Stress during gestation alters immune cell numbers but not immunoglobulins in mammary secretions of sow

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Stress during gestation alters immune cell numbers but not immunoglobulins in mammary secretions of sows

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Research has received funding from the EU FP7 Prohealth project (no. 613574)
Context: Prenatal stress, neonatal health and survival

Placental morphology *(pig)* and endocrine and trophic functions *(rodents, primates)*
Fetal ontogenic development *(rodents, primates, ungulates)*
Fetal growth *(rodents, primates)*
Humoral and cellular factors of maternal immunity in swine

- **Immunoglobulins**
  - Colostrum: mainly IgG derived from sow serum
  - Milk: mainly IgA synthesized locally in MG

- **Cells**
  - Polymorphonuclear cells (PMN)
  - T and B lymphocytes
  - Monocytes / macrophages

- **Immunomodulating and non-specific antimicrobial components**
  - Cytokines, chemokines
  - Milk: lactoferrin, transferrin, lysozyme, lactoperoxidase

- **From 0 to 24-36 h**
  - Transcytosis across the epithelial cells of the gut of colostral immunoglobulins, cytokines and immune cells

- **During lactation**
  - Protection from digestive pathogens
Context

Impact of stress of pregnant females on colostrum and milk production

- Effects of experimental or natural exposure to heat stress during gestation in cows
  - Decreased colostrum and milk yield,
  - Decreased fat and protein content
  - Decreased IgG and IgA content

- Effect in other animals but cows?

- Effects of other experimental stressors (more “psychological” stress factors)?

- And apart immunoglobulins?
Context: Stress of pregnant sows in intensive pig husbandry

- Consequences of these natural stressful conditions on passive immunity transfer?

- Housing during gestation
- Maternal welfare and health
- Passive immunity in colostrum and milk
Experimental design

- 3 successive batches of pregnant Large-White x Landrace sows

**Conventional housing**
- Group housed
- (2.4 m²/sow, on slat)
- C, n=50

**Enriched housing**
- Group housed
- (3.4 m²/sow, on deep straw)
- E, n=56

**Conventional farrowing crates**

Pre-weaning mortality 26%

18 C sows and 19 E sows
Experimental design

- Conventional farrowing crates
- Conventional housing
- Enriched housing

- Saliva at 8.00 a.m => cortisol
- Colostrum and milk => IgG, IgA, leukocytes analysis by flow cytometry
Greater stress level in the conventional system during gestation

Salivary cortisol

Sow physiological state

Conventional
Enriched

MERLOT ELODIE / Influence of gestation housing system on maternal stress and transfer of maternal immunity
## Colostrum and milk immune composition

Comparable immunoglobulin content in mammary secretions of C and E females.

<table>
<thead>
<tr>
<th></th>
<th>System</th>
<th></th>
<th>C</th>
<th>E</th>
<th>sem</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Colostrum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(lactation day 0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG, g/L</td>
<td></td>
<td></td>
<td>67</td>
<td>52</td>
<td>27</td>
<td>ns</td>
</tr>
<tr>
<td>IgA, g/L</td>
<td></td>
<td></td>
<td>9</td>
<td>8</td>
<td>4</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Milk</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(lactation day 4)</td>
<td></td>
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<tr>
<td>IgG, g/L</td>
<td></td>
<td></td>
<td>1.0</td>
<td>0.8</td>
<td>0.6</td>
<td>ns</td>
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<tr>
<td>IgA, g/L</td>
<td></td>
<td></td>
<td>2.5</td>
<td>2.1</td>
<td>0.8</td>
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</tr>
</tbody>
</table>

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Colostrum and milk cellular composition

Colostrum and milk sample analysis strategy

Raw acquisition

SSC = structure

FSC-A = size

Identification of polynuclear and mononuclear cell populations

Selection of DNA containing event (Vybrant DyeCycle Ruby stain)

5% in colostrum

14% in milk

APC fluorescence

R3

R2

FSC-A = size

FSC-A = size

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Relative proportions of immune cells in colostrum and milk

Comparable proportions of leukocytes in C and E milk and colostrum samples

65% of the cells are CD45+ (leukocytes)
Relative proportions of immune cells in colostrum and milk

Comparable proportions of monocytes and T lymphocytes

<table>
<thead>
<tr>
<th></th>
<th>L₀</th>
<th>L₄</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>R2 gate</td>
<td>C</td>
<td>E</td>
<td></td>
</tr>
<tr>
<td>% CD2⁺CD8⁺low (memory T cells)</td>
<td>27</td>
<td>24</td>
<td>27</td>
</tr>
<tr>
<td>% CD2⁺CD8⁺high (T CD8 lymphocytes)</td>
<td>28</td>
<td>33</td>
<td>44</td>
</tr>
<tr>
<td>% CD8⁻CD4⁺ (T CD4 lymphocytes)</td>
<td>22</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>% CD172⁺ (monocytes)</td>
<td>31</td>
<td>37</td>
<td>19</td>
</tr>
</tbody>
</table>
Relative proportions of immune cells in colostrum and milk

Comparable proportions of polynuclear cells in E and C samples

<table>
<thead>
<tr>
<th></th>
<th>L0</th>
<th>L4</th>
</tr>
</thead>
<tbody>
<tr>
<td>R3 gate</td>
<td>C</td>
<td>E</td>
</tr>
<tr>
<td>% CD172⁺ (polynuclear cells)</td>
<td>63</td>
<td>73⁺</td>
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<tr>
<td></td>
<td>58</td>
<td>51</td>
</tr>
</tbody>
</table>

FSC-A = size
SSC = structure

R3 gate

INRA
Total cell numbers in colostrum and milk

Greater total number of cells in E compared to C in milk samples.

**Cells /mL colostrum at farrowing**

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<thead>
<tr>
<th></th>
<th>Conventional</th>
<th>Enriched</th>
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</thead>
<tbody>
<tr>
<td>Value</td>
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<td></td>
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<tr>
<td>Units</td>
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</table>

**Cells / mL milk at L4**

<table>
<thead>
<tr>
<th></th>
<th>Conventional</th>
<th>Enriched</th>
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</thead>
<tbody>
<tr>
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<td>Units</td>
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*
What can be the mechanism for increased cell numbers in the milk of E sows?

Subclinical mastitis? Other health disturbance?

- No sign of mastitis
- Furthermore E sows had lower blood markers of inflammation during late gestation (data not shown)

An effect of milk “dilution”?

- But IgG, IgA and nutritional compounds (protein, lipid and lactose %) were unaltered…

Effect of stress on hormonal control of immune cell homing to the mammary gland?

- Prolactin can be increased by chronic stress (rodents) and controls the migration of immune cells to the MG in non pathogenic state
Conclusion

Thus, chronic stress of pregnant sows reared in a poor environment is associated to:

- increased neonatal mortality of the piglets
- lower transfer of maternal immune cells to the young in the milk

Whether there is a causal relationship between these phenomena is not known.

Thanks for your attention!