Myoepithelial cell contraction participates in mammary epithelial cell exfoliation in cow milk

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Milk yield depends on the number of Mammary Epithelial Cell (MEC) in the mammary gland.

Proliferation / Apoptosis Balance

MEC exfoliation into milk

Knight & Peaker (1984)
Capuco et al. (2003)
Boutinaud et al. (2004)
Herve et al. (2016)
MEC exfoliation process

= Shedding of MEC from the mammary epithelium into milk

Ruminant milk contains exfoliated MEC

Associated with a loss of mammary epithelium integrity (Herve et al., 2016)
At milking, oxytocin stimulates the myoepithelial cell contraction.
Hypothesis:

The myoepithelial cell contraction at milking stimulates MEC exfoliation into milk through mechanical forces and is associated with a loss of mammary epithelium integrity.
Strategy

Atosiban

Blood vessel

OT

OT

Oxytocin

Myoepithelial cell contraction
Animals and Treatments
8 multiparous dairy cows at morning milking - Crossover experimental design

Control + OT Treatment
- OT injection (5 IU) at time 0, 12, and 15
- Milking times at 0, 12, and 15
- Standard milk
- Residual milk

Ato + OT Treatment
- Ato injection (30 mg) at time -10, 0, and 12
- OT injection (5 IU) at time 0, 12, and 15
- Milking times at 0, 12, and 15
- Cisternal milk
- Alveolar milk

OT = Oxytocin  Ato = Atosiban
Milk MEC purification

1. Milk centrifugation

2. **Purification** of milk MEC using **magnetic beads** coated with an anti-cytokeratin antibody

3. Cell count determination

4. Determination of the % of apoptotic MEC by flow cytometry analysis

*Boutinaud et al. (2008)*
Animals and Treatments
8 multiparous dairy cows at morning milking - Crossover experimental design

Control + OT Treatment
- OT injection (5 IU)
- Time (min): 0, 12, 15
- Milking

Ato + OT Treatment
- Ato injection (30 mg)
- OT injection (5 IU)
- Time (min): -10, 0, 12, 15
- Milking

+ Blood sampling for the assessment of mammary epithelium integrity by plasma lactose concentration determination
Results: MEC exfoliation

<table>
<thead>
<tr>
<th>Type of milk</th>
<th>Control + OT</th>
<th>Residual + OT</th>
<th>Cisternal + OT</th>
<th>Alveolar + OT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk yield (kg)</td>
<td>22.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.3&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Milk MEC concentration (10&lt;sup&gt;3&lt;/sup&gt; cells/mL)</td>
<td>4.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>% apoptotic MEC</td>
<td>32.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>37.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

a, b, c, d, for P < 0.05
Results: MEC exfoliation

<table>
<thead>
<tr>
<th>Type of milk</th>
<th>Milk yield (kg)</th>
<th>Milk MEC concentration (10^3 cells/mL)</th>
<th>% apoptotic MEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control + OT</td>
<td>22.9^a</td>
<td>4.9^a</td>
<td>32.6^a</td>
</tr>
<tr>
<td>Standard</td>
<td>3.0^b</td>
<td>19.3^b</td>
<td>20.5^b</td>
</tr>
<tr>
<td>Residual Ato + OT</td>
<td>5.9^c</td>
<td>6.1^a</td>
<td>28.6^ab</td>
</tr>
<tr>
<td>Cisternal Ato + OT</td>
<td>13.3^d</td>
<td>4.3^a</td>
<td>37.6^a</td>
</tr>
</tbody>
</table>

*a, b, c, d, for P < 0.05
Results: MEC exfoliation

Number of exfoliated MEC (10^6)

<table>
<thead>
<tr>
<th>Type of milk</th>
<th>Control + OT</th>
<th>Residual + OT</th>
<th>Cisternal + Ato</th>
<th>Alveolar + OT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>110</td>
<td>10</td>
<td>32.6</td>
<td>28.6</td>
</tr>
<tr>
<td>Milk yield (kg)</td>
<td>22.9^a</td>
<td>3.0^b</td>
<td>5.9^c</td>
<td>13.3^d</td>
</tr>
<tr>
<td>Milk MEC concentration</td>
<td>4.9^a</td>
<td>19.3^b</td>
<td>6.1^a</td>
<td>4.3^a</td>
</tr>
<tr>
<td>(10^3 cells/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% apoptotic MEC</td>
<td>32.6^a</td>
<td>20.5^b</td>
<td>28.6^ab</td>
<td>37.6^a</td>
</tr>
</tbody>
</table>

a, b, c, d, for P< 0.05

Milk yield and MEC concentration are significantly different among groups.
### Results: MEC exfoliation

<table>
<thead>
<tr>
<th>Type of milk</th>
<th>Control + OT</th>
<th>OT</th>
<th>Ato + OT</th>
<th>Alveolar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>110 (a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual</td>
<td>70 (bc)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cisternal</td>
<td>30 (b)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alveolar</td>
<td>130 (c)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Milk yield (kg)
- Standard: 22.9<sup>a</sup>
- Residual: 3.0<sup>b</sup>
- Cisternal: 5.9<sup>c</sup>
- Alveolar: 13.3<sup>d</sup>

#### Milk MEC concentration (10<sup>3</sup> cells/mL)
- Standard: 4.9<sup>a</sup>
- Residual: 19.3<sup>b</sup>
- Cisternal: 6.1<sup>a</sup>
- Alveolar: 4.3<sup>a</sup>

#### % apoptotic MEC
- Standard: 32.6<sup>a</sup>
- Residual: 20.5<sup>b</sup>
- Cisternal: 28.6<sup>ab</sup>
- Alveolar: 37.6<sup>a</sup>

* a, b, c, d, for P < 0.05
Less exfoliation when myoepithelial cell contraction was inhibited.

More exfoliation when myoepithelial cell contraction was induced.
Results: Mammary epithelium integrity

Plasma lactose concentration (mg/L)

Time (min)

Standard milking

Control + OT

Ato + OT

a, b, c, d, for P < 0.05
Results: Mammary epithelium integrity

Plasma lactose concentration (mg/L)

Time (min)

Ato injection

Standard milking

Milking after Ato injection

Control + OT

Ato + OT

a, b, c, d, for P < 0.05
Results: Mammary epithelium integrity

Plasma lactose concentration (mg/L)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Standard milking</th>
<th>Milking after OT injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Control + OT</td>
<td>Ato + OT</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a, b, c, d, for P < 0.05
Results: Mammary epithelium integrity

Myoepithelial cell contraction was associated with a loss of mammary epithelium integrity.
Conclusions

1. Oxytocin stimulated MEC exfoliation whereas Atosiban inhibited it.

2. The MEC exfoliation process was associated with a loss of mammary epithelium integrity.
Thank you for your attention and great thanks to:

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