Effects of red-light photostimulation on the reproductive performance of extended boar semen samples

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Introduction
What is photobiology?

Photobiology is the use of light to affect/alter cell function

What is photostimulation?

Photostimulation consists of using the cell response to light in our own interest

What is phototherapy?

Phototherapy is the application of light benefits on cells for health purposes
Previous works about the effects of photo-stimulation (laser) sperm of different animal species (mouse, human, dog, bull, fish, sheep and rabbit):


In vitro experiments
Preliminary experiments

• Blue light
• Green light
• Red light
Conditions

• Light source: LED (Light Emission Diode)

• $\lambda=620-630$ nm (red)

• Room temperature

Treatments (light-darkness-light)

• Procedure #1 (10-10-10)

• Procedure #2 (15-10-15)

• Procedure #3 (20-10-20)
In vitro capacitation and progesterone-induced acrosome exocytosis

- Motility (CASA)
- Viability (SYBR14/PI)
- Membrane lipid disorder (M540/YO-PRO-1)
- Acrosome exocytosis (PNA-FITC/PI)
- Mitochondrial membrane potential (JC-1)

*P<0.05 vs. control

Progesterone addition
*P<0.05 vs. control

Figure showing the effect of different procedures on the percentage of true acrosome exocytosis over time. The y-axis represents the True acrosome exocytosis (%) and the x-axis represents Incubation time (h). The graph includes a line for Control, Procedure #1, Procedure #2, and Procedure #3. There is a significant increase in exocytosis at the Progesterone addition point. The asterisk indicates a P<0.05 vs. control.


*P<0.05 vs. control

Progesterone addition

% High MMP sperm

Incubation time (h)
Fertility trials
Fertility trials

Best treatment following in vitro capacitation tests (10-10-10) was selected for field fertility trials:

• Photostimulation (10-10-10) was conducted at 17ºC
• AI was performed immediately after photo-stimulation
• First data were obtained with post-cervical/IUI insemination
# Fertility trials

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Farrowing rate (%)</th>
<th>Total piglets at parturition</th>
<th>Live-born piglets at parturition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>800</td>
<td>83.7</td>
<td>13.5 ± 0.2</td>
<td>12.7 ± 0.2</td>
</tr>
<tr>
<td>Photo-stimulated</td>
<td>520</td>
<td>88.1*</td>
<td>14.9 ± 0.3*</td>
<td>14.0 ± 0.2*</td>
</tr>
</tbody>
</table>

*P<0.05 vs. control

Additional *in vivo* studies
<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Farrowing rate</th>
<th>Total piglets born</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8,258</td>
<td>87.4%</td>
<td>13.2 ± 0.8</td>
</tr>
<tr>
<td>maXipig</td>
<td>4,379</td>
<td>92.1%*</td>
<td>14.1 ± 0.6*</td>
</tr>
</tbody>
</table>

*P<0.05 vs. control

Fertility trials were conducted in the following countries:

- Spain
- Belgium
- Finland

(Unpublished)
Factors that could modulate the sperm response

- Fertility-range farm response
- Season
- Semen origin
Unpublished Fertility-range farm
Fertility-range farm

![Graph showing total piglets born in different farm fertility ranges. The graph compares Control and maXipig groups.](image-url)

Unpublished
Season

Unpublished
Season

Unpublished
Semen origin

![Graph showing the comparison of farrowing rate between Control and maXipig across different semen origins.](image-url)

- **Origin 1**: maXipig significantly higher than Control.
- **Origin 2**: maXipig slightly higher than Control.
- **Origin 3**: maXipig significantly higher than Control.

*Significant difference indicated by asterisk.*
Current efforts looking into:

- Breed/line
- Individual effect
- Extender
- Prolificacy-range farms
Can we explain the light-effects?
Can we explain the light-effects?

Two hypotheses:

- Via Opsins – Transducin (Protein G signalling cascade)
- Via Cytochrome C - electron transport chain
Via Opsins – Transducin (Protein G signalling cascade)

<table>
<thead>
<tr>
<th>RET-1</th>
<th>1D4</th>
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<tbody>
<tr>
<td>KO</td>
<td>WT</td>
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</table>

Presence and immunolocalization of transducin (G\textsubscript{αt1})

Via Cytochrome C - electron transport chain

Electron Transport Chain

Mitochondria, Krebs cycle input

CCO, Cytochrome C oxidase

H+ + 1/2 O2 → H2O

ATP synthase

H+ + H+ → H2O

630 to 900 nm light

2 copper atoms
2 iron atoms

H+ absorbed by light

NADH

Succinate

UQ

Co-Q10
Conclusions
Conclusions

- Red-light photostimulation improves the sperm ability to reach the full capacitation status
- Photostimulation immediately before AI improves the reproductive performance
- Different factors (including season, boar, semen origin…) may influence the effects of photostimulation on sperm
- Although red-light photostimulation exerts its effects via a yet-unidentified mechanism, current data suggest that opsins and/or electronic chain proteins could be involved
- Further should with cryopreserved sperm and with semen from other species is warranted
Program
Scientific and applied topics of boar semen preservation and artificial insemination

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Thank you for your attention!