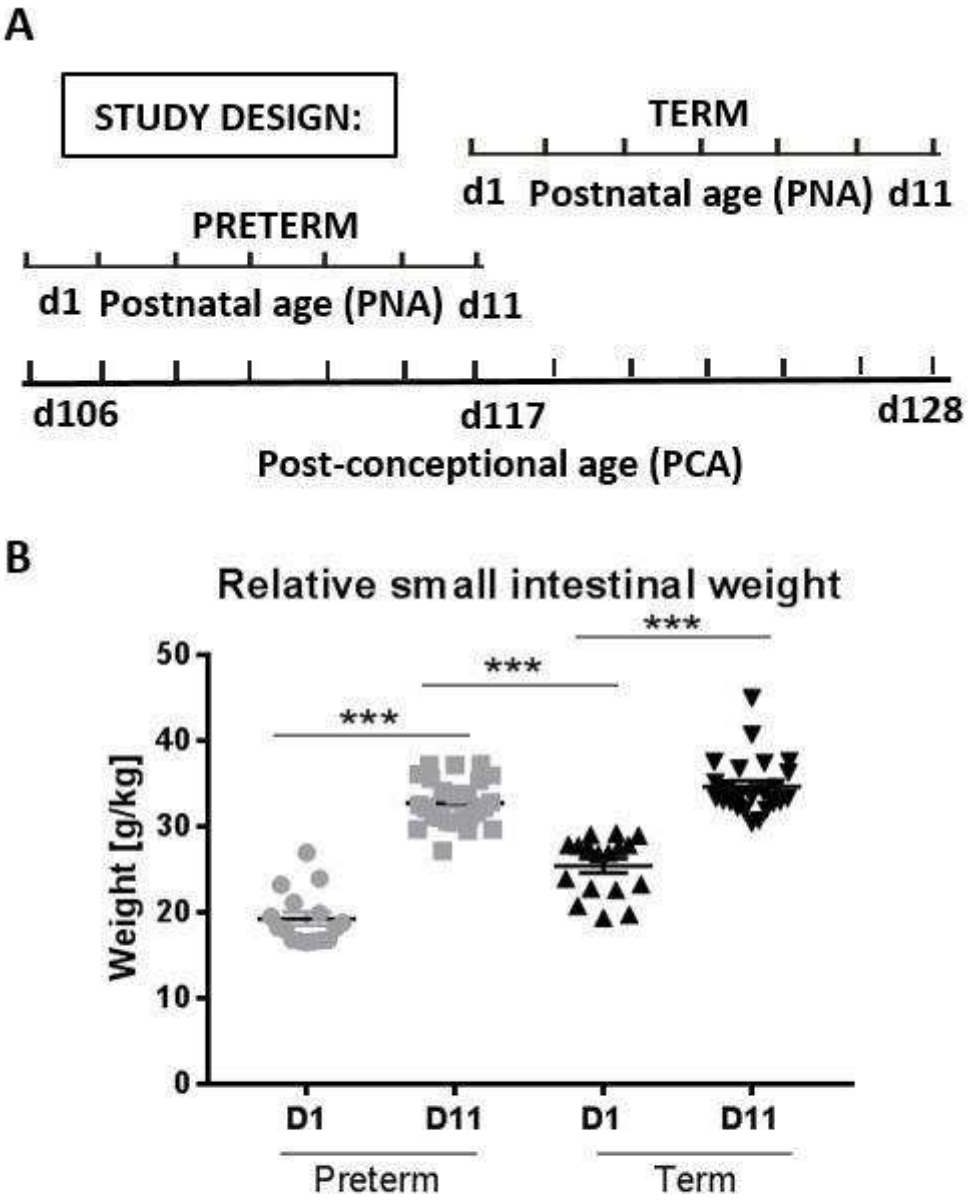

Fig. 1: Timeline of the study showing postconceptional- versus postnatal ages of preterm pigs (PRETERM) and term pigs (TERM) (A). Relative small intestinal weight on day 1 (d1) and day 11 (d11) (B). Data are expressed as means ± SE. Group differences are based on ANOVA anlaysis followed by Posthoc Tukey test. *** $P < 0.001$.

[Study design and relative small intestinal weight]

Preferred Presentation Type: **Oral Presentation**

Conference: 51st ESPGHAN Annual Meeting · Abstract: A-968-0002-00755 · **Status: Submitted**

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Abstract Preview - Step 3/4

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Topic: Neonatal and infant nutrition

Title: Effects of processing of bovine colostrum on levels of bioactive proteins

Author(s): Dereck E.W. Chatterton[1], Sasha Christina Aagaard[1,2], Tim Hesselballe Hansen[3], Duc Ninh Nguyen[2], Cristian De Gobba[1], Réne Lametsch[1], Per T. Sangild[2]

Organisation(s): [1]University of Copenhagen, Department of Food Science, Frederiksberg, Denmark, [2]University of Copenhagen, Comparative Pediatrics and Nutrition, Department of Veterinary Clinical and Animal Sciences, Frederiksberg, Denmark, [3]Biofiber-Damino A/S, Biofiber-Damino A/S, Gesen, Denmark

Text: **Objectives and Study:** Fresh bovine colostrum (BC) contains high levels of bioactive proteins, such as immunoglobulins (IG), lactoferrin (LF) and lactoperoxidase (LP). In preterm pigs, BC induces intestinal maturation, improves growth and protects against necrotising enterocolitis (NEC). Based on this, clinical trials using spray-dried, pasteurized BC to improve gut maturation in human preterm, have been initiated. It remains to be tested how different industrial processing may preserve the stability of colostrum proteins and help to secure high bioactivity of colostrum products used for infants.
Method: Untreated BC (control) from Danish dairy cows (Biofiber Damino, Denmark) was subjected to low-temperature long time pasteurization (LTLT, 62.5°C, 30 min) or high-temperature short time pasteurization (HTST, 72°C, 15 s). All products were then spray-dried (SD), with or without a final step of γ-irradiation (GI, ~14 kGy) to remove possible contamination during packaging. Samples were analysed for protein content and by nitrogen solubility index to determine protein denaturation (pH 4.6, removal of aggregated proteins and analysis of protein in the filtrate). IG and LF were determined by ELISA and Western blotting (WB). Proteins were separated by SDS-PAGE and compared with protein standards for identification and semi-quantified by densitometry, relative to values in a non-treated liquid BC sample. Proteomic analysis using LC-MS/MS of trypsin-treated samples was used to identify and quantitate the abundance of modifications.
Results: SD increased denaturation by 6% relative to non heat-treated BC. GI increased protein denaturation to 11%. LTLT increased denaturation to 19% which rose to 27% following GI. HTST increased denaturation to 48% with no further increment following GI (all $P < 0.05$).
LTLT resulted in 15% denaturation of total IgG compared to non-pasteurized BC, but not significantly. GI raised denaturation to 29%. HTST denatured total IgG by 34%, rising to 58% following GI (all $P < 0.05$, except for LTST alone (NS)). Compared to untreated BC, SD of unpasteurized BC had no effect on IgG1 levels, however GI increased denaturation of IgG1 (30%, $P < 0.05$). LTST caused no loss of IgG1 but GI increased denaturation, (38%, $P < 0.05$). HTST also increased denaturation to 40% but GI changed this to 60%. Levels of IgG2 mirrored those of IgG1, however it was more labile than IgG1 under LTST and HTST ($P < 0.05$). LTST led to a 21% reduction in LF and GI further reduced this (47%, $P< 0.05$). LTLT, and HTST, reduced the levels of LP (56 and 81% respectively) which were further reduced by GI. WB indicated aggregation of LF with HTST. Compared to unheated BC, differences in total methionine oxidation were observed, which were significant in the LTST plus GI treated BC ($P < 0.05$). No significant differences in total methionine oxidation were observed between untreated BC and LTST and HTST-treated BC.

Conclusion: BC proteins are highly sensitive to processing and the greatest effects are observed with HTST pasteurization. Further, high-dose GI treatment affects the stability of bioactive proteins, especially when combined with HTST processing. In conclusion, LTST followed by SD is an optimal way to preserve the levels of important colostrum bioactive proteins.

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Abstract Preview - Step 3/4

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Topic: Nutrition and health outcomes

Title: Cerebrospinal fluid and plasma proteomics to discover new biomarkers for sepsis and diet interventions in preterm neonates

Author(s): Tik Muk[1], Allan Stensballe[2], Anders Brunse[1], Per Sangild[1], Pingping Jiang[1]

Organisation(s): *[1]University of Copenhagen, Veterinary and Animal Science, Copenhagen, Denmark, [2]Aalborg university, Health Science and Technology, Aalborg, Denmark*

Text: **Objectives and Study:** Infection remains a leading cause of neonatal morbidity and mortality, particularly in preterm infants, and it is associated with neuroinflammation and brain injury. Immunomodulatory milk diets may attenuate peripheral inflammation. We hypothesized that systemic bacterial infection induces neuroinflammation in preterm neonates that is reflected by proteome changes in cerebrospinal fluid (CSF) and plasma. Using preterm pigs as a model for preterm infants, we also investigated if a bioactive milk diet, bovine colostrum, would ameliorate inflammation and infection-induced changes to CSF and plasma proteins.

Method: Immediately after birth, preterm pigs were intra-arterially administered 10^9 CFU/kg body weight of cultured *Staphylococcus epidermidis* (SE) a pathogen frequently causing sepsis in preterm infants. Pigs were fed parenterally (SE, n=15) or enterally with bovine colostrum (SE+COL, n=14). A third non-infected group was given parenteral nutrition and served as controls (CON, n=14). After 24 h, CSF and plasma samples were subjected to untargeted proteomic analysis on a Thermo q-Trap mass spectrometer. Protein identity and abundance were determined using the MaxQuant and Perseus software, and analysed by a linear mixed-effect model with Tukey post hoc test and FDR adjustment of P values. Detected protein differences (adjusted P≤0.1) were classified according to cellular functions: Neuroinflammation, brain function, transportation, complement system and metabolism.

Results: After 24 h, leukopenia, blood-brain barrier disruption and neuroinflammation were observed in SE-infected preterm pigs. Colostrum feeding reduced SE abundance ameliorated neuroinflammation and alleviated leukocyte response in blood and CSF. A total of 117 and 125 proteins with differential abundance were annotated in plasma and CSF, respectively. Neuropeptide Y, one of the most abundant peptides in the nervous system, showed lower abundance in CSF of SE vs. CON pigs, but increased in SE+COL pigs. Multiple inflammation-related proteins, including C-reactive protein, haptoglobin, LBP, CD109, Von Willebrand factor, increased in SE pigs, relative to CON, with more variable responses in SE+COL pigs. Complement proteins, including C1QC, C3 and C5, were increased in plasma and CSF of SE pigs but were lowered by colostrum feeding. ApoA4, a transcellular lipid transport modulator, was specifically increased in SE+COL pigs.

Conclusion: Systemic neonatal infection is associated with neuroinflammation and marked changes to CSF and plasma proteins, as detected by proteomics. These proteins associated with neuroinflammation and may serve as biomarkers for systemic infection in preterm neonates. Feeding an immunomodulatory milk diet may attenuate neuroinflammation but did not consistently affect the CSF and plasma biomarkers of systemic infection.

Preferred Presentation Type: Oral Presentation

Conference: 51st ESPGHAN Annual Meeting · Abstract: A-968-0019-00770 · **Status: Submitted**

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Abstract Preview - Step 3/4

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Topic: Neonatal and infant nutrition

Title: Human milk fortification affects gut microbiota in preterm pigs

Author(s): Jing Sun[1], Martin Mortensen[2], Søren Sørensen[2], Per Sangild[1]

Organisation(s): [1]University of Copenhagen, Department of Veterinary and Animal Sciences, Copenhagen, Denmark, [2]University of Copenhagen, Department of Biology, Copenhagen, Denmark

Text: **Objectives and Study:** Nutrient fortification of donor human milk (e.g. more protein, energy, minerals) is currently recommended to feed very premature infants to support growth and neurodevelopment. Yet, there are concerns that challenging the immature gut with formula-based fortifiers may induce gut dysfunction and inflammation. Undigested protein and nutrients in the hindgut could potentially interact with microbes to negatively affect gut microbiota composition and gut health (e.g. more protein fermentation, diarrhea and necrotizing enterocolitis, NEC). Using preterm pigs as a model for preterm infants, we investigated if nutrient fortification of human donor milk, using different fortification products, affects the gut microbiota, diarrhea and NEC.

Method: Using preterm pigs fed human donor milk, three types of commercial products were tested: BC (intact bovine colostrum, Biofiber Damino), ENF (intact and partially hydrolyzed whey protein plus vegetable oils, Mead Johnson/Nutrilon) and NAN (extensively hydrolyzed protein and maltodextrin, Nestlé). In Exp 1, cesarean-delivered preterm pigs were fed unfortified milk or milk fortified with BC or ENF (+4.6 g protein/kg/d) from day 3-8. In Exp 2, preterm pigs were fed milk fortified with BC, ENF or NAN from day 1-5. Diarrhea and NEC lesions were recorded. Gastric, small intestinal, caecal and colonic contents were collected at the euthanasia, and the microbiota was sequenced using 16S rRNA gene sequencing. PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) was used to predict the metagenome of each sample based on 16S rRNA sequencing.

Results: In Exp 1, both groups of fortified pigs (BC, ENF) had lower Shannon diversity and higher bacterial load in gastric contents. In the caecum and colon, fortification affected the contents of microbial genes that are related to several metabolic pathways (aminoacyl-tRNA, penicillin/cephalosporin and lipopolysaccharide biosynthesis, nitrogen metabolism, bacterial toxin). There were no differences among groups in NEC but ENF fortification induced more severe diarrhea than BC and unfortified groups. In Exp 2, the NAN-fortified pigs had more diarrhea, higher NEC incidence, and lower Shannon diversity of microbiota in gut contents than BC-fortified pigs. Colonic microbial community of NAN pigs also differed from that in BC (more abundant Haemophilus and Lactobacillus). Staphylococcus was less abundant in ENF and NAN pigs relative to BC pigs in the gut.

Conclusion: Nutrient fortification of human donor milk may increase NEC sensitivity. This may be associated with altered microbial homeostasis and protein fermentation in both the stomach and the hindgut regions. Further research are required to characterize metabolites in the gut contents and identify the type of nutrient fortifier that best supports growth of preterm infants without inducing adverse effects on the gut microbiota and immature gut functions.

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Abstract Preview - Step 3/4

- print version -

Topic: Basic science

Title: Pancreatic enzyme maturation is delayed and not affected by the first enteral nutrition in preterm pigs

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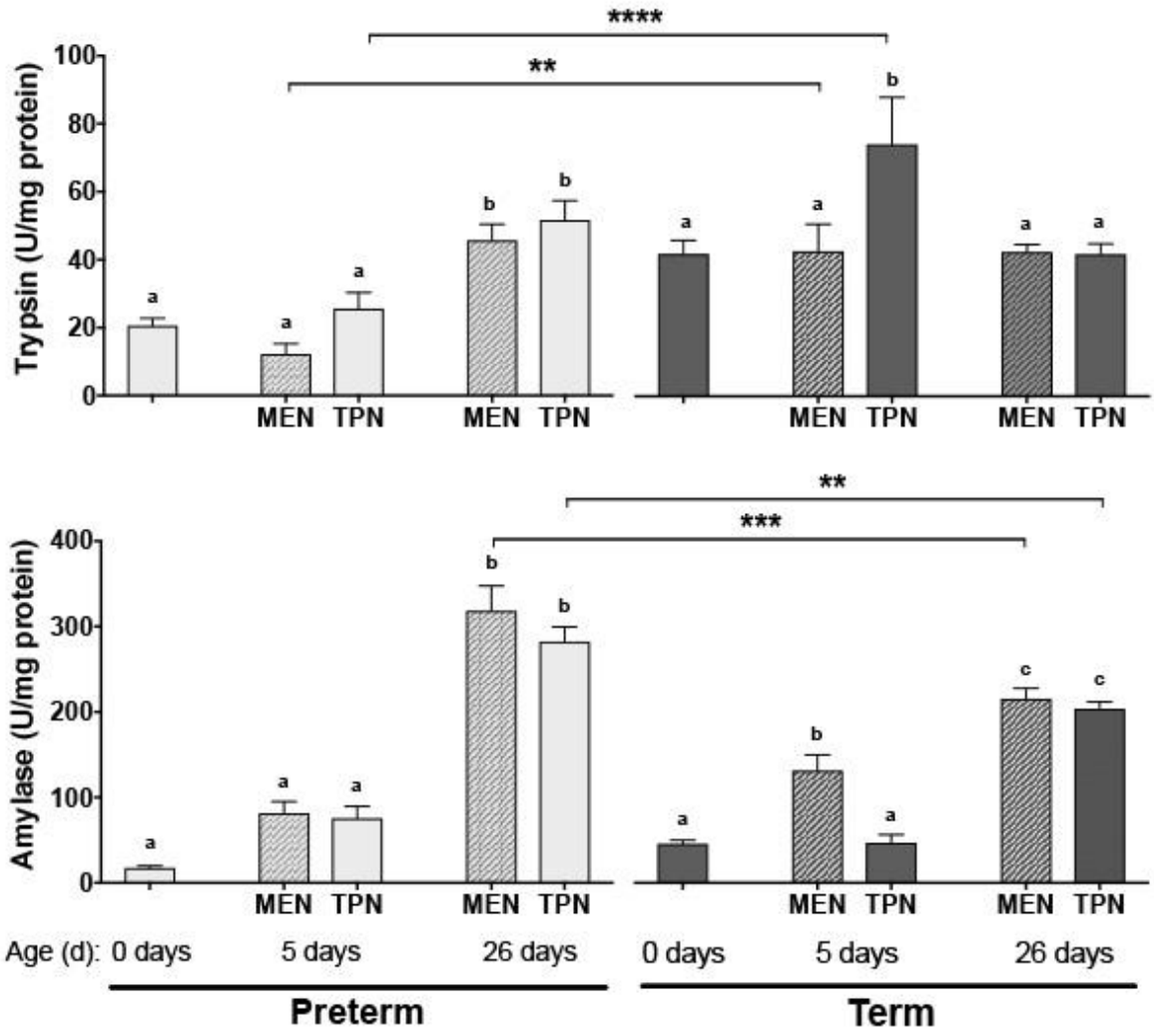
Organisation(s): [1]Lund University, Biology, Lund, Sweden, [2]University of Copenhagen, Comparative Pediatrics and Nutrition, Copenhagen, Denmark

Text: **Objectives and Study:** Preterm infants have an immature digestive system, predisposing them to short- and long-term complications, including intolerance to enteral feeding. Thus, optimal feeding strategies are required to prevent life-threatening conditions like severe diarrhoea and necrotising enterocolitis (NEC). Pancreatic enzymes are important for digestion but little is known about the postnatal development of the exocrine pancreas. Using preterm pigs as models for preterm infants, we hypothesized that: 1) postnatal development of the exocrine pancreas is delayed after preterm birth, and 2) minimal enteral nutrition during the first days of life provides better stimulation of the exocrine pancreas function relative to total parenteral nutrition.
Method: Pigs were delivered by caesarean section preterm (90% gestation) or at full term, and were nurtured during the first 5 days with total parenteral nutrition (TPN) or with parenteral nutrition plus minimal enteral nutrition (MEN) with bovine colostrum. From day 6, all pigs were fed full enteral nutrition with bovine milk until postnatal day 26. Pancreatic samples were collected on days 1, 5 or 26 from both preterm and term pigs (n=116). Trypsin and amylase activities were analysed in tissue homogenates. Protein content was measured and used to calculate the relative amount of the enzymes. Data were analyzed for the same gestational age between treatments (e.g. MEN *vs* TPN, Tukey's post-hoc test, $P < 0.05$ indicated with different letters) and within treatments between different gestational ages (e.g. Preterm *vs* Term, Sidak's post-hoc test, $P < 0.05$ indicated with *) (see Figure).
Results: Pancreatic trypsin and amylase concentrations increased with age in preterm piglets, especially by 26 days ($P < 0.05$), with no differences between the diet regimens (TPN vs MEN) (see Figure). Term piglets showed higher relative trypsin activity levels at birth and these were maintained with advancing age, but 5 d old TPN-fed term pigs had higher levels compared with MEN ($P < 0.05$). Amylase activity was low at birth in term piglets and increased with age ($P < 0.05$). At 5 days, term TPN-fed piglets failed to increase their amylase activity relative to birth. Pancreatic trypsin was lower in preterm than in term piglets at 5 days after birth ($p < 0.05$) but amylase was higher in preterm than in term piglets at day 26 ($P < 0.05$).
Conclusion: Pancreatic trypsin and amylase relative activities increased with age in preterm piglets and by day 26 the enzyme levels converged to those in term piglets. TPN or MEN nutrition for the first 5 days only had effects on enzyme levels in term piglets, indicating that the effects of the first diet depends on gestational age at birth. Increased trypsin activity in term, 5 d-old TPN-fed piglets may be explained by lack of enteral stimuli to stimulate pancreatic secretion. Conversely, amylase activity level was very low at birth and increased with age. Lower amylase activity in term, 5 d-old TPN-fed piglets may be explained by lack of enteral stimuli to increase enzyme production. We conclude that pancreatic trypsin and amylase activities depend on both gestational age at birth and postnatal age and have different sensitivities to the first feeding.



[Pancreatic trypsin and amylase enzyme levels in preterm and term pigs fed with TPN or MEN]

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Milk feeding reduces gut bacterial translocation but not responses to systemic infection in preterm pigs

A. Brunse, O. Bæk, D. Nguyen, A. Moodley, T. Thymann, P. Sangild

Bloodstream infection (BSI) is the most frequent severe complication afflicting preterm infants and often results from either prolonged use of indwelling catheters or gut microbial translocation. Breastfeeding preterm infants protects against BSI and sepsis, but it is unclear whether the effect is direct (systemic delivery of milk-derived antimicrobial factors) or indirect (improvement of gut integrity). We hypothesized that enteral feeding of preterm neonates diet-dependently affects both gut- and catheter-derived bacteremia. Hence, we compared the protective effects of milk and formula feeding against BSI.

Preterm, cesarean-delivered pigs were fed bovine colostrum (MILK) or infant formula (FORM) for 5 days. On day 3, animals received $5*10^9$ CFU/kg intravascular *Staphylococcus epidermidis* (SE) (SE-MILK, n=8; SE-FORM, n=8) or saline (SAL-MILK, n=4; SAL-FORM, n=7). On day 5, clinical status, organ pathology, bacteriology and hematological parameters were assessed.

Fewer MILK animals had gut lesions (9 vs. 80%, $p<0.001$) and translocation of enteric bacteria to the bone-marrow (33 vs. 80%, $p<0.05$), compared with FORM animals, irrespective of SE status. SE-infected animals were not clinically affected despite high densities of SE in blood and bone marrow, as well as markedly reduced circulating platelets and lymphocytes (both $p<0.01$), compared with controls (SAL). SE-infected animals also showed increased ex vivo blood neutrophil phagocytic function, indicating improved innate immune responses. Milk feeding did not alter neutrophil function nor improve SE clearance *in vivo*.

Milk feeding prevents gut inflammation and bacterial translocation compared with infant formula, but does not prevent catheter-induced BSI. In preterm infants, the beneficial effects of milk feeding on BSI may be mostly related to improvement of gut barrier integrity.

Patent ductus arteriosus and its relation to diet-induced necrotizing enterocolitis in preterm pigs

Xudong Yan, Kristine Bach Korsholm Knudsen, Inge Boetker Rasmussen Ifaoui, Lene Elisabeth Buelund, Thomas Thymann, Jørgen Agerholm, Fintan McEvoy, Per Torp Sangild

Background:

Patent ductus arteriosus (PDA) is the most common cardiovascular event in preterm infants (30-60% of very preterm infants). Preterm infants with PDA are more likely to develop severe complications, including feeding intolerance and necrotizing enterocolitis (NEC), but the links (direct/indirect) are poorly understood. An animal model in preterm pigs that incorporates all the physiological signs of immaturity (e.g. respiratory, gastrointestinal, cardiovascular, metabolic dysfunctions) may help to identify the relationship among PDA, prematurity at birth, complications such as NEC. We hypothesized that preterm piglets show delayed closure of the ductus arteriosus (DA) and this is related to NEC development.

Methods:

In experiment 1, preterm and term piglets were delivered by caesarean section at 106 and 116 d gestational age (GA), (n= 66 and n=44 respectively, 5 sows, term= 117 d). Piglets were euthanized for tissue collection at birth or 11 d of age after slow feeding with colostrum/milk (NEC-protective diet regimen). In experiment 2, preterm piglets (n=147 from 10 sows) were delivered at GA 100-105 or 110-115 d. Piglets were euthanized for collection of the DA and the gut at birth or 2 d of age following feeding with colostrum or infant formula (NEC-protective or NEC-inducing diet). In experiment 3, preterm GA 106 d preterm pigs (n=33) were fed with fast advanced infant formula and euthanized for tissue collection on d 3-5. In all three experiments, a functional PDA was diagnosed by color-flow Doppler echocardiography 1-5 d after delivery. The collected DAs were stained for structural inspection. Blinded to the PDA assessment, NEC lesions in the stomach, intestine and colon were scored according to increasing mucosal damage severity (1: healthy, 6: fulminant necrosis).

Results:

In experiment 1, incidence of PDA was detected in 20% of preterm pigs vs. 0% of near-term piglets 24 h after birth. There were no signs of NEC and no mortality. In subgroups (n=49), there were no correlations between PDA and PO_2, PO_2 and cortisol within 24 hours. In

experiment 2, GA 100-105 d piglets had immature respiration and high mortality, relative to GA 110-115 d piglets (44 vs. 10%). They also showed higher incidence of NEC at 2 d (8 vs. 0%), and this was accompanied with higher incidence of PDA (46 vs. 20%). NEC lesions were relatively mild and with no clear overlap between NEC and PDA piglets. In experiment 3, 42% (14/33) of piglets were diagnosed with PDA on d 3-5. Mild NEC lesions (grade 2-4) were detected in 66% (22/33) of the piglets and severe NEC lesions (grade 5-6) in 4 pigs (12%). Severe NEC lesions was related to the PDA ($p<0.05$ versus piglets without PDA). Histology showed that the DA had fewer and less advanced closure in immature piglets versus those in more mature piglets. These histological maturational changes were closely correlated with birth weight and gestational age ($p<0.05$).

Conclusion:

Preterm piglets show higher incidence of PDA and NEC than near-term piglets. Both flow turbulence and histological features of the DA tissue could be used to diagnose PDA in preterm pigs. Severe NEC lesions within the first days after preterm birth were associated with PDA in piglets. Preterm pigs can serve as an excellent model to study PDA, its comorbidities and possible treatments.

Abstract Preview - Step 3/4

- print version -

Topic: Clinical nutrition

Title: Refeeding with lactose and milk minerals alters the gut microbiota and expands plasma volume in a piglet model of moderate malnutrition

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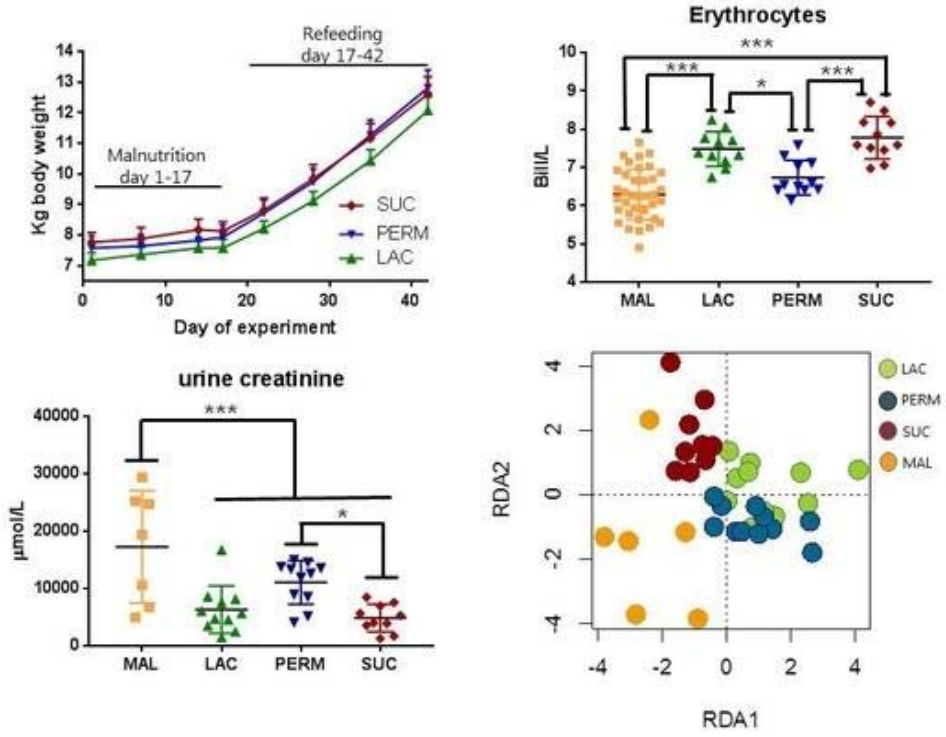
Organisation(s): [1]Univ. of Copenhagen, Veterinary and Animal Science, Frederiksberg, Denmark, [2]Univ. of Copenhagen, Dept. of Food Science, Frederiksberg, Denmark

Text: Objectives and Study
Moderate malnutrition includes stunted growth, wasting and disturbed electrolyte profile. The small intestine is atrophic and patients often present symptoms of gut dysbiosis and diarrhea. Refeeding of patients with moderate malnutrition is mostly based on corn-soy blends with added sucrose. It is however unclear if other sugars like lactose, or a combination of lactose and milk minerals can show superior effects with regard to growth, clinical and paraclinical endpoints and endpoints related to gut mucosal function and gut microbiota.

Method
Four-week old pigs were fed a pure low-protein maize diet for 17 days to induce symptoms of moderate malnutrition. Tissues were collected from a subsample of pigs at this stage to represent pigs with moderate malnutrition (MAL, n=7) whereas the rest were fed a corn-soy blend until day 42, with added sucrose (SUC, n=11), lactose (LAC, n=11) or whey permeate (a dairy product high in lactose and milk minerals, PERM, n=11). All three products were added at 10% of the total diet.

Results
Malnutrition was characterized by slow growth and low P, albumin and bilirubin, while Na, K, alanine aminotransferase and gammaglutamyl transferase were increased (all $P < 0.05$). Following refeeding, growth, body composition and organ weights were similar for all groups. Relative to baseline before malnutrition, MAL pigs showed decreased *in vitro* TNF-α response to TLR2 agonist (immune paralysis), and refeeding recovered the immune response to similar levels among the three refeeding groups (all $P < 0.05$). Refeeding was further associated with a marked reduction in creatine kinase and alkaline phosphatase in the three refeeding groups relative to MAL, whereas creatinine was decreased and albumin increased only in SUC and LAC (all $P < 0.05$). Refeeding was also associated with higher plasma Mg, P and K ($P < 0.05$) relative to MAL. Relative to SUC and LAC there was a lowering in PERM of both Ca, Mg, Fe, albumin, hematocrit, erythrocytes and hemoglobin (all $P < 0.05$), suggesting an expansion of plasma volume in PERM. This was also associated with an increased urine creatinine concentration in PERM versus LAC (tendency) and SUC ($P < 0.05$), indicating lower urine production. Likewise, the concentration of Na, K, Cl and protein were numerically elevated in PERM versus LAC and SUC indicatory of more concentrated urine. The brush border enzyme activity were largely similar for MAL, LAC, PERM and SUC. The gut microbiota (GM), in MAL and SUC showed differences ($P < 0.05$) in α- and β-diversity as compared to LAC and PERM in samples collected from rectal region. Furthermore, between LAC/PERM and MAL or SUC, significant differences (adjusted $P < 0.05$) in the prevalence of more than 30 bacterial species (many of them typically know as beneficial members) belonging to Bacteroidales, Lactobacillales, Clostridiales and Proteobacteria were determined.

Conclusion
Refeeding after moderate malnutrition with a corn-soy based diet with added permeate, induces altered gut microbiome, expands plasma volume and reduces urine production relative to diets enriched with sucrose or lactose. In patients with normal cardiac function but low blood pressure, expansion of plasma volume can be regarded as a benefit to support perfusion of peripheral tissues.



[Growth, erythrocyte count, urinary creatinine and the rectal microbiome]

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