



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

Sandra Gamboa^{1,2}, Andreia Quaresma¹, Fátima Castre¹, Pedro Bravo^{1,2}; Rosa Rebordão¹, António Rocha³

¹ Agricultural School, Polytechnic Institute, Bencanta, 3040-316 Coimbra, Portugal

² CERNAS – Natural Resources, Environment and Society Centre, Coimbra, Portugal

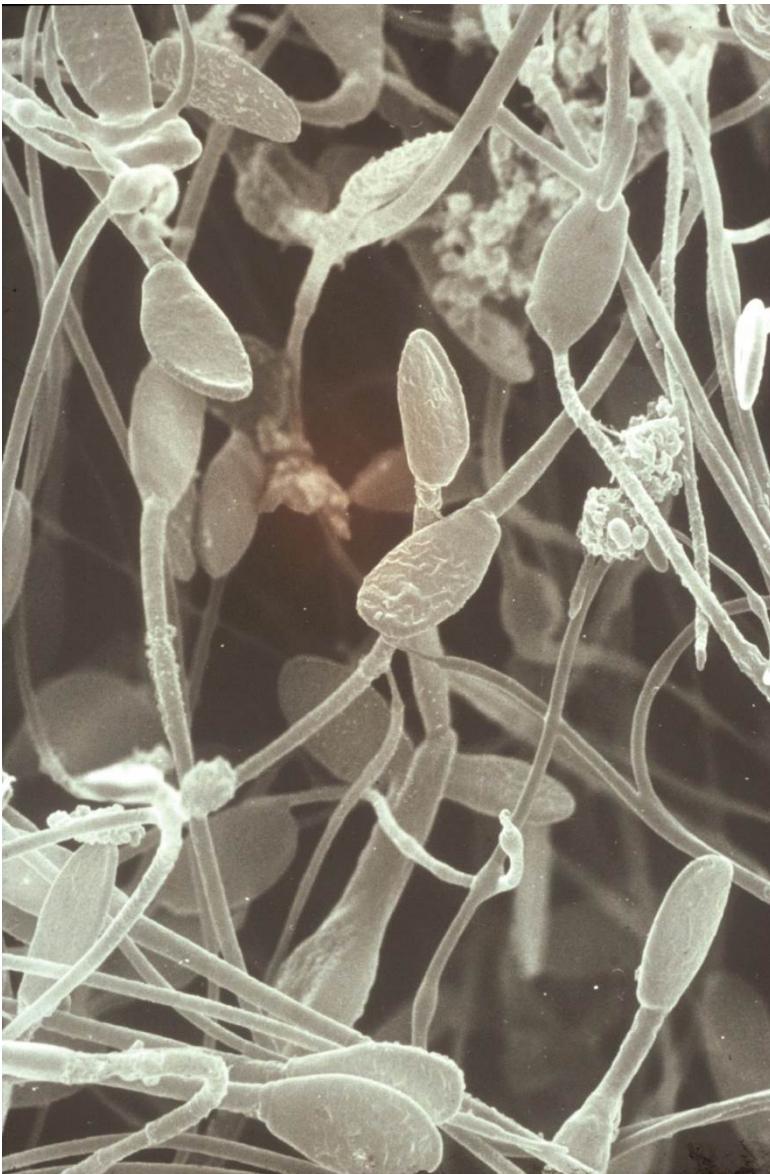
³ ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Porto, Portugal



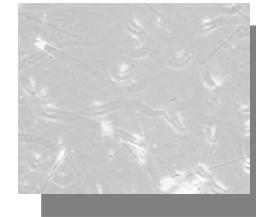
Quality of Stallion Sperm processed with Androcoll-E™ and their relation with *in vivo* fertility



Sperm



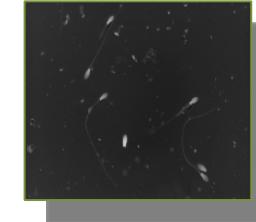
Motility



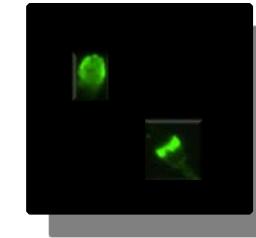
Vitality



Morphology



Acrosome



Animal Reproduction Science
Volume 116, Issue 1, Pages 119-128, November 2009

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

A. Johannsson, J.M. Morrell, J. Thorén, M. Jönsson, A.-M. Dalin, H. Rodriguez-Martinez

Reproductive BioMedicine Online

Reproductive BioMedicine Online
Volume 21, Issue 3, Pages 429-436, September 2010

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

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

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. MORRELL¹, H. RODRIGUEZ-MARTINEZ², A. JOHANNISSON¹

Article first published online: 5 JUL 2010

DOI: 10.1111/j.2042-3306.2010.00101.x

© 2010 EVJ Ltd

Issue



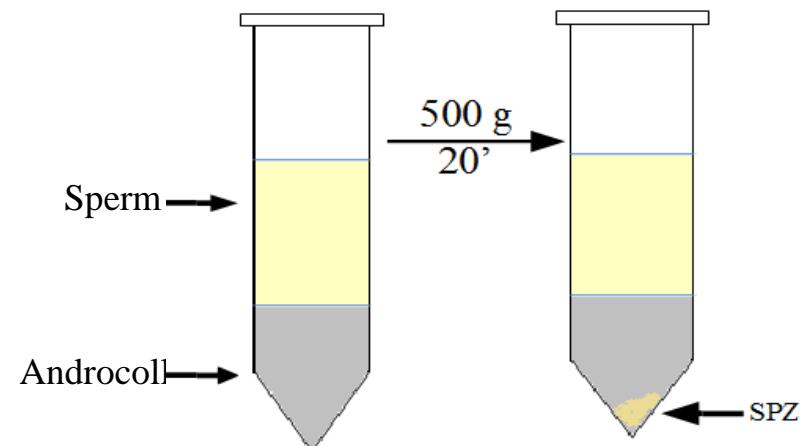
Equine Veterinary Journal
Volume 42, Issue 7, pages 579–585, October 2010

Vol.2, No.3, 159-165 (2012)
doi:10.4236/ojas.2012.23022

Open Journal of Animal Sciences

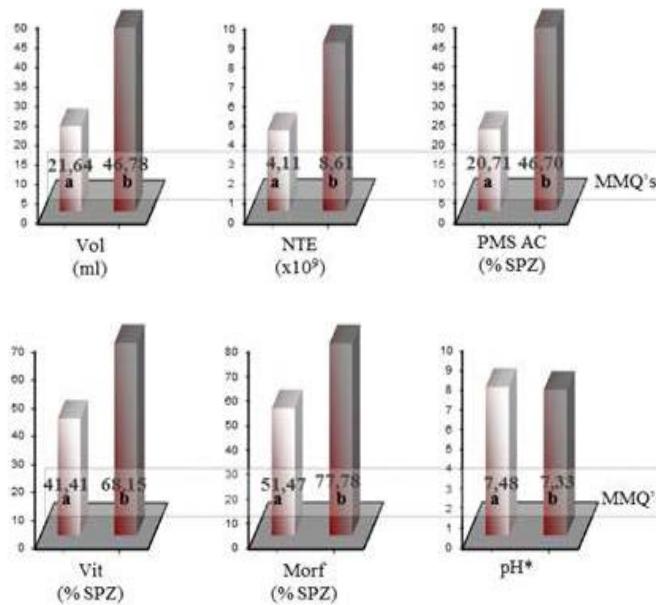
Single Layer Centrifugation with Androcoll-E™ improved progressive motility and percentage of live spermatozoa with intact acrosome of chilled stallion semen but did not have an effect on DNA integrity*

Ana Luisa Costa¹, Ana Martins-Bessa¹, Ana Rebello de Andrade², Tiago Guimarães³, Maria Rosa Rebordão⁴, Sandra Gamboa⁴, Pedro Pinto Bravo⁴, Maria José Correia⁵, Jorge Colaço¹, Isabel Galvão¹, António Rocha³

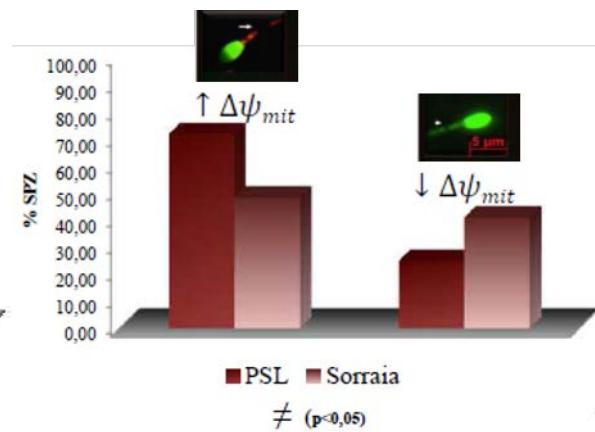
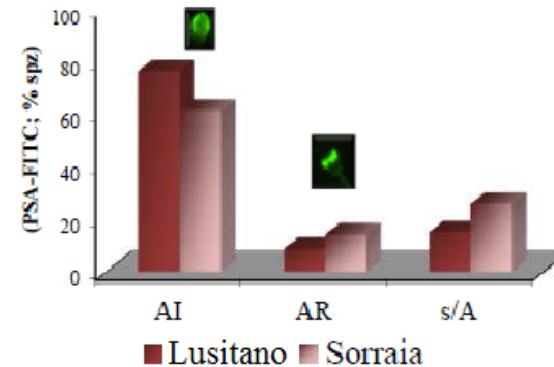


Lusitano vs Sorraia

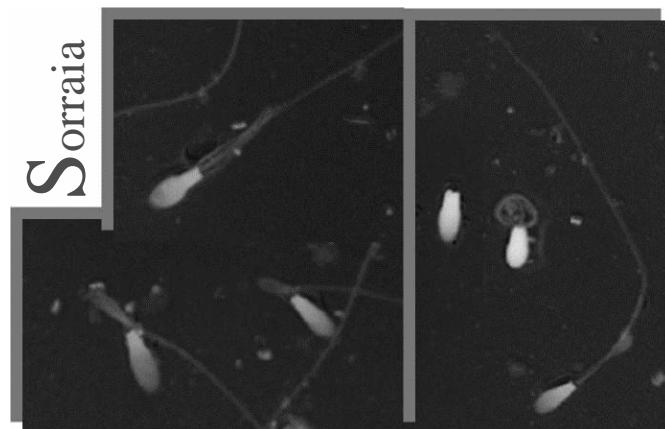
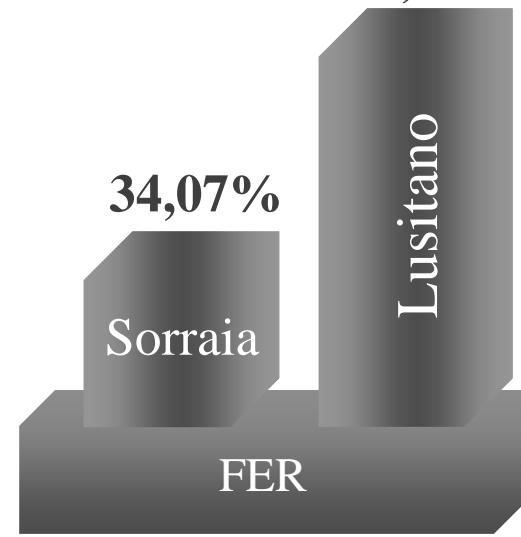
background



Sorraia Lusitano



85,68%



SLC with Androcoll™

Lusitano



Sorraia

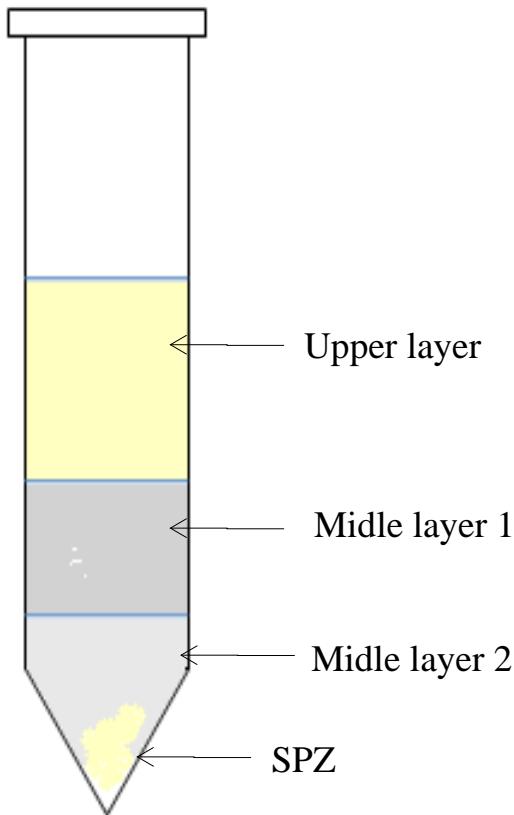


SLC with Androcoll™

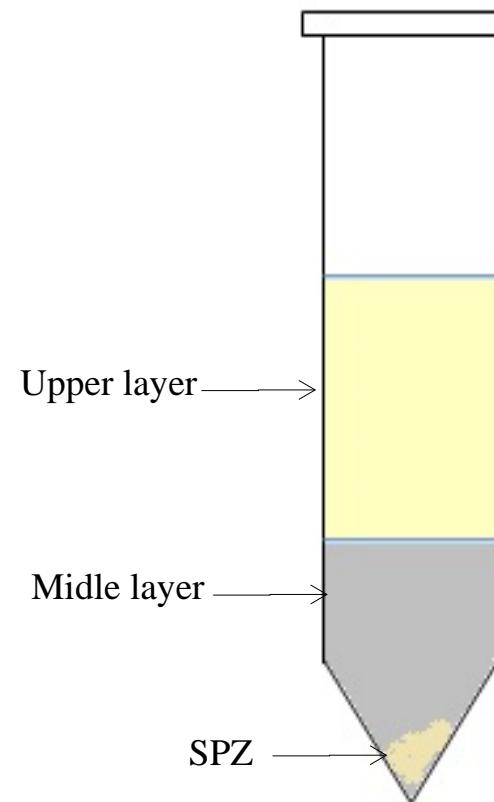
Lusitano



two
consecutive
seasons

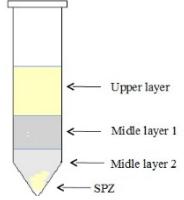


Sorraia



1 Motility

SLC-treatment



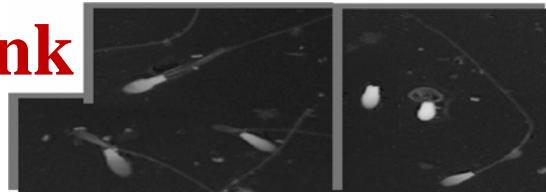
2 Viability

E-N



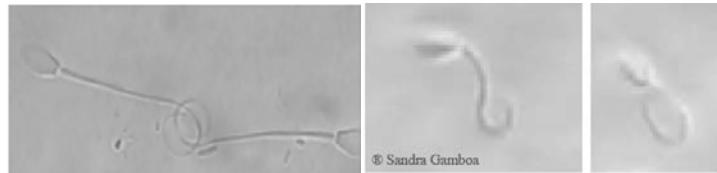
3 Morphology

India ink



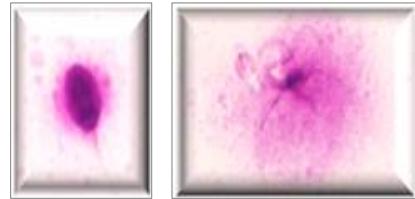
4 Plasmalemma functionality

HOS test



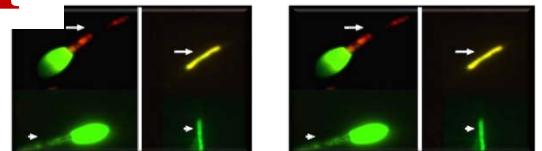
6 DNA integrity

SCD



5 Mitochondrial functionality ($\Delta\psi_m$)

JC-1



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

8 Pregnancy rates

AI

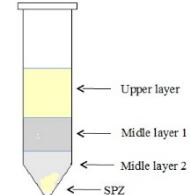
300×10^6 sperm in INRA96



11 mares

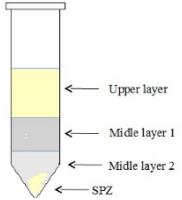


12 mares

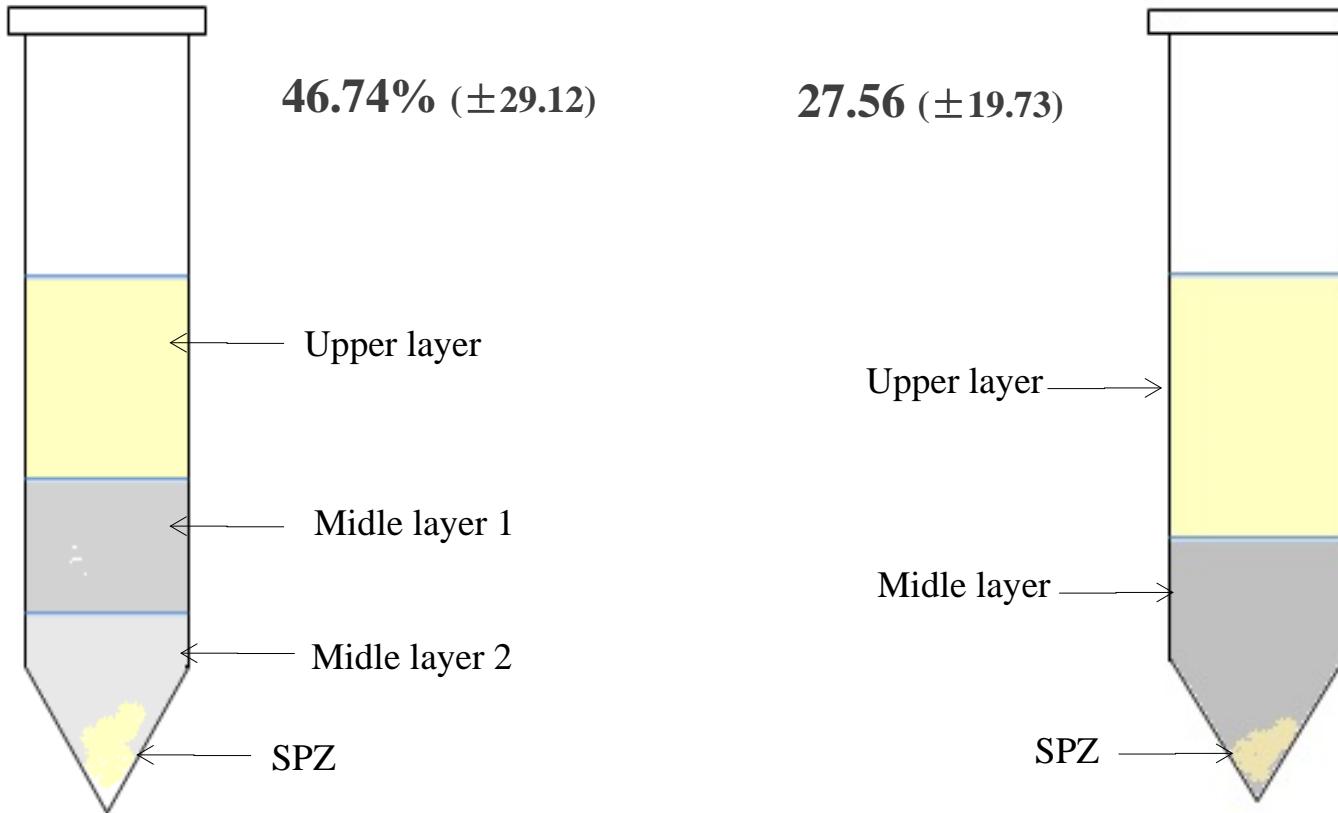


SLC with Androcoll™

SLC-treatment

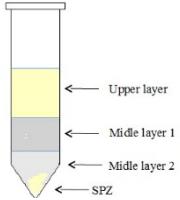


Sperm lost



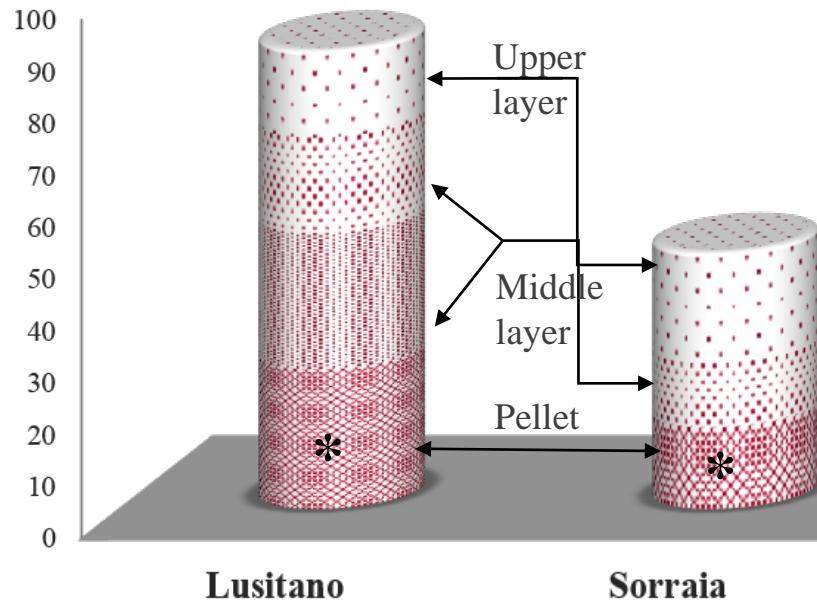
SLC with Androcoll™

SLC-treatment



Viability

% SPZ

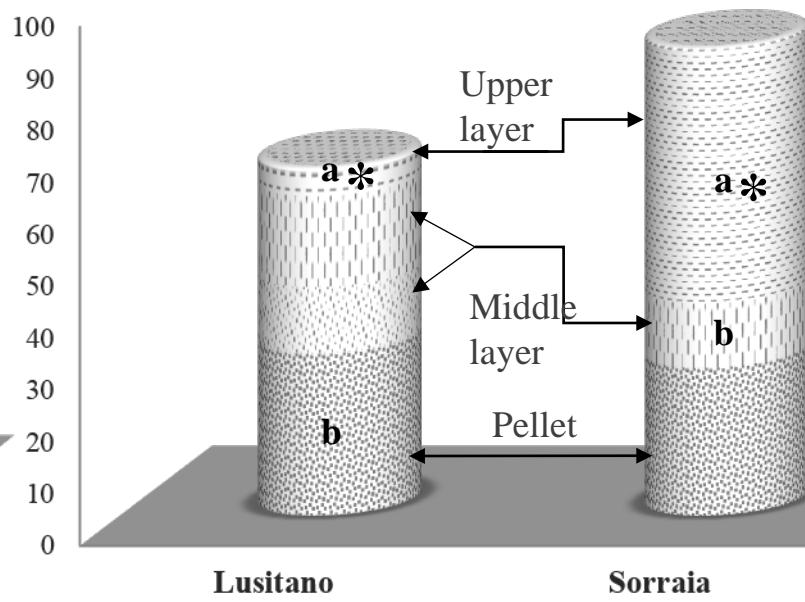


*— differences between stallions

Lusitano ≠ Sorraia

Raw semen
($p<0,001$)
Pellet
($p<0,01$)

% SPZ



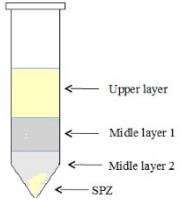
a, b – differences between layers
*— differences between stallions

Lusitano ≠ Sorraia

Raw semen
($p<0,05$)
Upper layer
($p<0,01$)

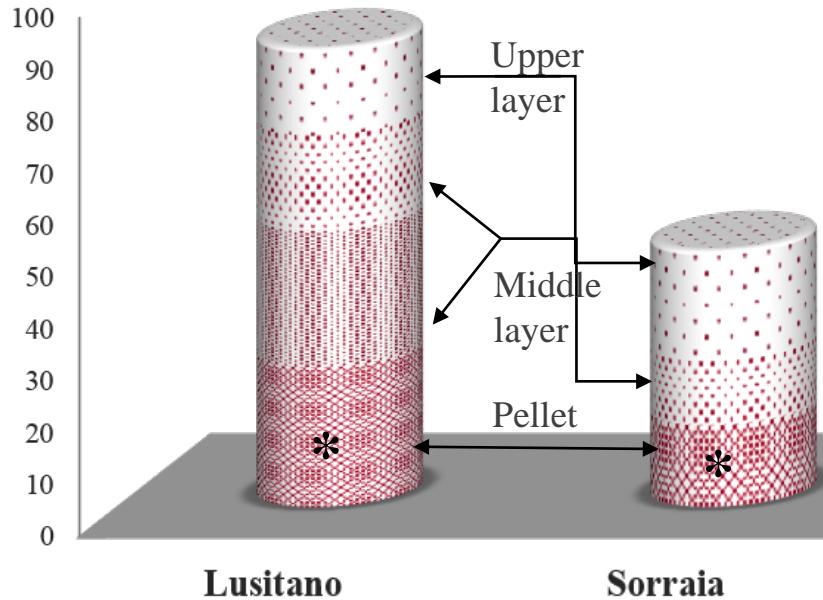
SLC with Androcoll™

SLC-treatment



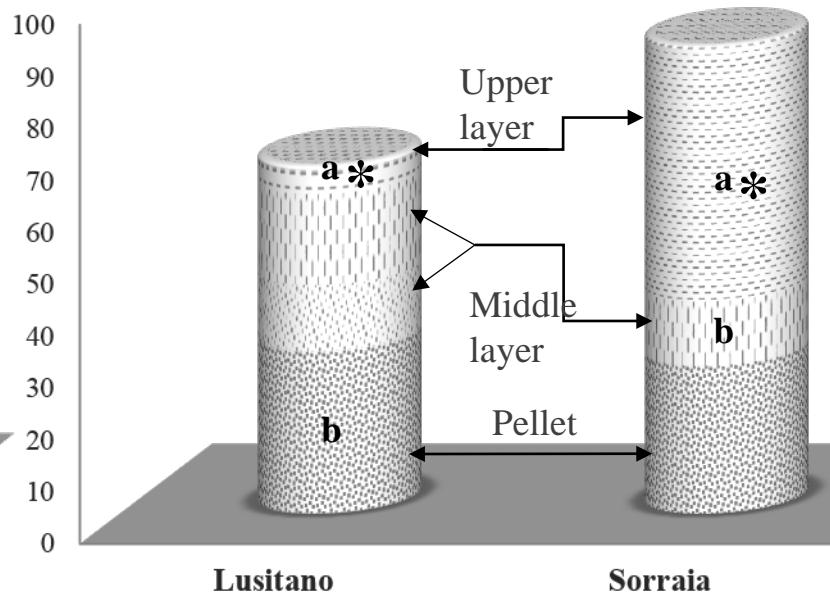
Viability

% SPZ



*— differences between stallions

% SPZ



a, b – differences between layers
*— differences between stallions

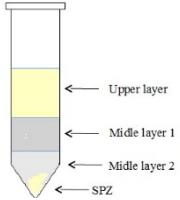
↑ % live and HOS⁺ SPZ

pellet
Lusitano

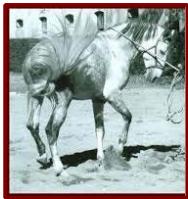
upper layer
Sorraia

SLC with Androcoll™

SLC-treatment

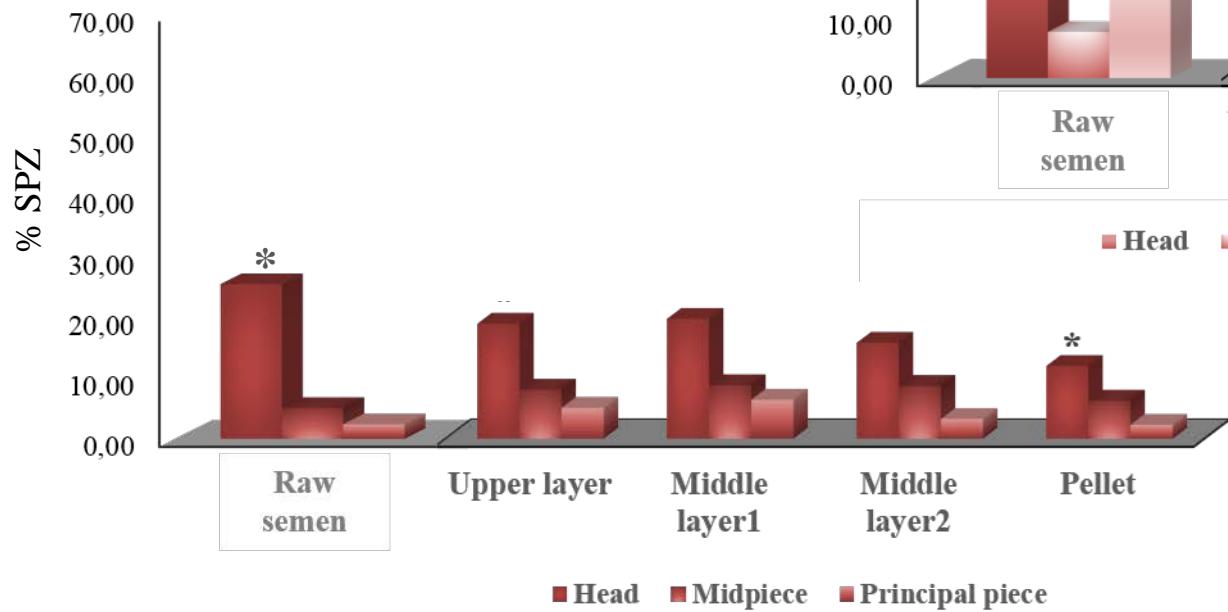


Sperm morphology



Lusitano ($p<0.05$)

SLC -better head shape morphology



% SPZ

70,00
60,00
50,00
40,00
30,00
20,00
10,00
0,00

Raw
semen

Upper layer

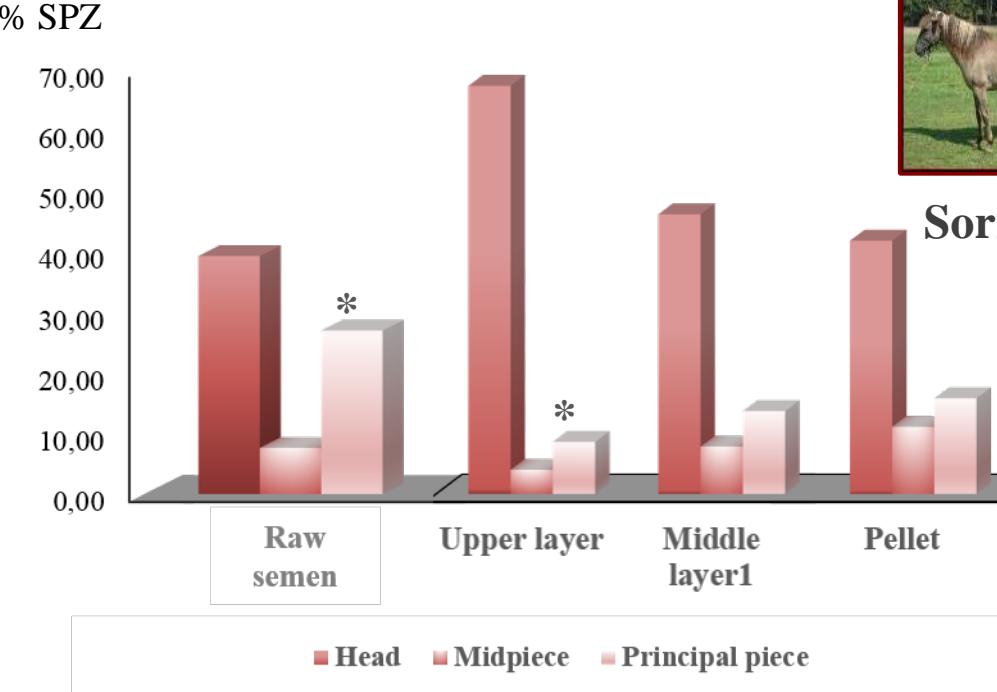
Middle
layer1

Pellet

■ Head ■ Midpiece ■ Principal piece



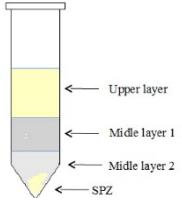
Sorraia (ns)



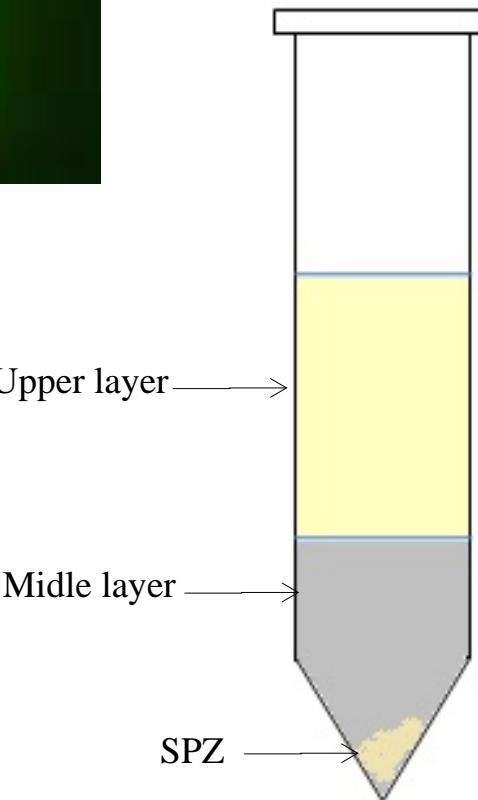
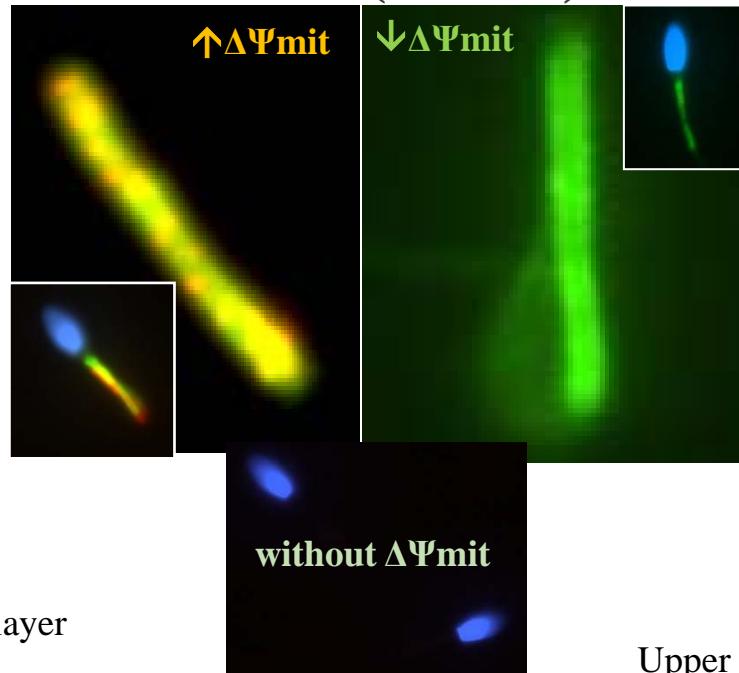
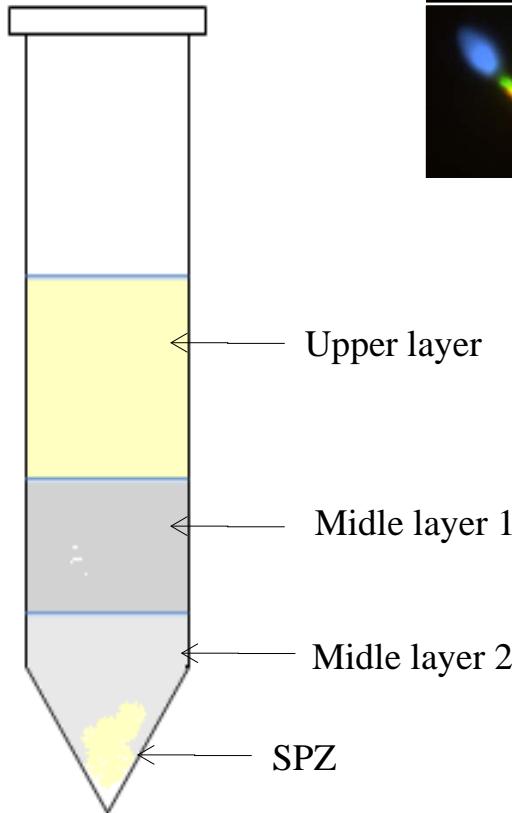
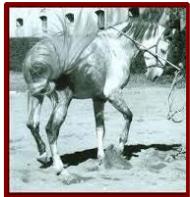
■ Head ■ Midpiece ■ Principal piece

SLC with Androcoll™

SLC-treatment

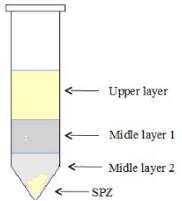


MMP (JC-1)

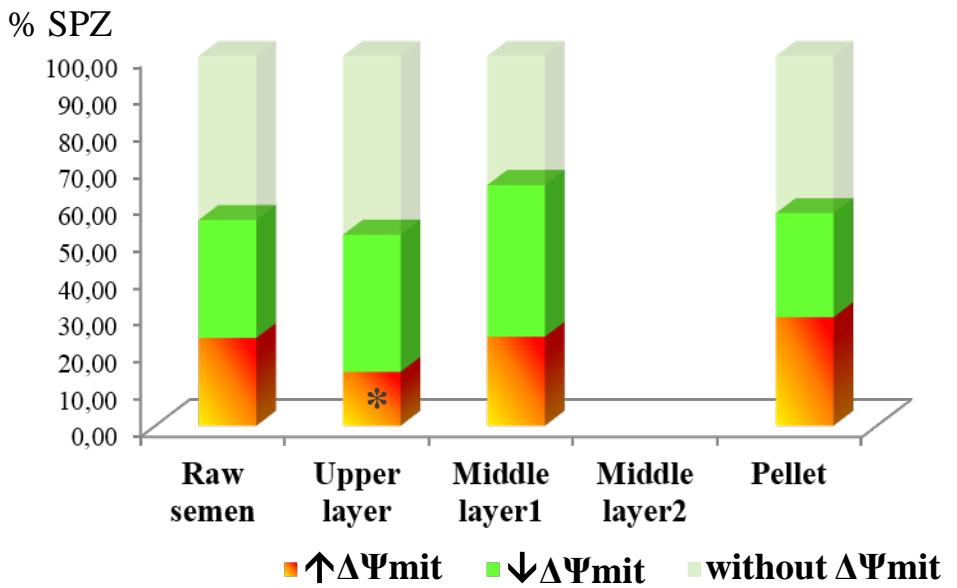
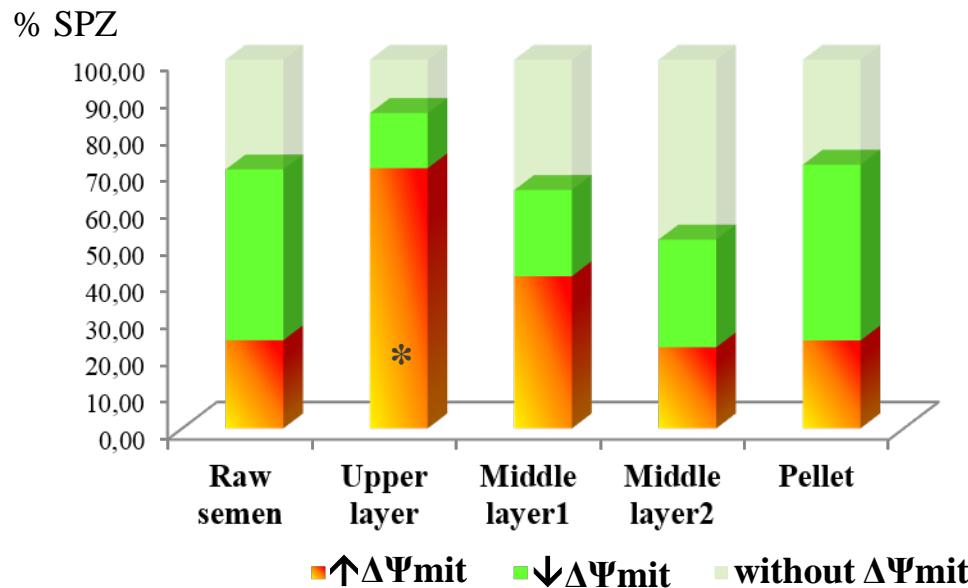
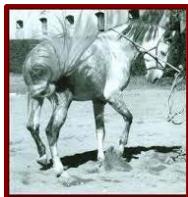


SLC with Androcoll™

SLC-treatment



MMP (JC-1)

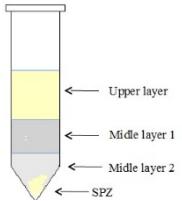


Lusitano \neq Sorraia

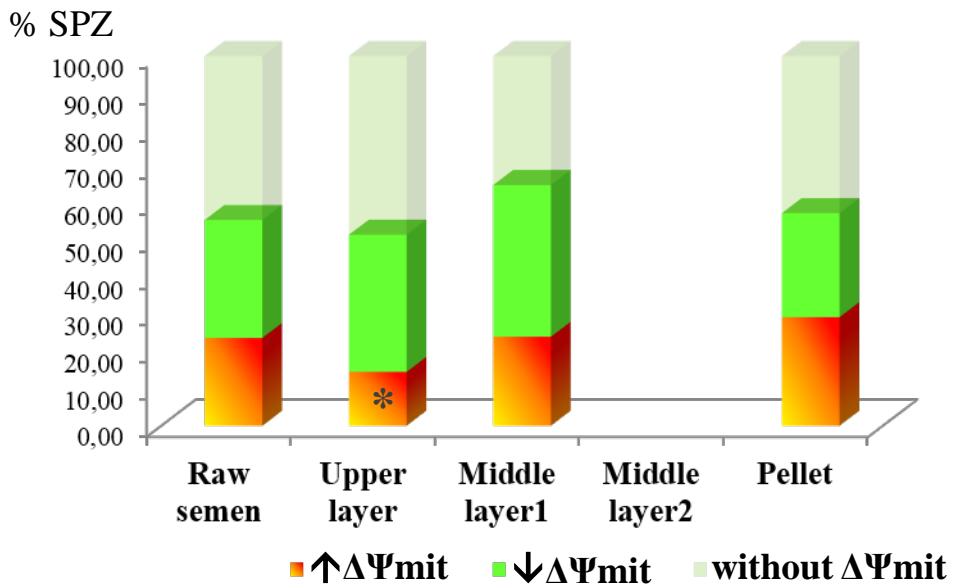
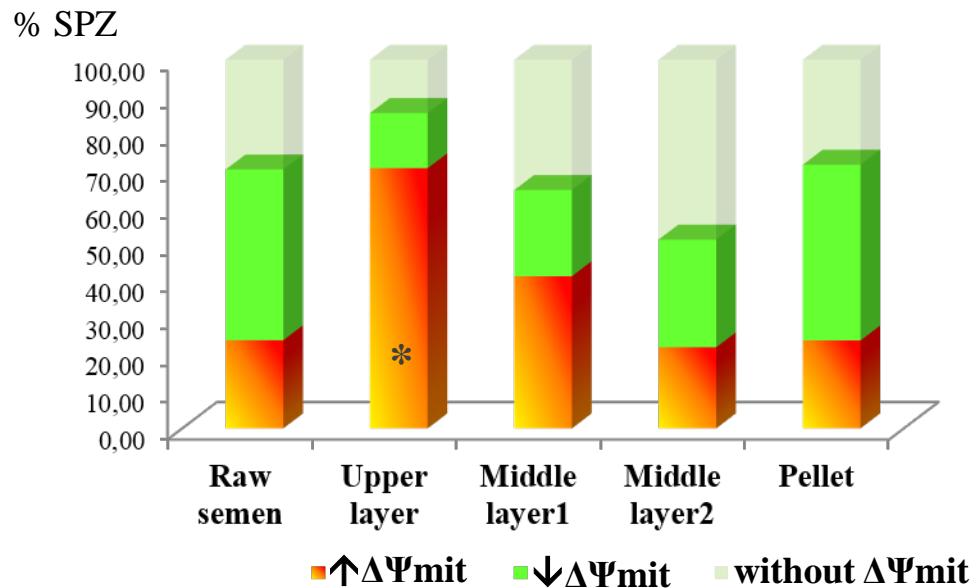
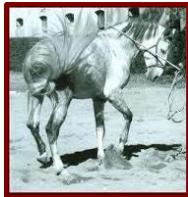
Upper layer
($p < 0,05$)

SLC with Androcoll™

SLC-treatment



MMP (JC-1)



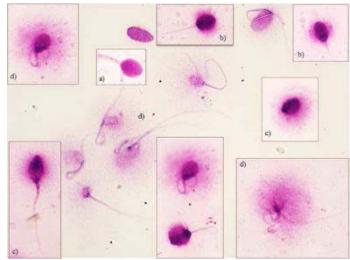
SLC selected SPZ = Raw semen

SLC with Androcoll™

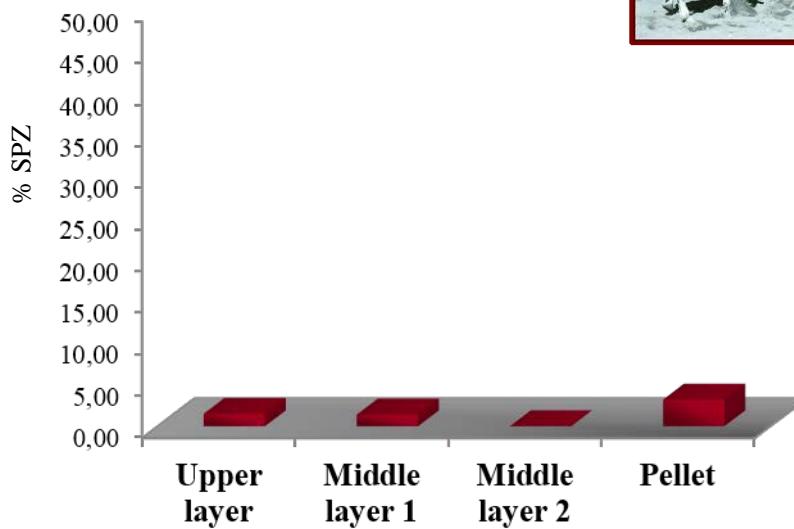
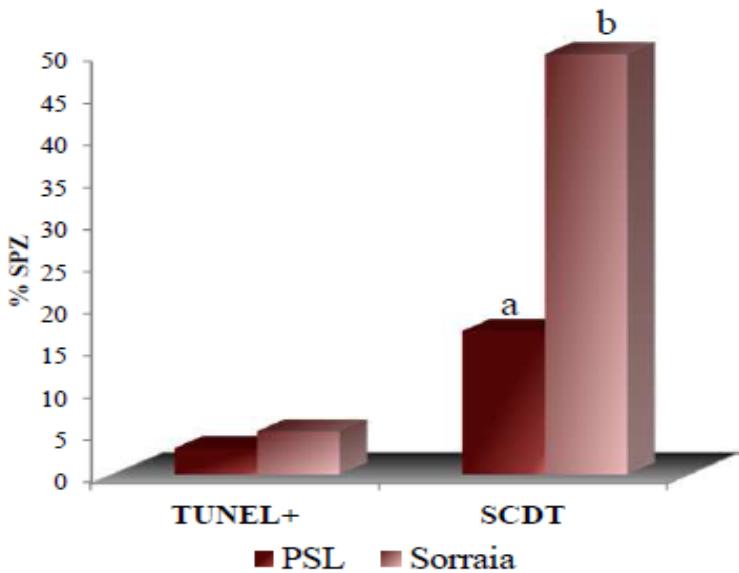
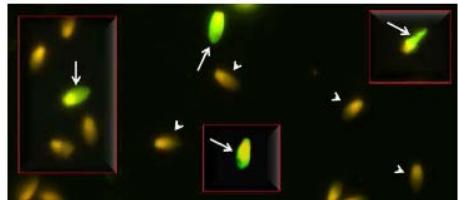
SLC-treatment

DNA integrity_(ns)

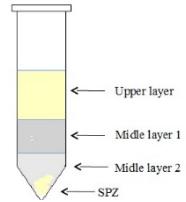
SCD test



TUNEL

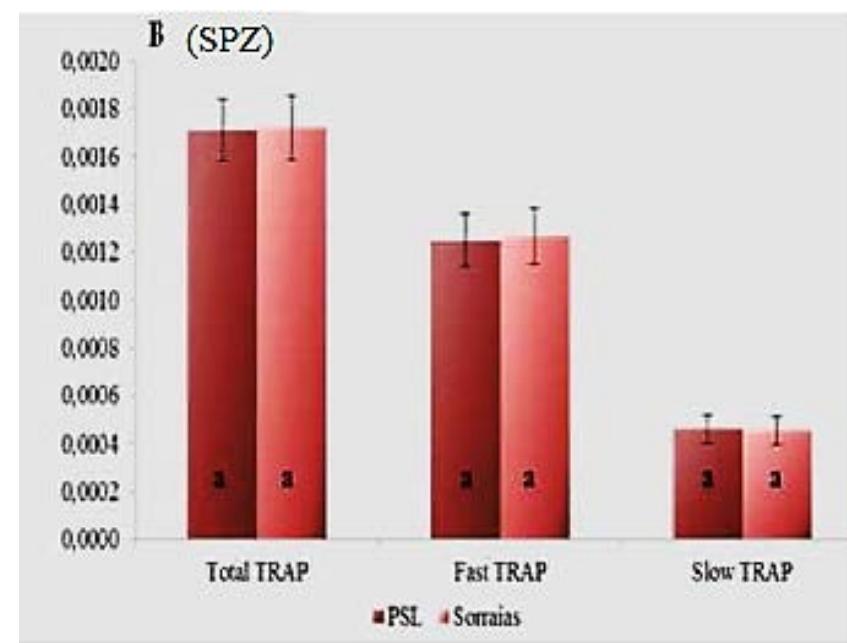
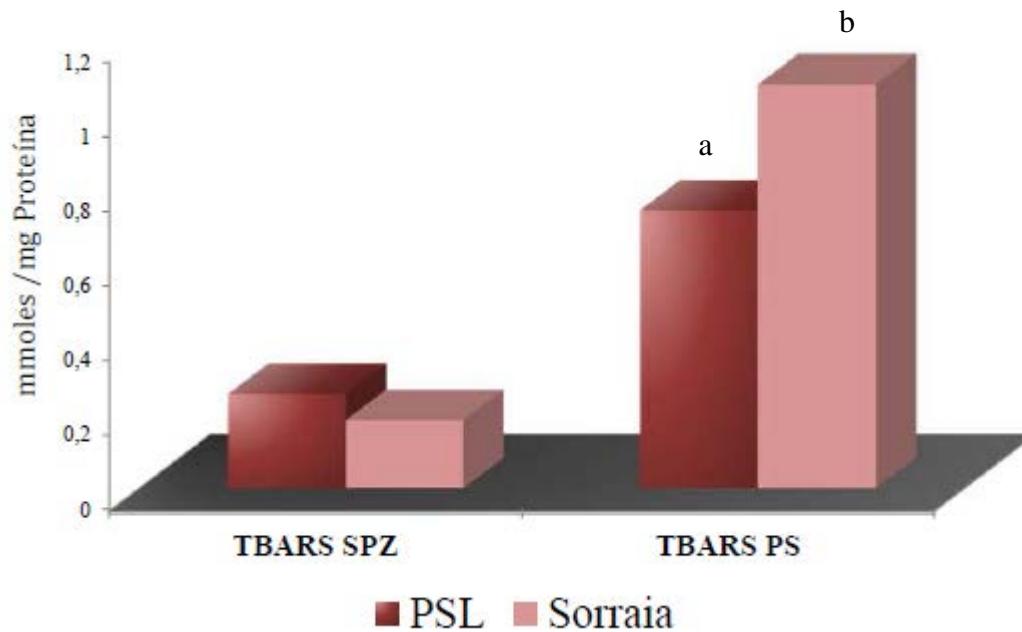


Oxidative stress ?



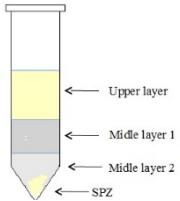
Lusitano vs Sorraia

Lipid - peroxidation



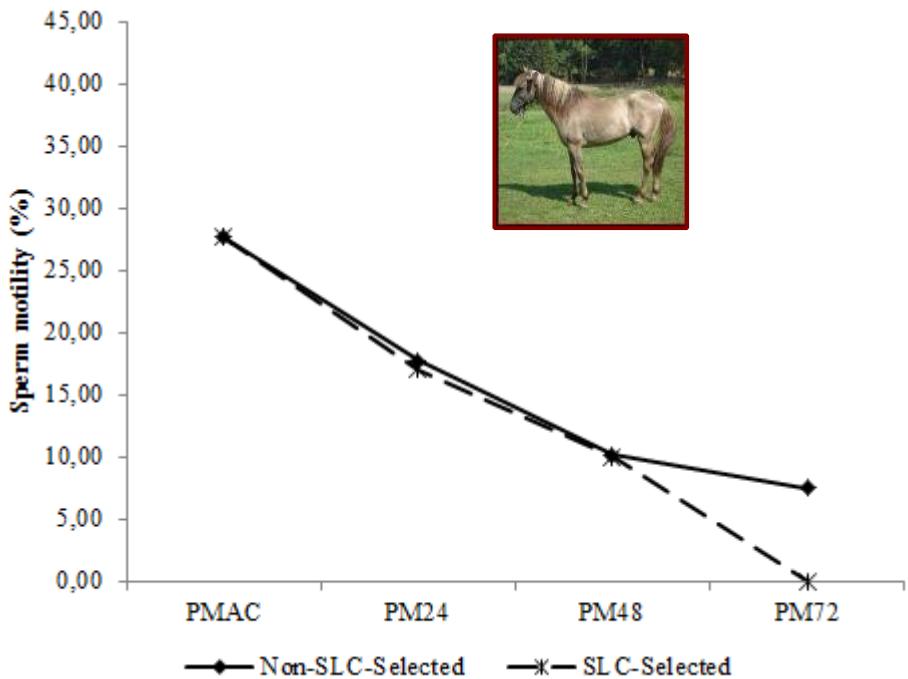
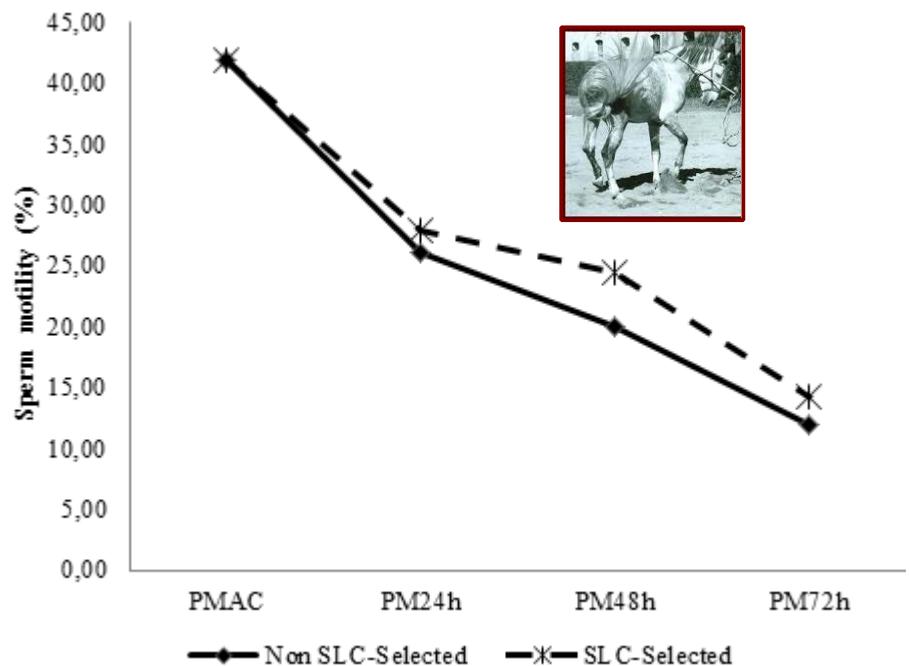
SLC with Androcoll™

SLC-treatment



Sperm Motility

Significant differences between stallions

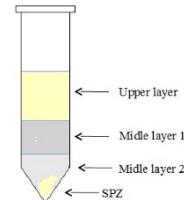


No differences between treatments

SLC with Androcoll™

SLC-treatment

AI



		DG+	DG-
SLC-treated	Lusitano	7	2
	Sorraia	0	6
non-SLC-treated	Lusitano	8	1
	Sorraia	0	9
Total	Lusitano	15	3
	Sorraia	0	15

Per cycle fertility



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

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

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

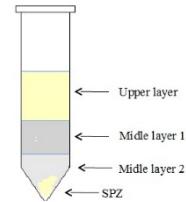
0.00% - non-SLC-selected sperm
(9 mares; 0 cycles DG+)

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

No differences between treatments

SLC with Androcoll™

SLC-treatment



- Androcoll™ 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 ($\uparrow\Delta\Psi_{mit}$; 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™

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

Fundaçao para a Ciéncia e Tecnologia (FCT), Portugal

(PTDC/CVT/108456/2008)

Escola Superior Agrária de Coimbra, IPC

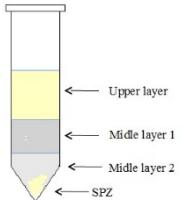
CERNAS (PEst-OE/AGR/UI0681/2011)



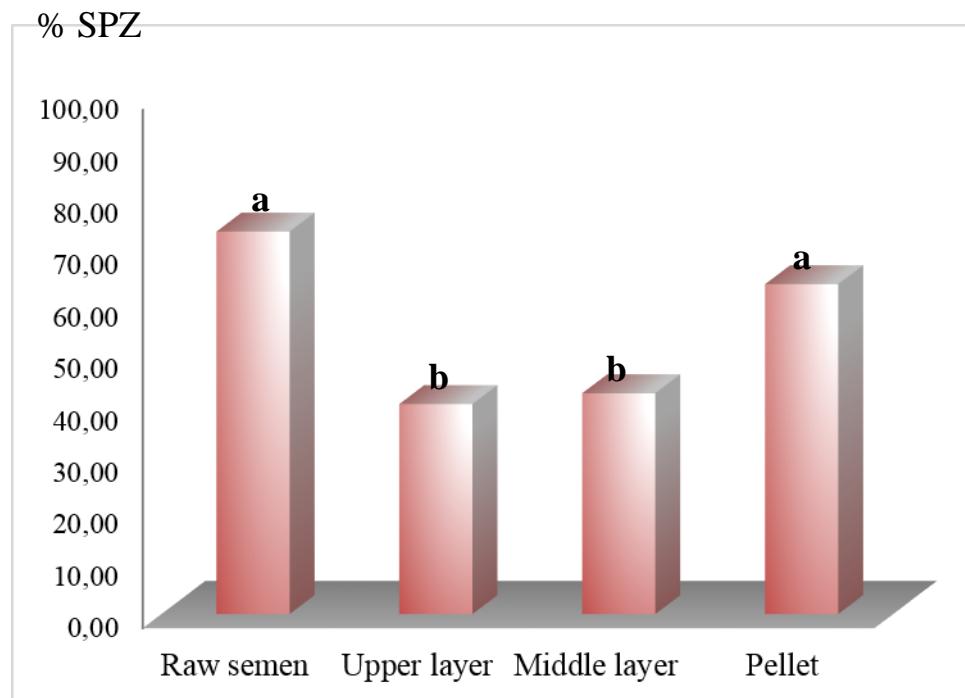
Thank you!

SLC with Androcoll™

SLC-treatment



Acrosome integrity



a,b – p<0,05 – differences between layers

