Gene expression profiles of immune cells under the influence of bovine trophoblast cell derived extracellular vesicles

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Embryonic and Early Foetal Losses in Cattle and Other Ruminants

MG Diskin and DG Morris

Teagasc, Animal Production Research Centre, Mellows Campus, Athlone, Co. Galway, Ireland
Embryonic loss is an important problem in the cattle industry.

Review article

Pivotal periods for pregnancy loss during the first trimester of gestation in lactating dairy cows

Milo C. Wiltbank, Giovanni M. Baez, Alvaro Garcia-Guerra, Mateus Z. Toledo, Pedro L.J. Monteiro, Leonardo F. Melo, Julian C. Ochoa, José E.P. Santos, Roberto Sartori

Departments of Dairy Science, University of Wisconsin–Madison, Madison, Wisconsin, USA
Department of Animal Science, University of São Paulo, Piracicaba, SP, Brazil
Department of Animal Sciences, University of Florida, Gainesville, Florida, USA
Embryonic loss is an important problem in the cattle industry. Understanding of mechanisms that regulate placental and embryonic development is relevant to this industry. Immune regulatory interactions between placental and maternal immune cells. Fetal-maternal cross-talk → Release and uptake of EVs.
Embryonic loss is an important problem in the cattle industry.

Understanding of mechanisms that regulate placental and embryonic development is relevant to this industry.

Immune regulatory interactions between placental and maternal immune cells.

Fetal-maternal cross-talk \(\rightarrow\) Release and uptake of EVs.

*Mongojo-Tortajada et al., 2014*
Hypothesis

Changes in the gene expression profile of maternal immune cells

- IL-1
- IL-2
- IL-4
- IL-5
- IL-6
- IL-8
- IL-10
- IL-12
- IL-13
- IL-15
- IL-17
- IL-18
- IL-23
- IFN-γ
- TNF-α
- TGF-β
- GM-CSF
- FoxP3
- T-bet
- GATA
- GATA-3
- CD25
- CD28
- CD152

- CD4+ γδ TCR+
- CD8+
- CD14+
- γδ TCR+
- CD14+
- CD14+
- CD14+
**Methods**

**Goal:** Determine *gene expression profile* of immune cells under influence of placental EV

- Isolation and culture of trophoblast cells
- EV isolation from conditioned media*

- Isolation of immune cells from blood
- Flow cytometry
  - Staining and sorting
  - Specific cell populations

- Culture with EVs
  - 48h

- Quantitative RT-PCR

*Théry et al., 2006
Experimental Design

CD4+/CD25+ Cells

- Trophoblast-derived EVs
- Whole supernatant
- Supernatant without EVs
- Negative control

CD4+/CD25- Cells

- Trophoblast-derived EVs
- Whole supernatant
- Supernatant without EVs
- Negative control

CD8+ Cells

- 1 Animal

Monocytes

γ/δ-T cell
Results

**Gata3 Gene Expression**

<table>
<thead>
<tr>
<th>Condition</th>
<th>CD4+CD25-</th>
<th>CD4+CD25+</th>
<th>CD8+</th>
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</thead>
<tbody>
<tr>
<td>no EV</td>
<td>-0.091</td>
<td>-0.32</td>
<td>-0.53</td>
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<tr>
<td>EV</td>
<td>-1</td>
<td>-0.8</td>
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<td>no EV</td>
<td>0.11</td>
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**Foxp3 Gene Expression**

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<th>CD4+CD25+</th>
<th>CD8+</th>
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<tbody>
<tr>
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<td>0.66</td>
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<tr>
<td>EV</td>
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<tr>
<td>no EV</td>
<td>-1.5</td>
<td>-1</td>
<td>1.41</td>
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**Suppress cell-mediated immunity**

<table>
<thead>
<tr>
<th>Condition</th>
<th>CD4+CD25-</th>
<th>CD4+CD25+</th>
<th>CD8+</th>
</tr>
</thead>
<tbody>
<tr>
<td>no EV</td>
<td>Downregulated</td>
<td>Downregulated</td>
<td>Upregulated</td>
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</table>
Results

Suppress cell-mediated immunity

<table>
<thead>
<tr>
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<th>CD4+CD25-</th>
<th>CD4+CD25+</th>
<th>CD8+</th>
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<tbody>
<tr>
<td>no EV</td>
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<td>Downregulated</td>
<td>No change</td>
</tr>
<tr>
<td>EV</td>
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</table>
**Results**

**Stimulate cell-mediated immunity**

<table>
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<th>CD4+CD25-</th>
<th>CD4+CD25+</th>
<th>CD8</th>
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<tbody>
<tr>
<td>No change</td>
<td></td>
<td>Downregulated</td>
<td>Upregulated</td>
</tr>
</tbody>
</table>

**IL2 Gene Expression**

**IL17 Gene Expression**
Summary

Trophoblast-derived EVs modulate immune cells gene expression.

Trophoblast-derived EVs are potentially related to shift from a stimulant to a suppressive immune response.

Trophoblast-derived EVs most likely are important agents to regulate a successful pregnancy.

Perform quantitative RT-PCR of four more animals.

If results are consistent, design and perform *in vivo* experiment.

Future directions
Acknowledgements

Committee
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John Stevens, PhD
Zhongde Wang, PhD

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