Quality of Stallion Sperm processed with Androcoll-E™ and their relation with *in vivo* fertility

Sandra Gamboa¹,², Andreia Quaresma¹, Fátima Castro¹, Pedro Bravo¹,², Rosa Rebordão¹, António Rocha³

¹ Agricultural School, Polytechnic Institute, Bencanta, 3040-316 Coimbra, Portugal
² CERNAS – Natural Resources, Environment and Society Centre, Coimbra, Portugal
³ ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Porto, Portugal
Quality of Stallion Sperm processed with Androcoll-E™ and their relation with in vivo fertility
Sperm

Motility

Vitality

Morphology

Acrosome
Colloidal centrifugation with Androcoll-E™ prolongs stallion sperm motility, viability and chromatin integrity


Single layer centrifugation of stallion spermatozoa improves sperm quality compared with sperm washing

J.M. Morrell, H. Rodriguez-Martinez, A. Johannisson

Single layer centrifugation of stallion spermatozoa consistently selects the most robust spermatozoa from the rest of the ejaculate in a large sample size

J. M. Morrell, H. Rodriguez-Martinez, A. Johannisson

Equine Veterinary Journal
Volume 42, Issue 7, pages 579–585, October 2010

Sperm

500 g

20°

Androcoll

SPZ
Lusitano vs Sorraia

Sorraia  

Lusitano

85.68%

34.07%

FER

Lusitano

Sorraia
SLC with Androcoll™

**Lusitano**

**Sorraia**
SLC with Androcoll™

Lusitano

two consecutive seasons

Sorraia

Upper layer
Midle layer 1
Midle layer 2
SPZ

Upper layer
Midle layer
SPZ
1. Motility

2. Viability

3. Morphology

4. Plasmalemma functionality

Methods:
- SLC-treatment
- E-N
- India ink
- HOS test
6 DNA integrity

5 Mitochondrial functionality ($\Delta \psi_m$)

7 Sperm motility: 72h of conservation at 4°C

8 Pregnancy rates

$300 \times 10^6$ sperm in INRA96

11 mares

12 mares
SLC with Androcoll™

Sperm lost

46.74% (± 29.12)  

27.56 (± 19.73)

Upper layer

Midle layer 1

Midle layer 2

SPZ

Upper layer

Midle layer

SPZ
SLC with Androcoll™

Viability

% SPZ

Lusitano  Sorraia

Upper layer

Middle layer

Pellet

* – differences between stallions

Lusitano ≠ Sorraia

Raw semen  (p<0,001)

Pellet  (p<0,01)

HOS +

% SPZ

Lusitano  Sorraia

Upper layer

Middle layer

Pellet

a, b – differences between layers

a, b – differences between stallions

Lusitano ≠ Sorraia

Raw semen  (p<0,05)

Upper layer  (p<0,01)
SLC with Androcoll™

Viability

SLC-treatment

HOS +

<table>
<thead>
<tr>
<th>Lusitano</th>
<th>Sorraia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper layer</td>
<td>Upper layer</td>
</tr>
<tr>
<td>Middle layer</td>
<td>Middle layer</td>
</tr>
<tr>
<td>Pellet</td>
<td>Pellet</td>
</tr>
</tbody>
</table>

% SPZ

↑ % live and HOS + SPZ

pellet Lusitano

upper layer Sorraia

*– differences between stallions

a, b – differences between layers

*– differences between stallions
SLC with Androcoll™

Sperm morphology

SLC - better head shape morphology

Lusitano (p<0.05)

SLC-treatment

Sorraia (ns)

*% SPZ

EAAP Annual Meeting 2013 – session 03
SLC with Androcoll™

MMP (JC-1)

Upper layer
Midle layer 1
Midle layer 2
SPZ

Upper layer
Midle layer
SPZ
SLC with Androcoll™

MMP (JC-1)

Upper layer (p<0.05)

Lusitano ≠ Sorraia

EAAP Annual Meeting 2013 – session 03
Nantes 2013
SLC with Androcoll™

MMP (JC-1)

SLC-treatment

SLC selected SPZ = Raw semen
SLC with Androcoll™

DNA integrity

Results and discussion

SLC-treatment

SCD test

TUNEL

DNA integrity

Oxidative stress

?
Lusitano vs Sorraia

Lipid - peroxidation

- Bar chart showing TBARS SPZ and TBARS PS with comparative mmoles/mg protein for PSL and Sorraia.

- Additional bar chart showing B (SPZ) with comparisons for Total TRAP, Fast TRAP, and Slow TRAP between PSL and Sorraia.
SLC with Androcoll™

Sperm Motility

Significant differences between stallions

No differences between treatments
SLC with Androcoll™

<table>
<thead>
<tr>
<th></th>
<th>DG+</th>
<th>DG-</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SLC-treated</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lusitano</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Sorraia</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td><strong>non-SLC-treated</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lusitano</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Sorraia</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>15</td>
<td>3</td>
</tr>
</tbody>
</table>

Per cycle fertility

78% - SLC-selected sperm
(9 mares; 7 cycles DG+)

89% - non-SLC-selected sperm
(9 mares; 8 cycles DG+)

\[ \chi^2(2)=0.400; \ p=0.527; \ N=18 \]

0.00% - SLC-selected sperm
(6 mares; 0 cycles DG+)

0.00% - non-SLC-selected sperm
(9 mares; 0 cycles DG+)

No differences between treatments
SLC with Androcoll\textsuperscript{TM}

- Androcoll\textsuperscript{TM} do not selected a high population of viable, morphological normal and osmotically active spermatozoa in Sorraia stallion

- In fertile stallion, the highest percentage of sperm with high mitochondrial potential (\( \uparrow \Delta \Psi \text{mit} \); JC-1) and DNA integrity (SCD test) was retained in the upper layer.

- Motility in cooled sperm is not significantly improved by SLC

- Fertility is independent of treatment with Androcoll-E\textsuperscript{TM}
Acknowledgements

Conceição Cravo
Miguel Moura
Fábio Alexandre
Manuel Vaz de Castro

Prof. Maria do Mar Oom (FCT, UNL, Portugal)
Technical Secretary of the Sorraia Horse Studbook.

Author Contributions:

S.G. planned the experiment for sperm fractions and AI trial; S.G., A.Q. and F.C. performed the experiments; S.G. and A.Q. analyzed the data; P.B. performed the ultrasonographic examinations of the mares; A.R. is the PI of the project PTDC/CVT/108456/2008

This work was supported by

Fundação para a Ciência e Tecnologia (FCT), Portugal
(PTDC/CVT/108456/2008)
Escola Superior Agrária de Coimbra, IPC
CERNAS (PEst-OE/AGR/UI0681/2011)
Thank you!
SLC with Androcoll<sup>TM</sup>

Acrosome integrity

<table>
<thead>
<tr>
<th></th>
<th>Raw semen</th>
<th>Upper layer</th>
<th>Middle layer</th>
<th>Pellet</th>
</tr>
</thead>
<tbody>
<tr>
<td>% SPZ</td>
<td>80</td>
<td>50</td>
<td>50</td>
<td>80</td>
</tr>
</tbody>
</table>

a, b – p<0.05 – differences between layers