Effect of MAPK Inhibitors on Meiotic Maturation and Embryonic Development in Pigs

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“Reprogramming of somatic cell differentiation by nuclear transplantation”

Oocytes matured in vitro

Embryo transfer

Cell culture

Enucleation and nuclear transfer

Cloned piglets:
- transgenic animals
- disease models
- xenotransplantation

Embryonic stem cell lines

Regenerative medicine
Treatment of nuclear donor cells with modifiers of epigenetic factors increases development of nuclear transfer embryos

(Research being conducted by Limei Che)
**Hypothesis and objectives**

**Hypothesis:**
- The blockage of the meiotic resumption for 22 h with inhibitors of MAPK pathway will improve developmental rate of porcine embryos in vitro

**Objectives:**
- Verify whether inhibitors of MAPK pathway (*U0126 and SB203580*) prevent meiotic resumption of pig oocytes
- Assess the development of embryos produced from oocytes treated with MAPK inhibitors in the presence (or not) of follicular fluid
Experimental protocol

Blastocyst stage embryos at D-7

Control  U0126  SB203580

Meiotic stage after 22 hrs IVM

Meiotic stage after 48 hrs IVM

Parthenogenetic Activation
# Effect of MAPK Inhibitors on Meiotic Maturation

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Meiotically Arested oocytes at 22hrs</th>
<th>Meiotically matured oocytes at 48hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>31.5%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.4%</td>
</tr>
<tr>
<td>MEK1/2 (U0126)</td>
<td>79.0%&lt;sup&gt;b&lt;/sup&gt;</td>
<td>57.4%</td>
</tr>
<tr>
<td>p38MAPK (SB203580)</td>
<td>37.0%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>66.7%</td>
</tr>
</tbody>
</table>

* Different letters in the same column denotes statistical significance (P<0.05)*
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Follicular fluid (20 %)</th>
<th># oocytes</th>
<th>Blastocysts (%)</th>
<th>Cell#/embryo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>+</td>
<td>61</td>
<td>45.9</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>73</td>
<td>45.2</td>
<td>45.7</td>
</tr>
<tr>
<td>U0126 [10µM]</td>
<td>+</td>
<td>58</td>
<td>36.2</td>
<td>35.1</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>51</td>
<td>43.1</td>
<td>39.6</td>
</tr>
<tr>
<td>SB203580 [10µM]</td>
<td>+</td>
<td>46</td>
<td>41.3</td>
<td>32.7</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>55</td>
<td>49.1</td>
<td>57.9*</td>
</tr>
</tbody>
</table>

In vitro development to blastocyst stage at D-7
Conclusions

• MEK1 (U0126) inhibitor prevents meiotic resumption of porcine oocytes

• Oocytes treated with inhibitors of MEK1 (U0126) and p38MAPK (SB203580) complete meiotic maturation similarly to untreated oocytes

• Development to blastocyst stage was not significantly affected by oocyte treatments
Thanks!

• To NSERC for providing me with this wonderful opportunity!

• To Dr. Bordignon for allowing me to work in his amazing lab and to learn so much!