Results

The main goal of this thesis project was to create a NEC mouse model by combining Maternal Antibiotics Treatment with Formula-Feeding. This hypothesis was tested in a series of experiments (Exp 1-4), each successive experiment building on the findings from the former. The following is a description of each experiment. An abridged version of each experiment is provided, followed by a detailed description of experimental setup and results.

Proinflammatory cytokines

	Treatment groups (N total = 30)				
Cytokines	**AB-BF**, N = 8[1]	**AB-FF**, N = 11[1]	**CON-BF**, N = 8[1]	**CON-FF**, N = 3[1]	**p-value**[2]
IL-10	1.32 (1.2, 1.6)	0.00 (0.0, 0.5)	1.16 (0.7, 1.8)	2.12 (1.2, 2.3)	0.012
IL-12p70	13.60 (10.4, 18.7)	15.96 (9.4, 17.0)	10.30 (7.4, 12.4)	14.32 (14.1, 15.8)	0.3
IL-1b	3.81 (2.8, 4.6)	2.64 (2.4, 3.9)	2.61 (2.0, 6.6)	3.10 (2.5, 9.1)	>0.9
IL-2	1.41 (1.3, 1.7)	1.22 (1.1, 1.3)	1.46 (1.3, 1.6)	1.14 (1.0, 1.3)	0.053
IL-4	0.98 (0.9, 1.1)	0.52 (0.5, 0.6)	0.72 (0.6, 1.0)	0.77 (0.7, 0.9)	0.004
IL-5	2.01 (1.5, 2.2)	0.89 (0.6, 1.1)	1.72 (1.6, 2.1)	1.34 (1.2, 1.7)	<0.001
IL-6	14.97 (14.1, 17.9)	24.06 (21.8, 30.2)	12.34 (10.8, 16.7)	39.42 (32.2, 40.6)	0.002
KCGRO	7.76 (5.7, 10.4)	7.20 (6.0, 9.4)	8.09 (4.5, 25.1)	8.91 (7.9, 17.6)	0.9
TNFa	2.64 (2.4, 3.3)	1.45 (1.4, 1.8)	2.98 (2.2, 3.7)	1.84 (1.5, 2.4)	0.004

[1] Median (IQR)
[2] Kruskal-Wallis rank sum test
* P-value indicates overall group differences

Matrinem round 1

Figure 1: Experimental protocols

Exp 1: Model feasibility

Rationale:

- *Chen et al.*, showed that treatment with antibiotics during pregnancy(Maternal Antibiotics Treatment, **MAT**) "resulted" in mild NEC-like symptoms in the offspring.
- XXX *[Find paper]* found formula-feeding to be damaging to the intestine.
- One of the primary causal factors of NEC is formula-feeding (paper on what is thought to cause NEC).
- We tested the hypothesis that MAT combined with formula feeding (**FF**) would result in a worsened phenotype - even more NEC-like than either treatment on it's own.

Aim:

- To test whether it was possible to keep mice alive from postnatal day 3 and 48 hours onwards, while being separated from the mother.
- Whether combining a broad spectrum antibiotic with formula-feeding will result in NEC-like symptoms such as:

- Visible signs of intestinal inflammation and / or greater levels of pro-inflammatory cytokines expressed in tissue or serum

Conclusion:

- Survival rate: 29 of 38 (76.3157895%)
- Visual inspection: no clear signs of intestinal inflammation
- Cytokine expression: Statistically significant differences in the level of STATISTICAL-SIGNIF-CYTOKINE-NAMES.

Detailed description of Exp 1. Date of exp

Antimals and experimental setup
The experimental setup has been described in detail in the methods section.
Briefly, six male and 18 female C57BL/6N mice were co-housed for 24 hours in 6 cages (1 male + 3 female each). Two weeks after mating, pregnant females were separated evenly into maternal antibiotic treatment (MAT) and water-only controls (CON).
Antibiotics (Gentamicin, Vancomycin, Neomycin, and Ampicillin (all 0.5 g/L)) were mixed into the drinking water of the MAT mice while controls received water only. - Antibiotic treatment commenced on gestational day 15 and continued until delivery. - Each mother was housed with her offspring in separate cages during the first 2 days after birth. - On day 3, mice from water or antibiotics treated mothers were separated and assigned to either formula feeding (FF) or breastfeeding (BF) forming four experimental groups: AB + formula-feeding (AB-FF, N = 13) AB + breastfeeding (AB-BF, N = 8), Water + formula-feeding (CON-FF, N = 9), and CON + breastfeeding (CON-BF, N = 8).

- Formula-feeding was performed as per METHODS AND MATERIALs. In Exp-1 we administered 0.08 ml formula for pups >2g, and 0.06 for pups 2g every 3 hours throughout the entire study. In later experiments this dose was changed, and feeding intervals were increased to every 4 hours between midnight and 08 AM.
- 29 of 38 pups survived the treatments.
- Those that did not survive were euthanize because of esophageal perforation during feeding.
- After fine-tuning of the feeding methods we were able to feed the pups without further harm.

- We never had to euthanize a pup that reached our humane endpoints, since all the surviving pups were in generally good shape.
- While significantly lighter than their breastfed controls, all pups increased their bodyweight during the experiment, attesting to the efficacy of our feeding regiment.
- The pups were physically active and responded to changes in light and physical stimuli.
- Skin color remained bright purple and fur development was visible throughout the experiment.
- No signs of intestinal inflammation were evident from the visual inspection of intestines during sampling. Our subjective observation of the intestines were small degrees of bloating in formula-fed pups.