September 23rd, 2023 09:00 - 11:00

PARALLEL SESSION 33 - NUTRITION 5

ID 406. Alternating feeding with colostrum and formula affects necrotising enterocolitis and gut microbiota in preterm pigs

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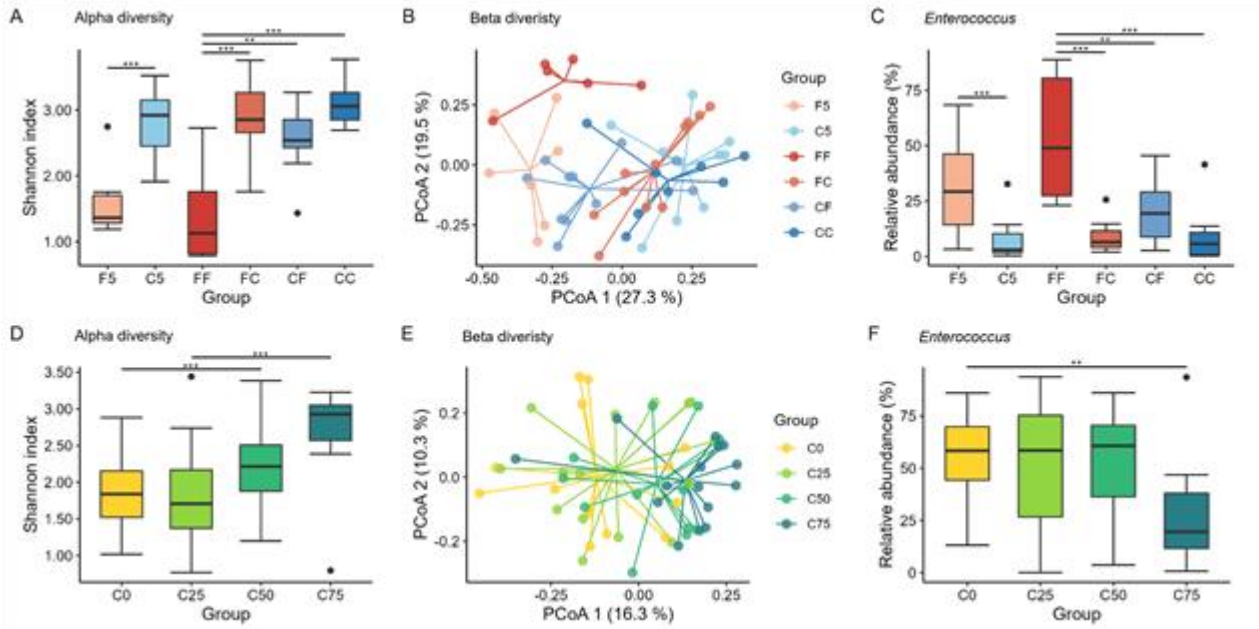
Background: Exclusive feeding with bovine colostrum protects against necrotising enterocolitis (NEC) in preterm pigs, used as model for infants. If to be used in clinical practice, colostrum needs to be fed together with infant formula to avoid protein overload. It is not clear how mixed colostrum–formula feeding regimens affect NEC sensitivity and the gut microbiota. We hypothesized that mixed colostrum–formula feedings induce differential gut microbiota, potentially predisposing to NEC.

Methods: Colonic gut microbiota, profiled with 16S ribosomal RNA gene amplicon sequencing, was compared in pigs receiving exclusive colostrum (C) or formula (F) until day 5 of life (C5, F5, n = 7–10), followed by feeding with C or F until day 9 (CF, CC, FC, FF, n = 6–11, Exp1). Other preterm pigs received either 0, 25, 50 or 75% C (C0, C25, C50, C75), with remaining diet being F (n = 13–15, Exp2). Incidence of NEC was determined based on macroscopic scoring of the gut.

Results: In Exp1, pigs fed any C had higher species diversity (alpha diversity) and lower abundance of Enterococcus than pigs fed F only (CC, CF, FC vs FF; C5 vs F5,

p<0.05). Pigs fed any C had similar microbial community structure (beta diversity, FC or CF vs CC, p>0.05) that differed from pigs fed exclusive F (CF or FC vs FF, p<0.05). In Exp2, pigs fed >50% C had higher species diversity than pigs fed less C (C50 or C75 vs C0 or C25, p<0.05). C75 pigs had lower relative abundance of Enterococcus than C0 ones. Feeding regimen did not clearly affect NEC incidence, although groups receiving any C tended to have less NEC (67 vs 83%, p>0.05).

Conclusion: Preterm pigs fed minimum 50% C, relative to F, have a gut microbiota that differs from that in pigs fed exclusive F (e.g., higher diversity, less enterococci). Diet–induced changes in the gut microbiota that may not be able to explain the high NEC sensitivity in preterm pigs.



Mixed feeding with colostrum and formula demonstrated different effects on the gut microbiota. Minimum 50% C feeding increased alpha–diversity (A, D), altered beta–diverstiy (B, E), and reduced enterococci (C, F).

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The University of Copenhagen (Per T. Sangild) holds a patent on the use of colostrum for preterm infants.

ID 220. Transplantation of viable faecal virome is necessary and sufficient for reducing necrotizing enterocolitis severity

Miss Malene Roed Spiegelhauer[1], Miss Simone Margaard Offersen[1], Miss Xiaotian Mao[2], Miss Michela Gambino[3], Mr Thomas Thymann[1], Mr Dennis Sandris Nielsen[2], Mr Duc Ninh Nguyen[1], Mr Anders Brunse[1]

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Background:

Necrotizing enterocolitis (NEC) is a severe gastrointestinal disease affecting premature infants, usually preceded by gut dysbiosis and increased relative abundance of Proteobacteria. We recently found that transferring donor faecal filtrate (FFT), where bacteria were removed by filtration, could prevent NEC and reduce the relative abundance of mucosa-associated Proteobacteria. We propose that this is due to bacteriophages infecting intestinal bacteria in a host-specific manner, but we cannot exclude the potential effect of faecal metabolites. In this study we compared the NEC-preventive capacity of native FFT versus inactivated FFT, and provide evidence that intact faecal bacteriophages are necessary for NEC prevention.

Methods:

Colon content from four healthy 10-day-old suckling piglets was filtered at 0.45µm to remove bacteria (FFT). An aliquot of FFT was exposed to UV-irradiation at 265nm to target nucleic acids and inactivate faecal viruses (iFFT). Premature caesarean-born piglets were fed increasing volumes of formula for NEC induction, and were orally administered saline (CON), FFT or iFFT during the first two days of life. The

animals were closely monitored, and clinical and faecal assessments were performed twice daily. Animals were euthanized on day five or when presenting clinical signs of NEC. Macroscopic NEC–like lesions were graded in a blinded fashion, and organs were collected for analysis and microscopic scoring of intestinal pathology.

Results:

Reduced colon pathology score was observed in animals receiving FFT ($p<0.05$) relative to controls, whereas no reduction was seen in the iFFT group (Figure 1). The iFFT group displayed an increased stomach pathology score relative to FFT ($p<0.05$) and controls ($p=0.06$). No differences were found for small intestinal pathology scores. Additionally, earlier onset of diarrhoea was observed in the FFT group but not in the iFFT group ($p<0.05$).

Conclusion:

We demonstrated that the NEC preventive effect of FFT requires viable donor faecal viruses. Moreover, when the FFT is UV–irradiated, inactivating viruses, the residual faecal metabolites appear incapable of preventing NEC. Paradoxically, the earlier onset of diarrhoea occurring in the FFT group is likely a result of donor–derived porcine viruses also susceptible to UV–irradiation. Further refinement is necessary to develop an effective and safe virome–based treatment against NEC.

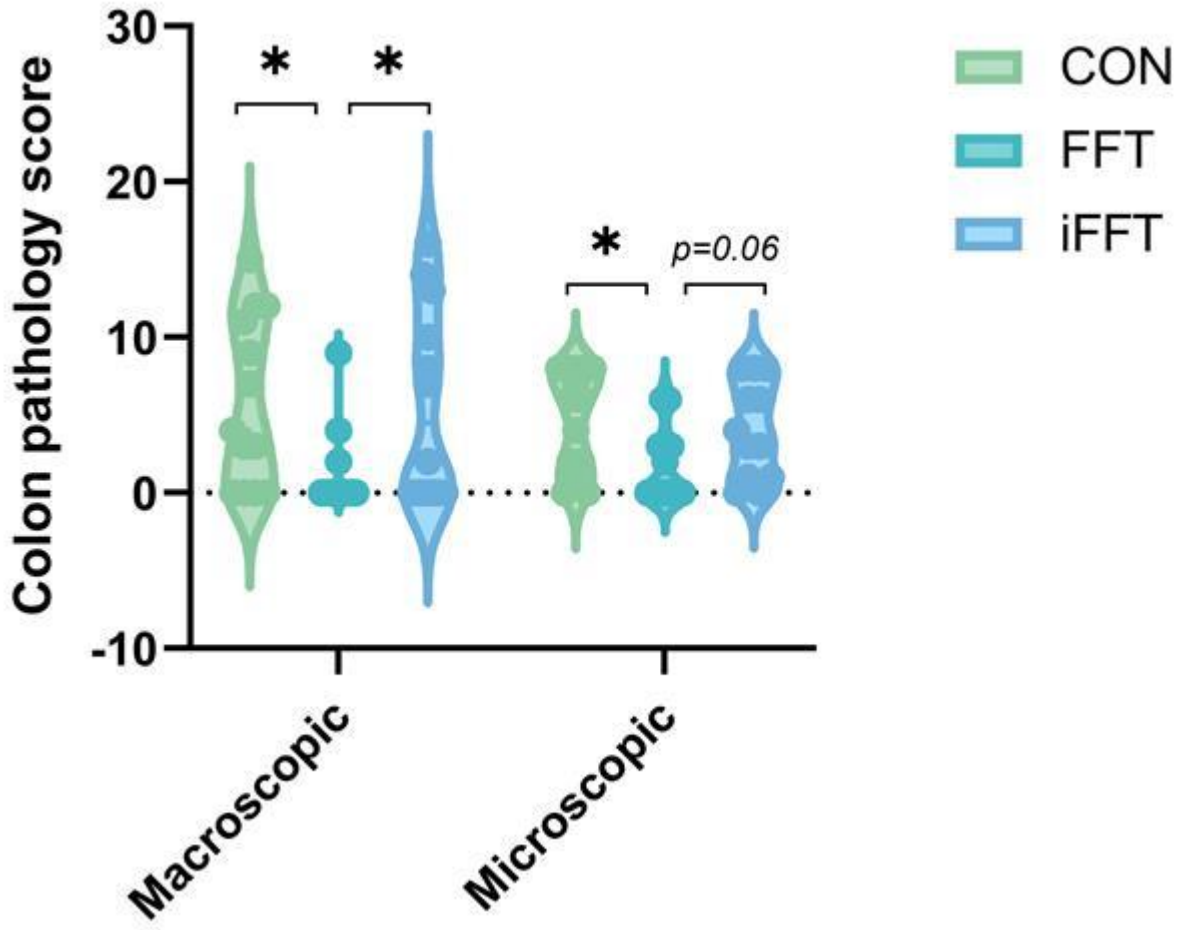

Figure 1. Colon pathology scores. * p < 0.05

Statistical analysis: Kruskal–Wallis nonparametric test followed by post–hoc pairwise comparison by Dunn test with Benjamin Hochberg p–value adjustment

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Statistical analysis: Kruskal–Wallis nonparametric test followed by post–hoc pairwise comparison by Dunn test with Benjamin Hochberg p–value adjustment

None declared

ID 102. Downregulation of miR-155-5p ameliorates intestinal injury in necrotizing enterocolitis by suppressing ferroptosis and inflammation

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Background: Necrotizing enterocolitis (NEC) is an acquired disorder of mucosal damage, especially in premature infants. MicroRNAs (miRNAs) have been found to play an important role in the progression of NEC. Using bioinformatics analysis, we found that the level of miR–155–5p was significantly increased in intestinal tissues from infants with NEC. However, the mechanism of miR–155–5p in the progression of NEC remains unclear.

Methods: Human normal colorectal cells (FHCs) were stimulated with lipopolysaccharide (LPS) to mimic NEC in vitro; an NEC rat model was constructed by artificial feeding and hypoxia stimulation for in vivo study. Then, the inflammatory cytokine levels, cell viability and apoptosis rate were detected. Biochemical indices, including GSH, SOD, CAT and MDA, were detected by specific detection kits. ROS and MMP in FHC cells were detected by flow cytometry using the corresponding probes. Intracellular iron was detected quantitatively and qualitatively by a kit and probe, respectively. The expression of ferroptosis–related proteins in FHC cells and intestinal tissues of NEC rats was detected by Western blotting. The pathological changes in the intestinal tissues of NEC rats were evaluated by H&E staining.

Results: Downregulation of miR–155–5p increased the viability of LPS–stimulated FHC cells, inhibited cell apoptosis, inflammation and ferroptosis, and alleviated intestinal injury in NEC rats. SLC7A11 was identified as a direct target of miR–155–5p.

The miR-155-5p inhibitor inhibited LPS-induced inflammation and ferroptosis in FHC and NEC rats by upregulating SLC7A11, which was manifested as upregulation of the ferroptosis-related proteins FTH1 and GPX4 and downregulation of COX-2 and ACSL4; downregulation of the lipid peroxidation product MDA; downregulation of the antioxidant indices GSH, SOD and CAT; downregulation of the proinflammatory cytokines IL-6 and TNF-α; and inhibition of the IκBα/NF-κB p65 pathway.

Conclusion: Collectively, the downregulation of miR-155-5p was able to ameliorate intestinal injury in NEC by suppressing ferroptosis and inflammation. These findings might provide some theoretical support for exploring new treatments for NEC.

None declared

ID 706. The incidence of necrotizing enterocolitis during the COVID-19 pandemic in Sweden: A population-based cohort study

Doctor Elena Palleri[1], Doctor Laszlo Markasz[2], Doctor Anna Svenningsson[1], Professor Helene Engstrand Lilja[1]

[1]Karolinska University Hospital, Stockholm, Sweden, [2]Department of Women's and Children´s Health, Uppsala University, , Uppsala, Sweden

Introduction: COVID–19 pandemic has changed routines in Neonatal Intensive Care Units (NICUs), mostly by stricter infection control measures and restricting visitor presence. The effect of the pandemic restrictions in the NICUs has not yet been studied. Necrotizing enterocolitis (NEC) is a disease characterized by intestinal inflammation and bacterial invasion. The objective of this study was to investigate whether the incidence of NEC has changed during the COVID–19 pandemic in Sweden and if it was associated with a change in the frequency of extremely preterm births.

Methods: Data was retrieved from the Swedish Neonatal Quality Register (SNQ) for infants registered from 1 January 2017 to 31 December 2021 born below a gestational age of 35 weeks. The registry completeness is 98–99%. The diagnosis of NEC was the primary outcome. Generalized linear model were used to calculate the risk ratio for NEC.

Results: A total of 13239 premature infants born were included in the study. 235 (1.8%) infants developed NEC, 91 of which (38% of all NEC cases) required surgical treatment. 8967 infants were born before COVID–19 pandemic restrictions and 4272 during. Median gestational age at birth was 32,8 weeks in both periods. The table shows patients characteristics. The incidence of NEC was significantly lower during

COVID–19 pandemic compared to the prior period (1.43% vs 1.94% p value 0.037), but not the incidence of surgical NEC (0.89% vs 1.14%, p value 0.192). The crude Risk ratio (RR) of developing NEC was (0.74 95% CI(0.55–0.98) p value 0.038), but when adjusted for region, gestational age at birth, probiotics, intrauterine growth restriction and Chorioamnionitis the RR was 0.80 95%CI (0.60–1.06), p value 0.112. The incidence of late onset sepsis with positive culture was also significantly lower during COVID–19 pandemic (3.21% vs 4.15, p value 0.008).

Conclusion:

Under the COVID–19 pandemic the incidence of NEC was significantly diminished compared to the previous period despite the same frequency of extremely preterm births. This could partially be explained by the simultaneous introduction of Probiotics and the normal variation of NEC over time. The incidence of late onset sepsis was also decreased during the COVID–19 pandemic.

Table 1: Cohort of preterm newborns (born <35weeks of gestation) in Sweden 2017-2021

	Infants born during Covid-19 pandemic (n=4272)	Infants born before Covid-19 pandemic (n=8967)	P value
C-section, n(%)	2530(59.22)	5053(56.35)	**0.002**
Acute section among sections,n(%)	2376(93.91)	4686 (92.74)	0.056
Ablation, n(%)	377(8.94)	801(9.46)	0.344
*Preeclampsia/eclampsia, n(%)	650(15.42)	1239(14.64)	0.243
*PPROM(<v37) n(%)	715(16.96)	1410(16.66)	0.680
*Chorioamnionitis, n(%)	111(2.63)	213(2.52)	0.700
Gestational diabetes, n(%)	158(3.75)	271(3.20)	0.108
Number of feti 1,singletone 2,twins 3,triples 4,quadruples	 3188(74.63) 1013(23.71) 71(1.66) 0(0.00)	 6634(73.98) 2159(24.08) 170(1.90) 4(0.04)	0.457
*Antenatal steroids, n(%)	2455(69.35)	4951(69.35)	0.847
IUGR, n(%)	474(11.25)	999(11.80)	0.357
Female, n(%)	2001(46.84)	4114(45.88)	0.300
Gestational age, median (IQR)	32.86(30.29-34.14)	32.86(30.14-34.14)	0.925
Infants born <28 weeks,	571(13.37)	1244(13.87)	0.428
Birthweight, median(IQR)	1860(1370-2263)	1865(1335-2260)	0.547
APGAR, median (IQR)	9(8-10)	9(7-10)	0.375
RDS, n(%)	1236(28.93)	2503(27.91)	0.223
IVH Grade 1,n(%) IVH Grade 2, n(%) IVH Grade 3, n(%) IVH Grade 4, n(%)	148(3.77) 61(1.55) 40(1.02) 60(1.53)	263(3.17) 165(1.99) 78(0.94) 99(1.19)	0.085
hsPDA medically treated, n(%)	228(5.34)	516(5.75)	0.330
hsPDA, surgically treated, n(%)	31(0.73)	100(1.12)	**0.034**
Parenteral nutrition, n(%)	1643(38.46)	3368(37.56)	0.318
Days of parenteral nutrition, median(IQR)	7(4-12)	7(4-13)	0.123
Early onset sepsis (positive culture),n(%)	22(0.52)	61(0.68)	0.256
Early onset infection(positive culture)	40(0.94)	86(0.96)	0.889
Late onset sepsis(positive culture), n(%)	137(3.21)	371(4.15)	**0.008**
Late onset infection(positive culture), n(%)	252(5.91)	640(7.17)	**0.007**
NEC, n(%)	61(1.43)	174(1.94)	**0.037**
Surgical NEC verified, n(%)	29(0.68)	66(0.74)	0.715
SIP, n(%)	14(0.33)	39(0.43)	0.361
Probiotics, n(%)	499(11.68)	232(2.59)	**0.000**
Oxygen need at 36 weeks, n(%)	282(6.60)	681(7.59)	**0.040**
Death, n(%)	165(3.86)	336(3.75)	0.745

(*= not complete data, missing data <5%)(PPROM = Premature rupture of membranes, IUGR= Intrauterine growth restriction, RDS= respiratory distress syndrome, IVH= intraventricular hemorrhage, hsPDA= hemodynamically significant patent ductus arteriosus, NE= necrotizing enterocolitis, SIP= spontaneous intestinal perforation)

None declared