Effect of sow antibiotic treatment and offspring diet on microbiota and gut barrier throughout life

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BACKGROUND:

• intestinal function

• intestine and body health
The ‘golden triangle’ of intestinal interactions

Intestinal physiology and digestion → Host health & performance → Intestinal mucosal immunity → Intestinal Ecology

(Smidt, 2012. DPP, Keystone, CO, USA)
Functions of the intestine

**Physiology**
- to absorb nutrients, electrolytes, and water
- to secrete electrolytes & water
- to act as a **selective barrier** (control of harmful antigens & pathogens entry)

**Microbiology**
- to harbour & feed commensal bacteria
- to limit pathogen growth/effects (colonisation resistance)

**Immunology**
- to tolerate self- and harmless antigens (food, commensal bacteria)
- to mount appropriate immune responses to pathogens and harmful antigens

(http://www.vetmed.vt.edu/education/curriculum/vm8054/Labs/Lab19/Lab19.htm)
Importance and complexity of gut barrier function

<table>
<thead>
<tr>
<th>Protection Mechanisms</th>
<th>Secreted into the lumen</th>
<th>Epithelial barrier</th>
<th>Lamina propria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucus</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Defensins</td>
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<tr>
<td>Immunoglobulin A (IgA)</td>
<td></td>
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<tr>
<td>Digestive enzymes and peptides</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Epithelium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- enterocytes/colonocytes</td>
<td></td>
<td></td>
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<tr>
<td>- tight junctions/proteins (e.g. occludin, claudins, zonula occludens)</td>
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<tr>
<td>- cell protection systems (e.g. HSP)</td>
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<tr>
<td>Immune cell network (intra-epithelial lymphocytes, dendritic cells, T and B lymphocytes, mast cells, etc.)</td>
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</tr>
</tbody>
</table>

Developmental Origin of Health & Disease (DOHaD)

Common phenotype → NO GIT on this picture!

Role of GIT in food-induced metabolic disorders and obesity


→ Critical role of the GIT in the onset and development of metabolic diseases and obesity!

Questions

What are the physiological consequences of neonatal disturbances in gut colonization in pigs:

• Before and after weaning (short-term)?

• In adulthood (long-term; model for humans)?
  • With a balanced diet
  • With an unbalanced diet (e.g. high fat)?

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Antibiotic Administration Early in Life Impairs Specific Humoral Responses to an Oral Antigen and Increases Intestinal Mast Cell Numbers and Mediator Concentrations7

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→ Indication from rodent studies of short-term alterations in barrier function
→ Very little data on long-term consequences of early disturbances (imprinting)
Intestinal and colonic physiology

Small intestine & colon

Permeability (para-and trans-cellular)

Absorptive & secretory capacities

Key-enzyme activities

Ussing chambers
THE SWINE MODEL OF ANTIBIOTIC-INDUCED GUT DISTURBANCES
Protocol for short-term and long-term effects of perinatal antibiotic treatment in pigs

CTL sows (n=12)

- Amoxicillin (40 mg/kgBW/d)

ATB sows (n=11)

- Pre-weaning
- Post-weaning
- Growing

Short-term

Long-term

CTL – Low fat

ATB – Low fat

CTL – High fat

ATB – High fat

(JPL, 7/13)
Antibiotic treatment affects sows’ faecal microbiota composition

Amoxicillin
d -10 to d +21 around parturition
SHORT-TERM EFFECTS OF MATERNAL ANTIBIOTIC TREATMENT ON GUT FUNCTION
DISTAL ILEUM
Maternal antibiotic treatment reduces microbial diversity of offspring ileum transiently

Microbial diversity in ileum of ATB offspring is lower at day 14 (P<0.05)
Maternal antibiotic treatment impacts ileal microbiota composition of offspring

Microbial composition in ileum of ATB offspring is affected
Ileal paracellular permeability is transiently increased in offspring born to antibiotic-treated sows

Effect of peripartum antibiotic previously studied in a rat model: a mixture of antibiotics (mainly against Gram-negative bacteria and anaerobes) given to dams during late gestation and lactation increases in vivo permeability to macromolecules in rats pups aged 14d (Fak et al., AJ P 2008)
Ileal IAP is transiently decreased in offspring born to antibiotic-treated sows

IAP of ileal mucosa (µg/g tissue)
(A,B \ P < 0.01)
(Treat. \ P=0.12, \ Age \ P<0.003, \ T*A \ P=0.003)
Ileal HSP70 is transiently decreased in offspring born to antibiotic-treated sows

Treatment: $P=0.003$; Age: $P=0.072$; $T\times A$: $P=0.009$

<table>
<thead>
<tr>
<th></th>
<th>d14</th>
<th>d28</th>
<th>d42</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls sows</td>
<td>a</td>
<td>ab</td>
<td>bc</td>
</tr>
<tr>
<td>Antibiotics-treated sows</td>
<td>bc</td>
<td>c</td>
<td>bc</td>
</tr>
</tbody>
</table>

HSP70/bActin, Ileum
Intestinal immune responses and transcriptomics in offspring born to antibiotic-treated sows

- Gut-associated lymphoid tissue (GALT) in ATB offspring is influenced by maternal antibiotic treatment (at d21)

- Genes are differentially expressed in ileal tissue of ATB offspring (d21 & d42)

→ See oral presentation by Dr S. Ferret-Bernard in this session
PROXIMAL COLON
Maternal antibiotics does not affect microbial diversity of offspring colon

Microbial diversity in colon of ATB offspring is NOT affected

→ Microbial diversity in colon of ATB offspring is NOT affected
Maternal antibiotic treatment impacts colonic microbiota composition in offspring (ST)
Under oxidative stress, colonic transcellular permeability is transiently increased in ATB offspring

Treatment x Age: P=0.066

Controls sows
Antibiotics-treated sows
Colonic HSP70 is increased in offspring born to antibiotic-treated sows

Treat. : P=0.0027 ; Age : P=0.7082 ;
Treat.*Age : P=0.5688

Same results for inducible HSP27
Conclusions on short-term effects

Transient and successive modifications in gut physiology:

• Possibly detrimental early in life (d14)
• Oriented towards lower (bacterial) stress on the ileum
• Oriented towards higher (bacterial) stress on the colon
LONG-TERM EFFECTS OF MATERNAL ANTIBIOTIC TREATMENT ON GUT FUNCTION
Maternal antibiotic treatment has little effects on ileal and colonic microbiota in adult offspring

➔ Tendency for an interaction between ATB treatment and offspring adult diet (P=0.065):

➔ No effects on colonic microbiota
DISTAL ILEUM
Ileal paracellular permeability is increased in CTL offspring but not in ATB offspring

- Absence of change in gut permeability under a high fat diet linked to differences in gut microbiota already demonstrated in rodents:
  - increased permeability induced by a HF diet during 4 weeks in mice prevented by the use of broad spectrum antibiotics (Cani et al., Diabetes 2008),
  - rats resistant to obesity under 8 weeks of a HF diet characterized by absence of gut barrier defaults and reduced levels of Proteobacteria compared to rats prone to obesity (de La Serre et al., AJ P 2010)

- Here we show for the first time that early life colonization can also impact the gut barrier function response to a high fat diet in adults
Jejunal (but not ileal) IAP is lower in adult offspring born to antibiotic-treated sows.

Data suggest regional imprinting of intestinal functions (e.g. IAP).
NO long-term effects of maternal antibiotic treatment on offspring ileal HSPs
PROXIMAL COLON
Colonic paracellular permeability is decreased with HF diet in CTL but not ATB offspring

Treatment X Diet: $P = 0.009$

FD4 - Colon (ng/cm²/h)

Control sows

Antibiotics-treated sows
Under oxidative stress, colonic transcellular permeability is lower in ATB offspring

Treatment: $P = 0.047$

(Other factors & interactions: NS)
NO long-term effects of maternal antibiotic treatment on offspring colonic HSPs

Colonic HSP70

All factors: NS

HSP70/βActin, Colon

Controls sows
Antibiotics-treated sows

LF
HF
Conclusions on long-term effects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Site</th>
<th>Perinatal antibiotics</th>
<th>Adult diet (HF)</th>
<th>ATBQ * AD interaction</th>
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</thead>
<tbody>
<tr>
<td>Villus-crypt architecture</td>
<td>J,I</td>
<td>no</td>
<td>no</td>
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<tr>
<td>Alkaline phosphatase</td>
<td>J,I</td>
<td>YES</td>
<td>no</td>
<td>YES</td>
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<tr>
<td>Aminopeptidase N</td>
<td>J,I</td>
<td>no</td>
<td>no</td>
<td>no (≠J)</td>
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<td>Dipeptidyl-peptidase IV</td>
<td>J,I</td>
<td>YES</td>
<td>no</td>
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<tr>
<td>Sucrase</td>
<td>J,I</td>
<td>no</td>
<td>YES</td>
<td>YES</td>
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<tr>
<td>HSP 27 and HSP 70</td>
<td>I</td>
<td>no</td>
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<td>Paracellular perm.</td>
<td>I,Co</td>
<td>no</td>
<td>no</td>
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<tr>
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<td>I,Co</td>
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<td>Co</td>
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<td>no</td>
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<tr>
<td>PC/TC perm. Ratio</td>
<td>Co</td>
<td>YES</td>
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<td>Basal Isc current</td>
<td>I</td>
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<tr>
<td>Na⁺-Glucose absorpt.</td>
<td>I</td>
<td>no</td>
<td>no</td>
<td>YES</td>
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<tr>
<td>Carbachol-Cl⁻ secretion</td>
<td>I</td>
<td>no</td>
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</table>

OxS: oxidative stress (monochloramine)

→ Perinatal disturbances in mother’s microbiota DO HAVE long-lasting SELECTIVE and REGIONAL effects on key functions of offspring gut
CONCLUSIONS AND PERSPECTIVES
Conclusions

1/ Early disturbances in GIT bacterial colonisation (induced by maternal treatment with antibiotics) have significant consequences on various facets of GIT function during GIT development and also later in life.

2/ The affected functions include:
• Ileal and colonic permeability
• Key-intestinal enzymes (e.g. IAP)
• Cytoprotection systems (e.g. HSP; short-term only)
• Intestinal immunity & transcriptomics (short-term)
1/ Correlations between microbiota composition and physiology in small intestine and colon (coll. WUR)

2/ Underlying regulatory pathways
   * target genes: mRNA levels
   * epigenetic modifications (e.g. IAP, Klf4)

3/ Can probiotics (e.g. L. amylovorus) restore normal gut physiology after antibiotic-induced disorders (ongoing Interplay exp. 2)?
Thank you for your attention!

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