universidad de león

# Trends on sperm processing for improving preservation and fertility

Felipe Martínez Pastor

Universidad de León

felipe.martinez@unileon.es

reprobio.unileon.es

bianorbiotech.es

NeoGiANT https://neogiant.eu

Horizon 2020
European Union Funding
for Research & Innovation

# The power of grape extracts: antimicrobial and antioxidant properties to prevent the use of antibiotics in farmed animals

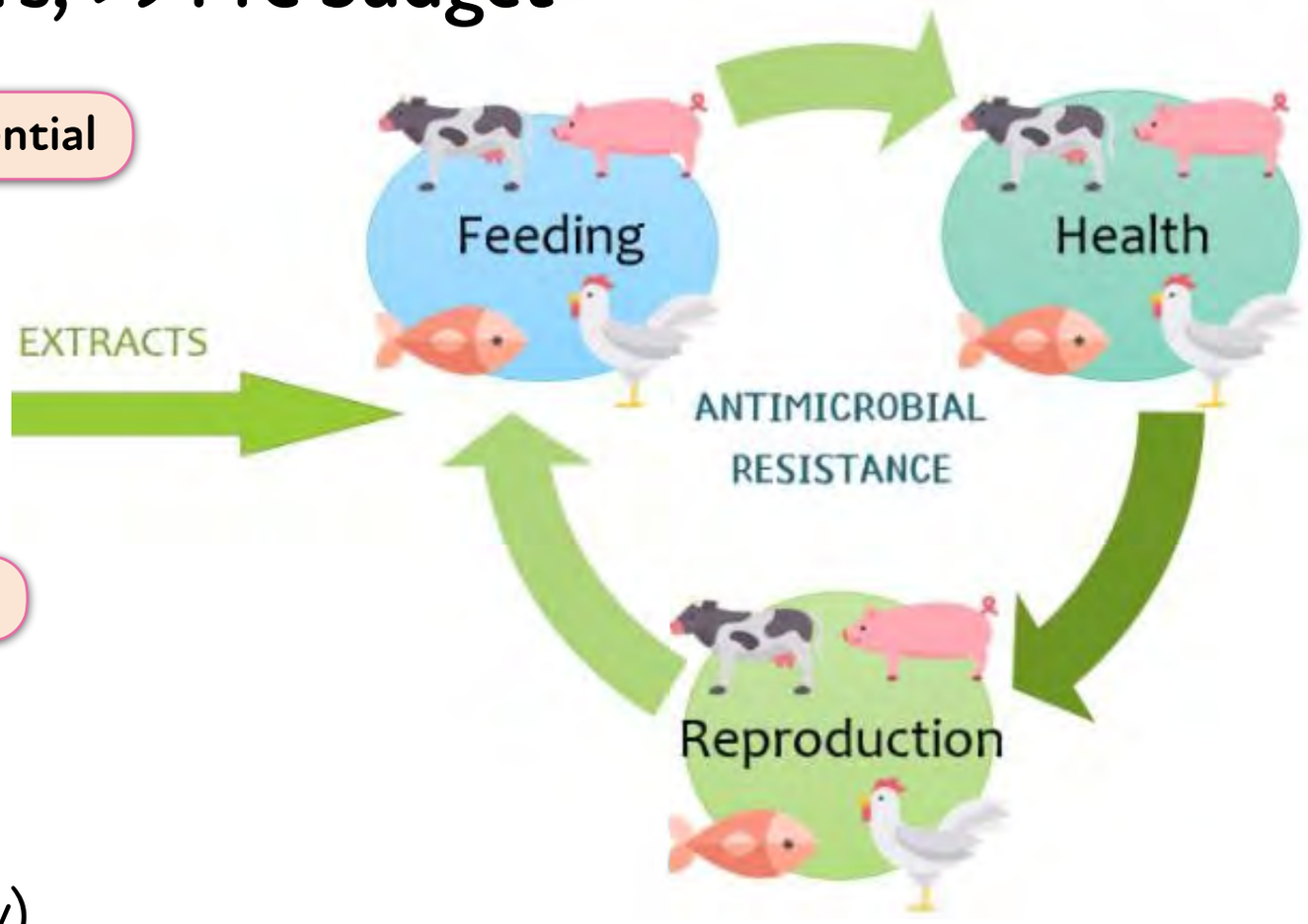
## H2020 project, 20 members, >9 M€ budget

High antimicrobial potential

LDSA
Laboratorio de Investigación y Desarrollo de Soluciones Analíticas

University of Santiago de Compostela, Spain

Low cytotoxicity




Simple and sustainable obtention of antimicrobial, polyphenolics-rich natural extract from grape marc (winery by-product, circular economy)

# Perspectives for antibiotic replacement in semen diluents and the NeoGiANT approach

Horizon 2020
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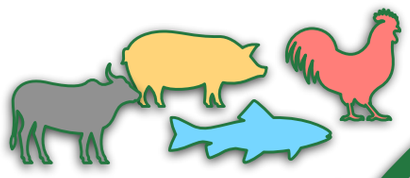
## Why are antibiotics a problem in animal reproduction?

**>90%**

Sows, dairy cows and turkeys are inseminated in EU and USA, growing elsewhere.

**BUT**


Semen diluents contain **antibiotics**



Continuous leak of broad-spectrum antibiotics to the environment.

The pig industry alone uses

***8 000 000 L***

of antibiotic-containing semen extenders per year in the EU

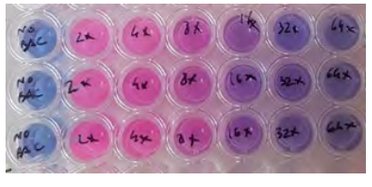
NeoGiANT

Antimicrobial natural extracts from grape marc for replacing antibiotics in animal production.

**WP6 - Assessment of the antimicrobial formulation for sperm for preservation**

Currently testing →

Antimicrobial potential



Formulation compatibility

Safety for spermatozoa

Jihočeská univerzita v Českých Budějovicích
University of South Bohemia in České Budějovice

Bianor Biotech
Science & Innovation

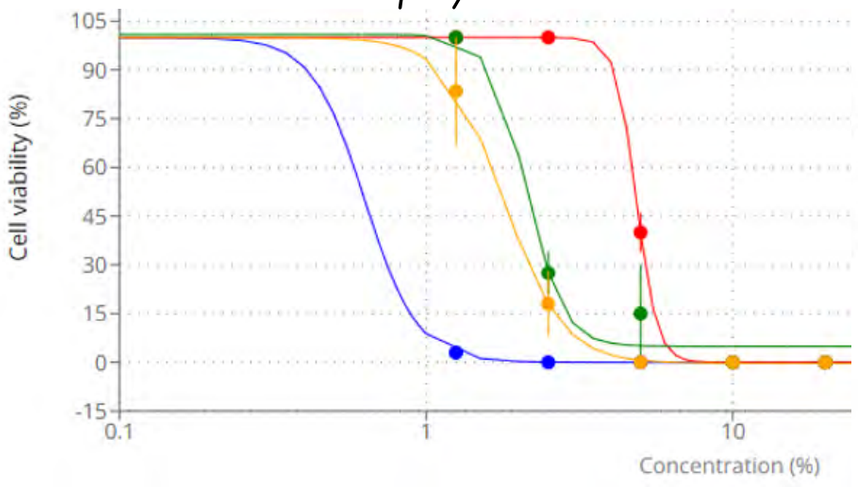
VRI VETERINARY RESEARCH INSTITUTE

Magapor

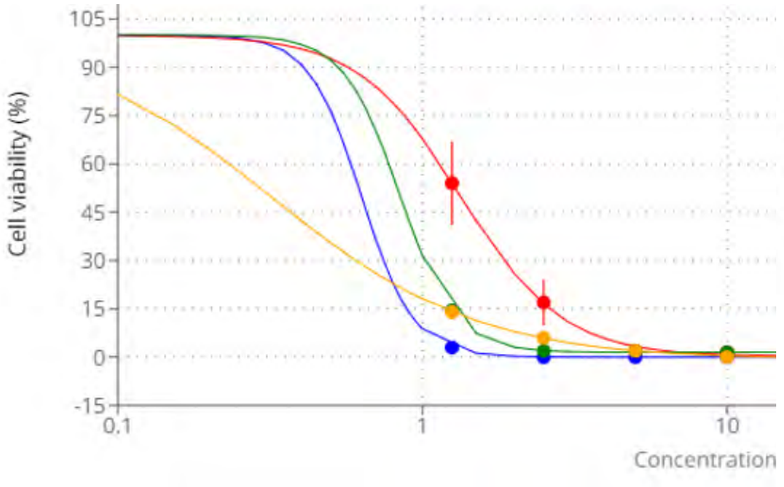
# Some preliminary results

Extracts **very effective** against bacteria relevant for semen storage and artificial insemination.

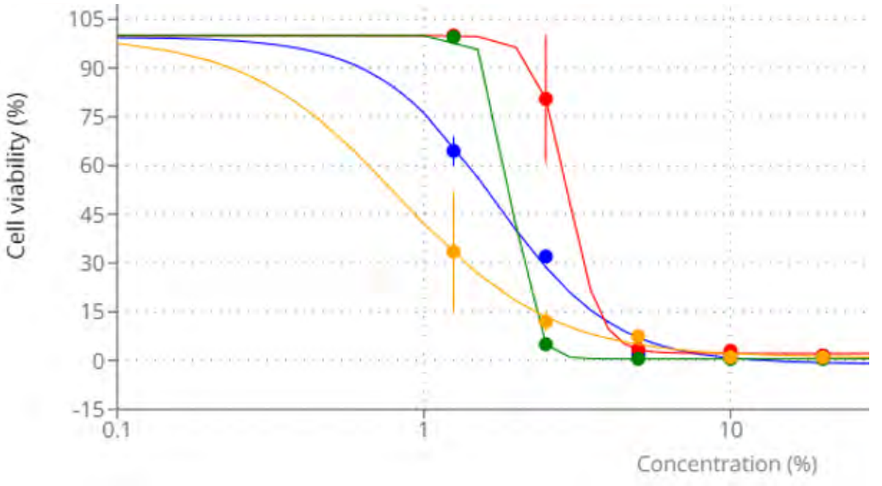
*Staphylococcus*



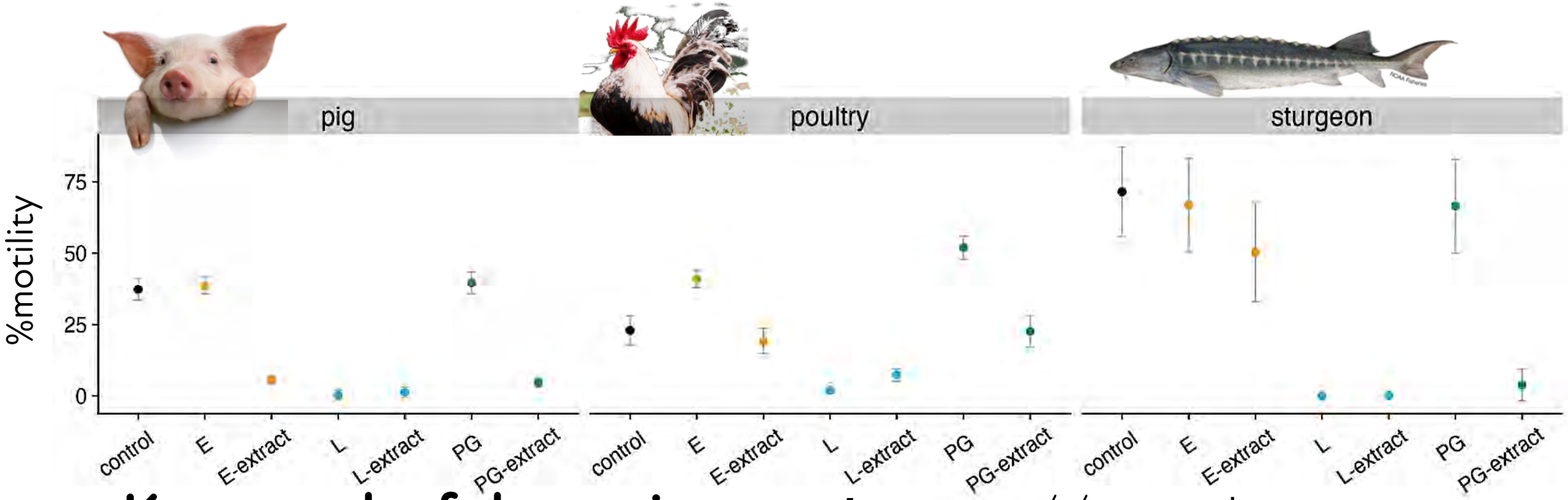

*Proteus*




*Salmonella*




Diverse effects depending on species: working on adjusting concentrations for best sperm quality/antimicrobial activity balance




**Keep track of the project at:** `https://neogiant.eu`

# Why using sperm selection/improvement?

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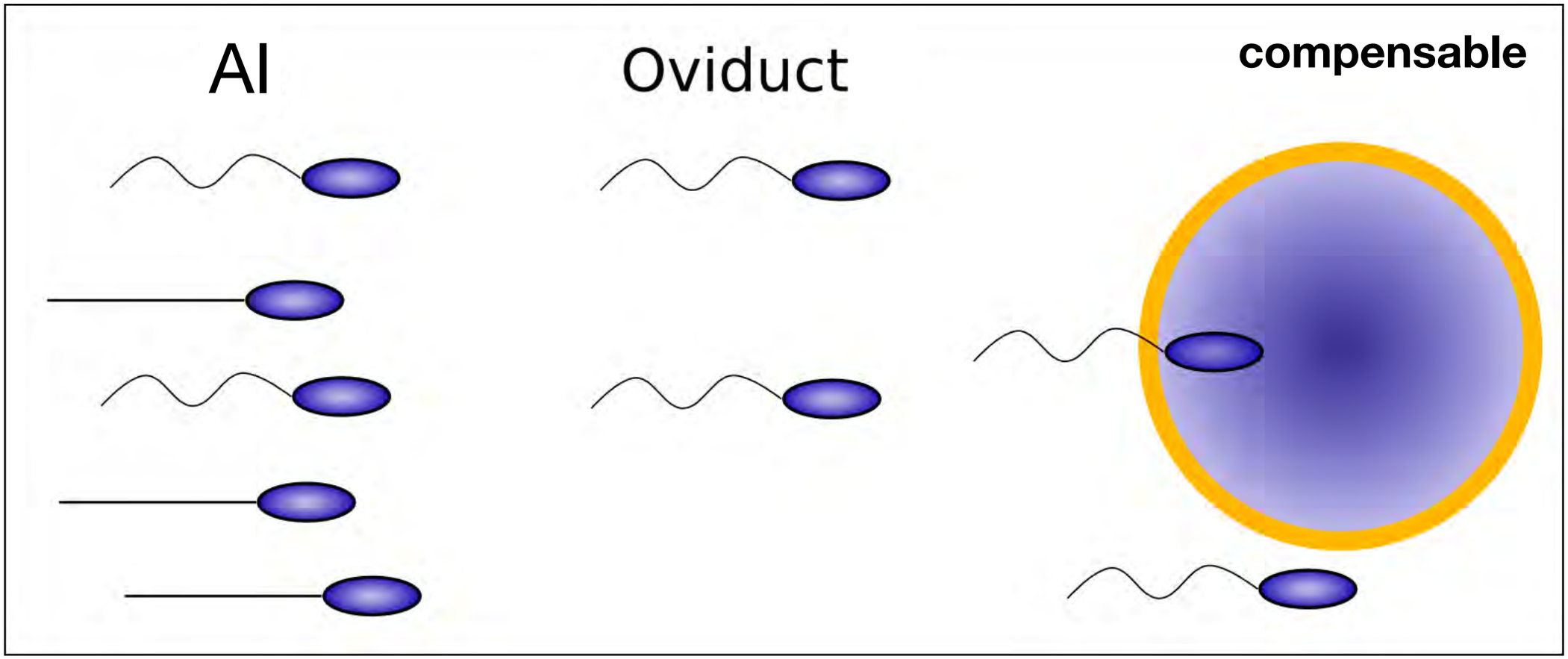
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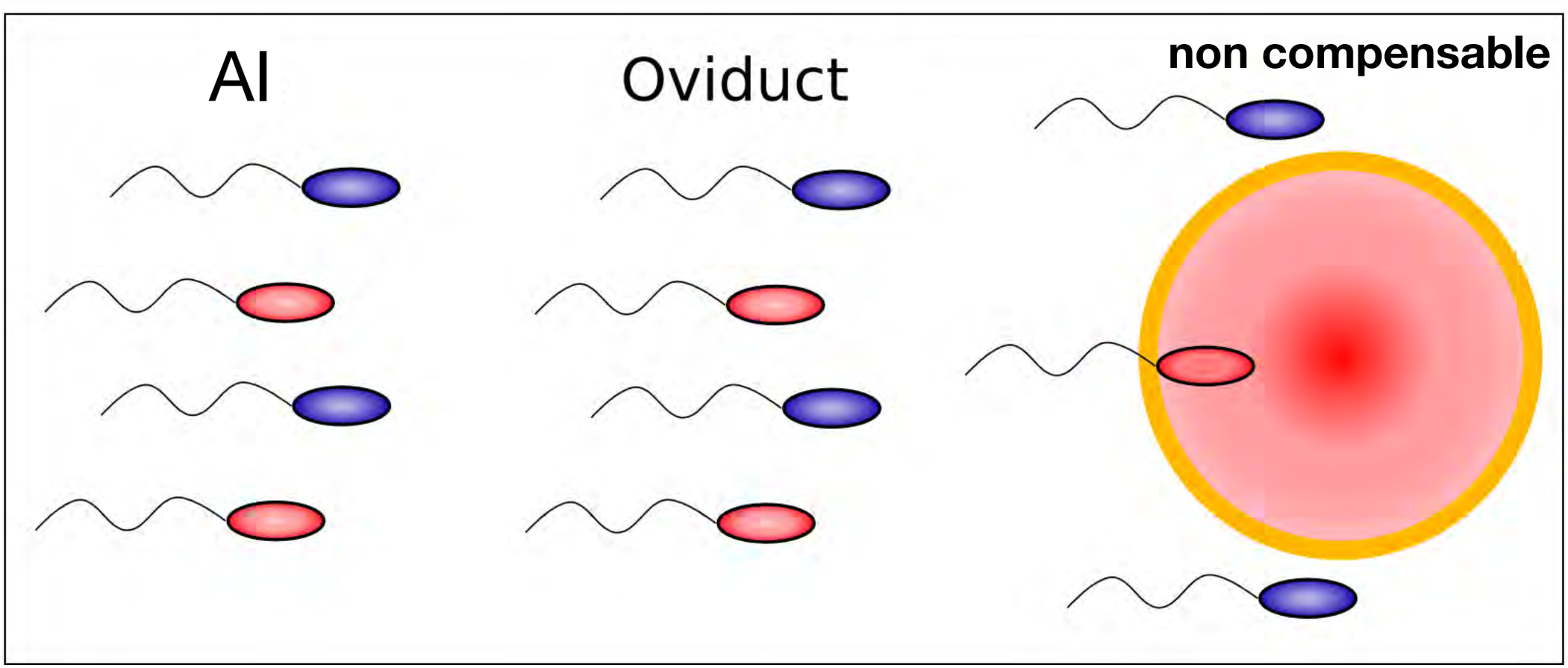
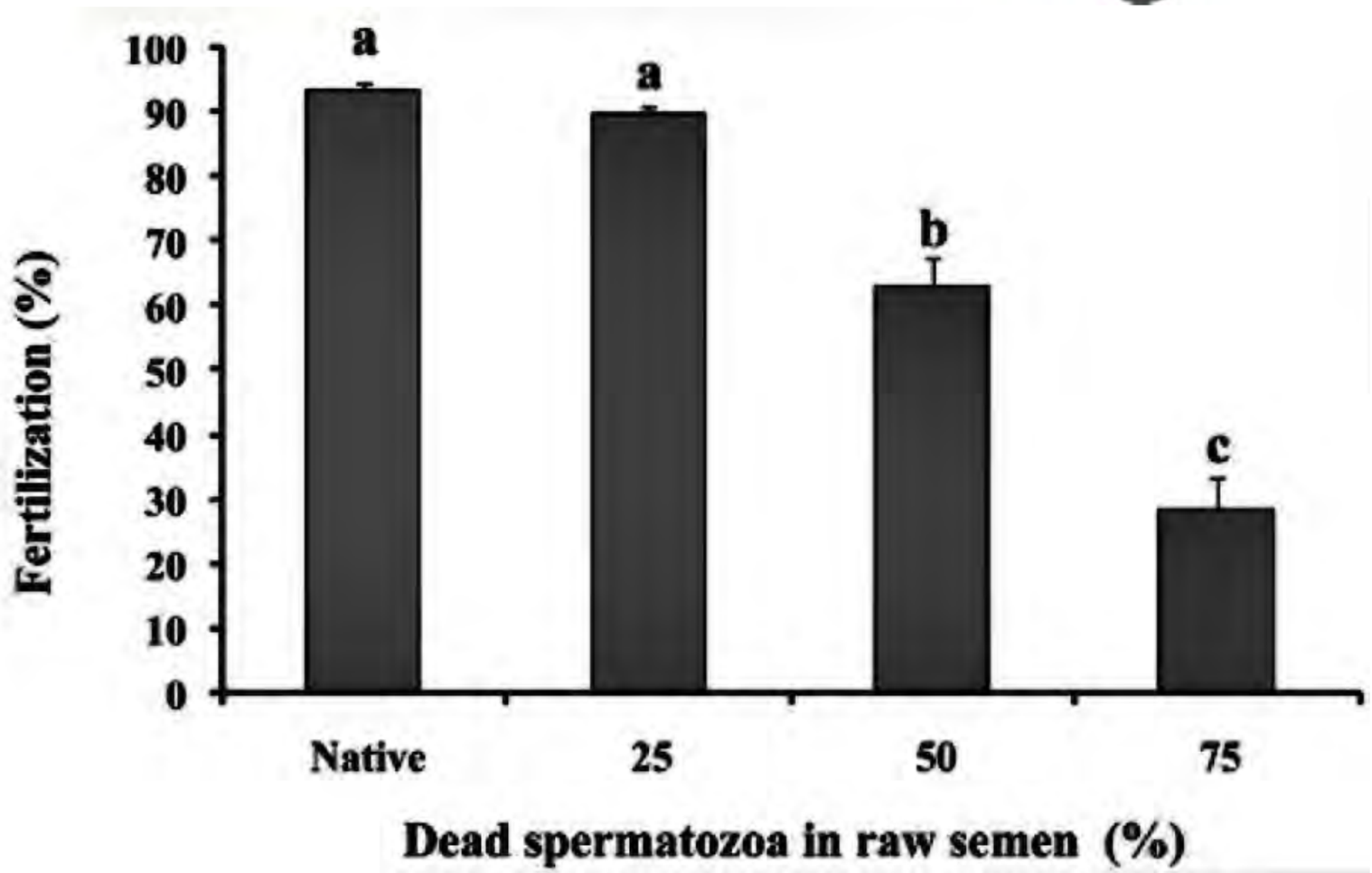
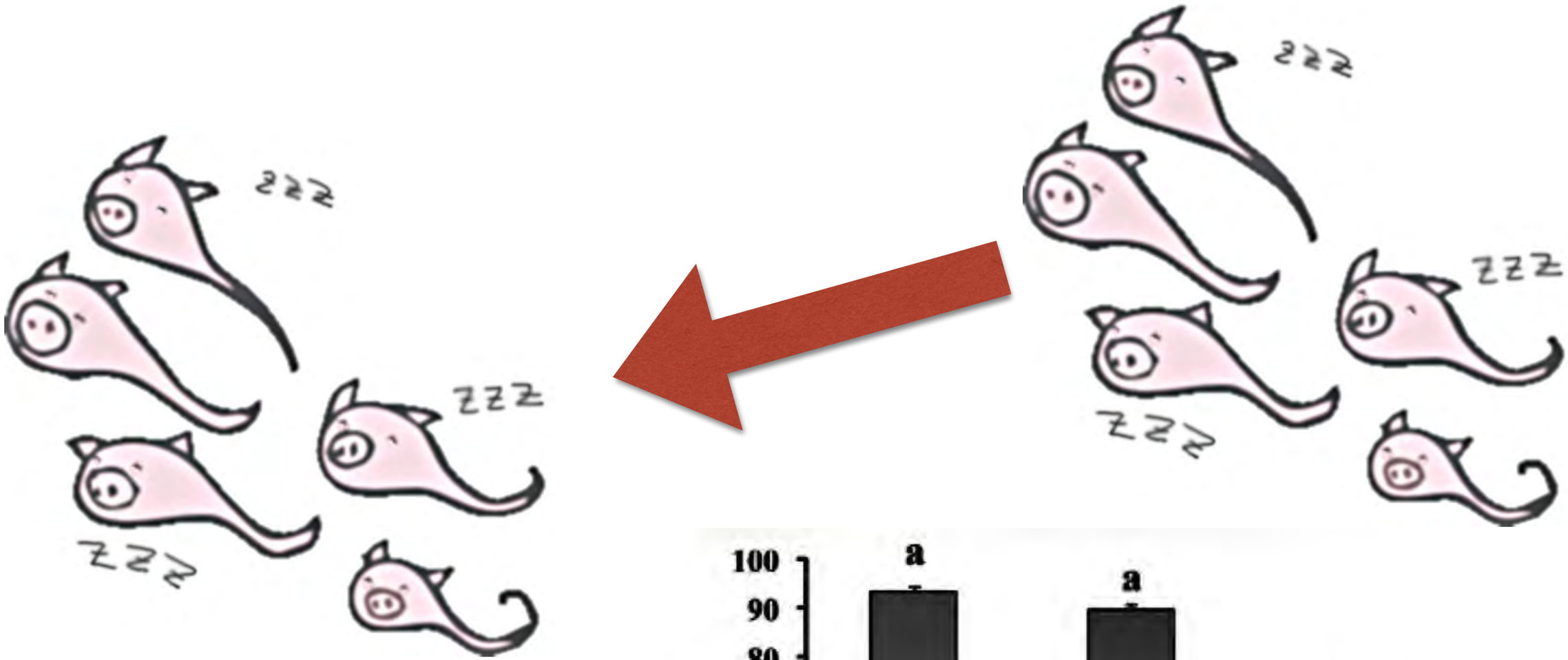
Oviduct
compensable

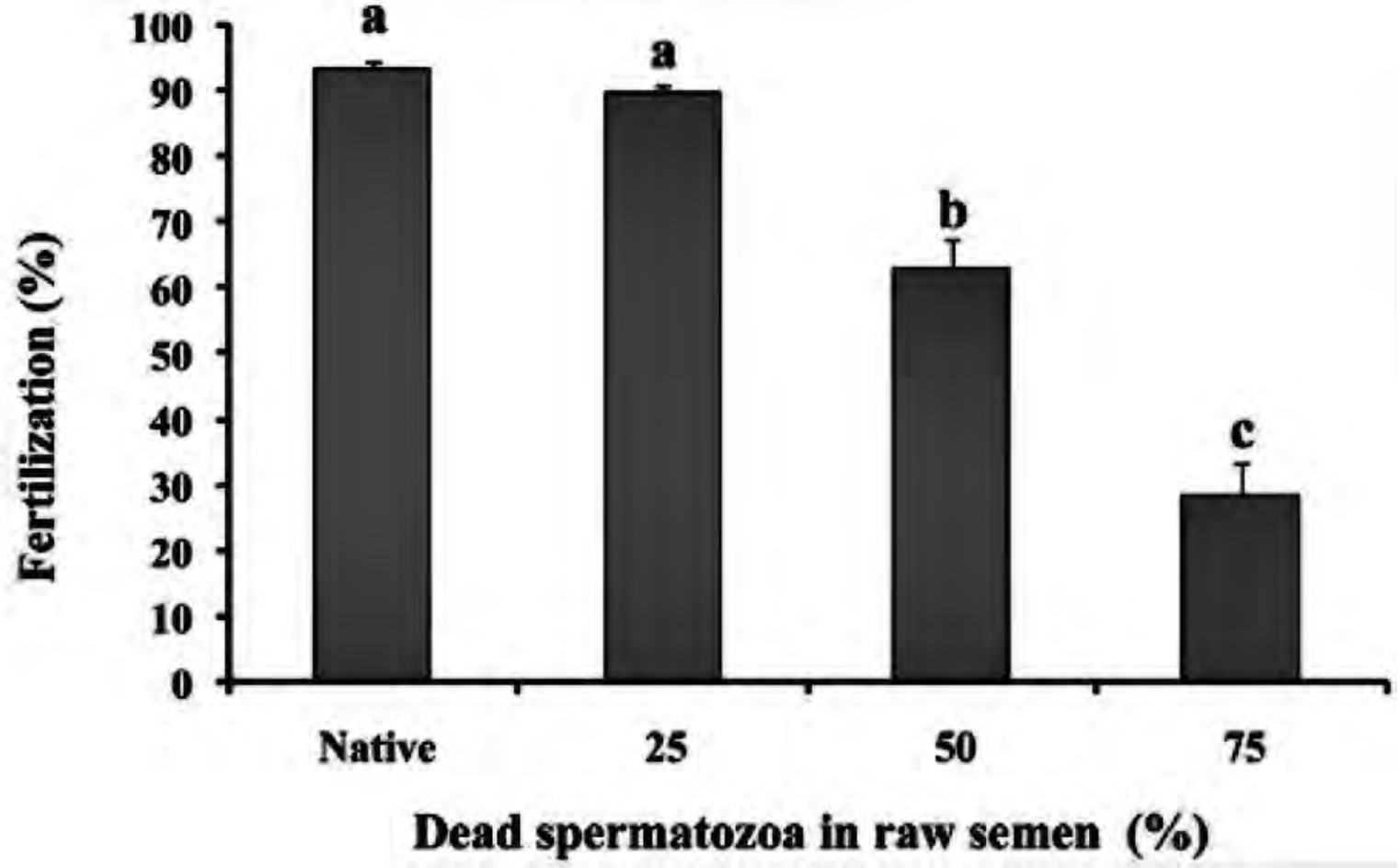

AI
Oviduct
non compensable

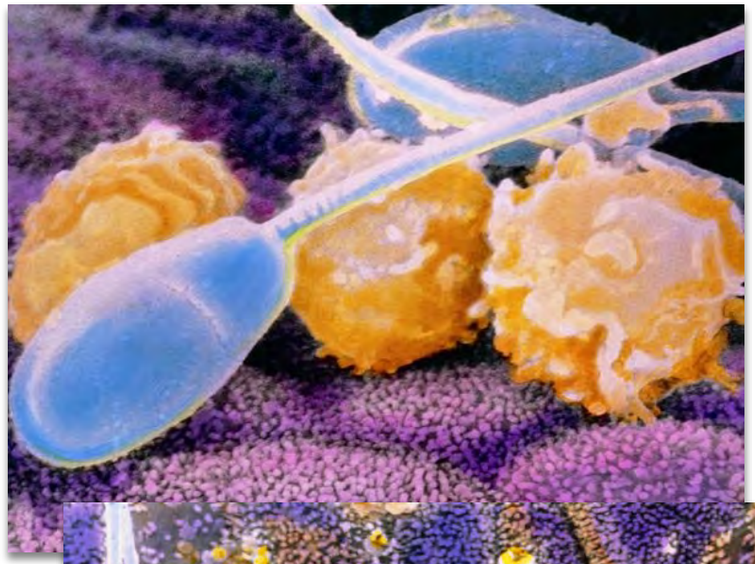
Roca, J. et al. Fertility and Sterility 100, 875–881

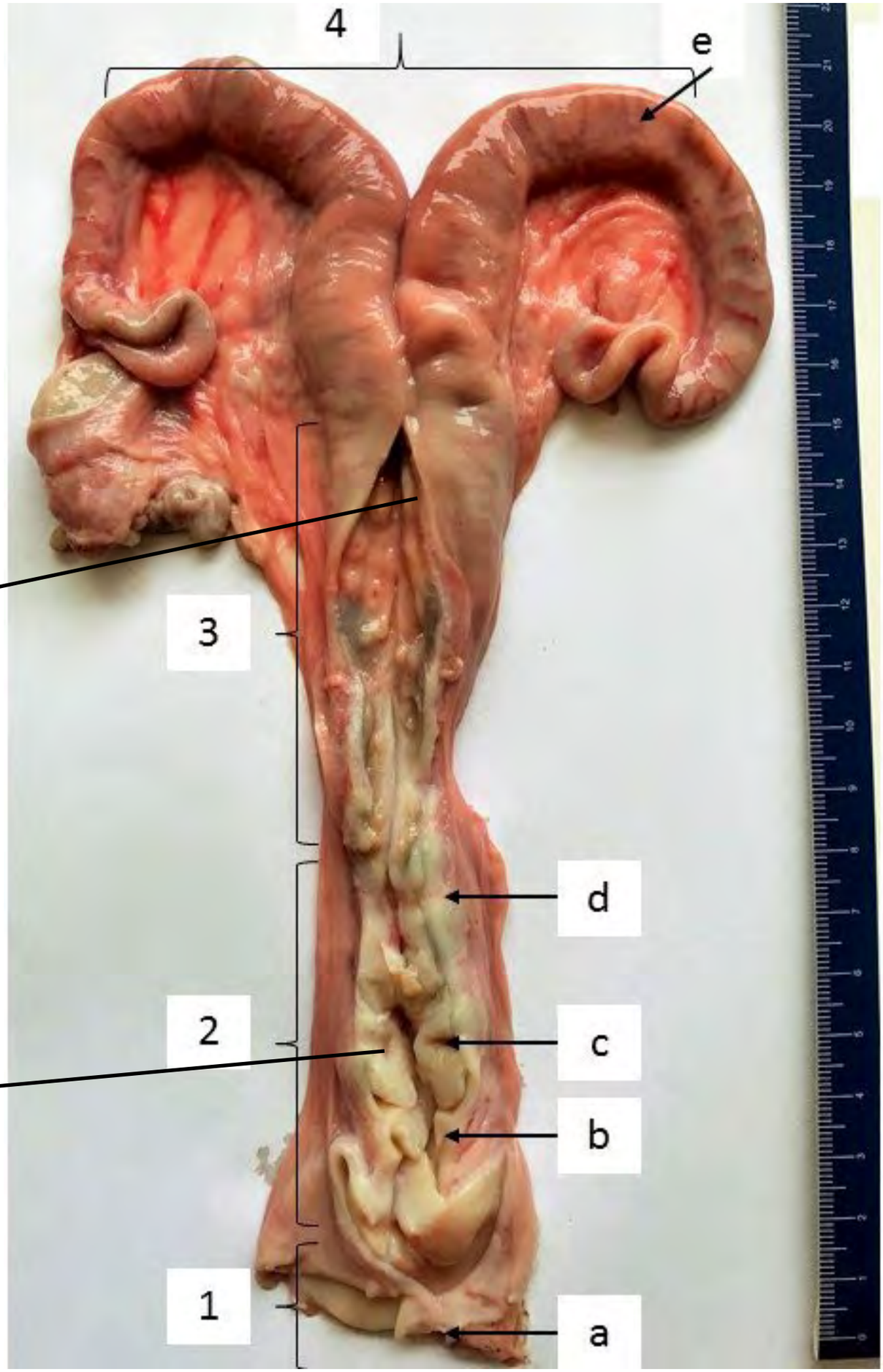



Roca, J. et al. Fertility and Sterility 100, 875–881





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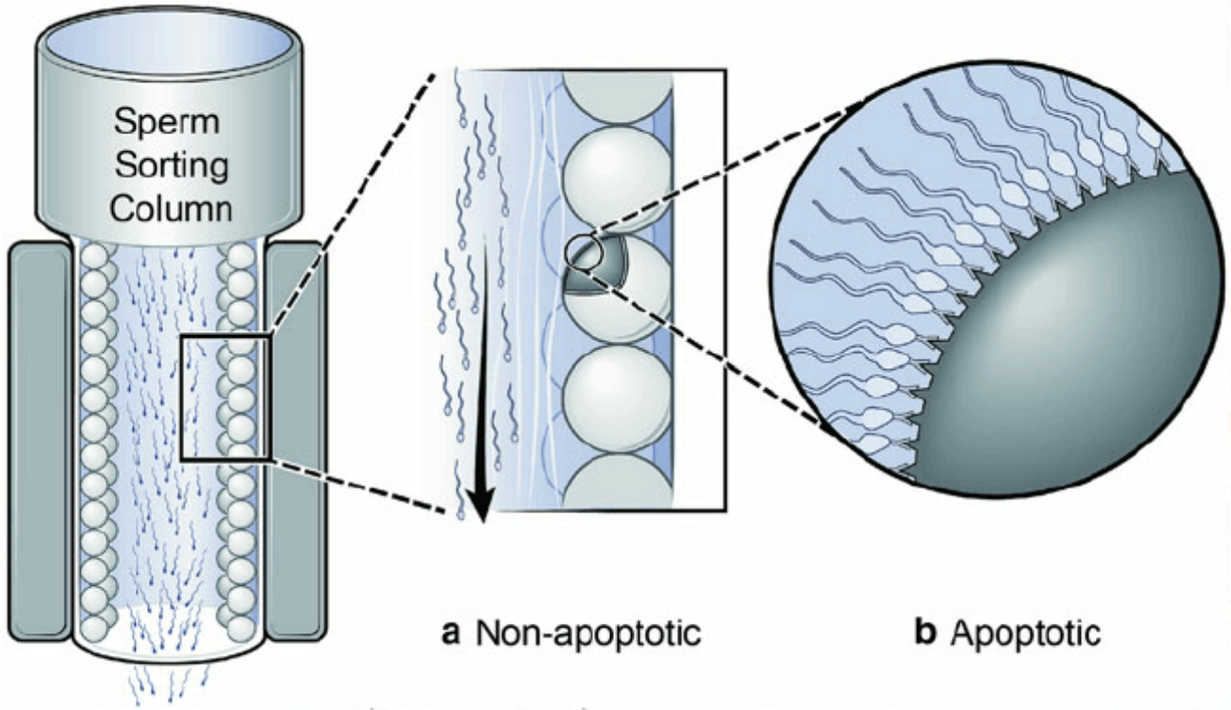
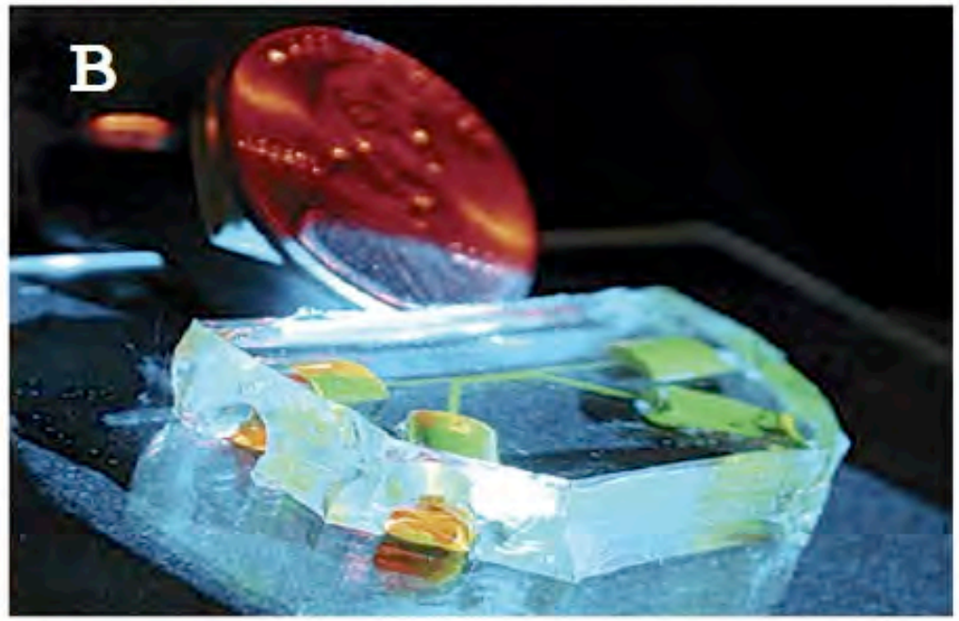
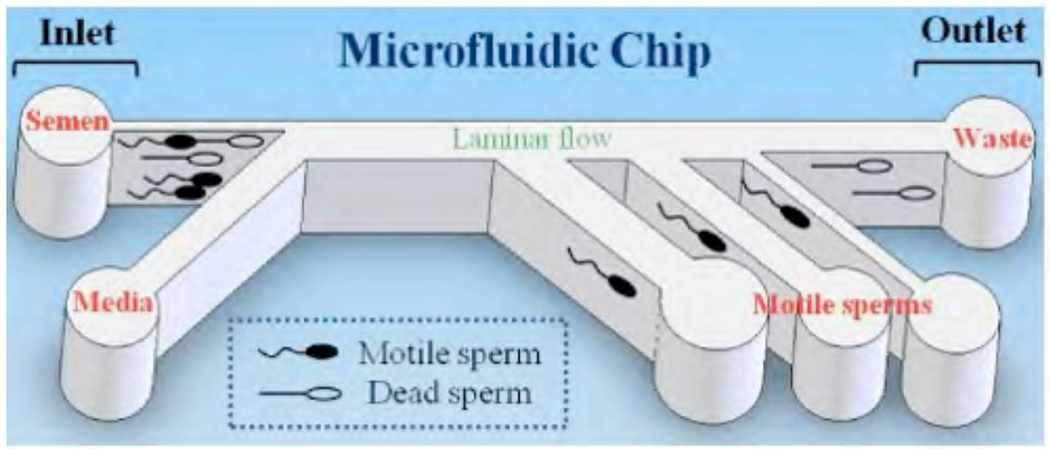
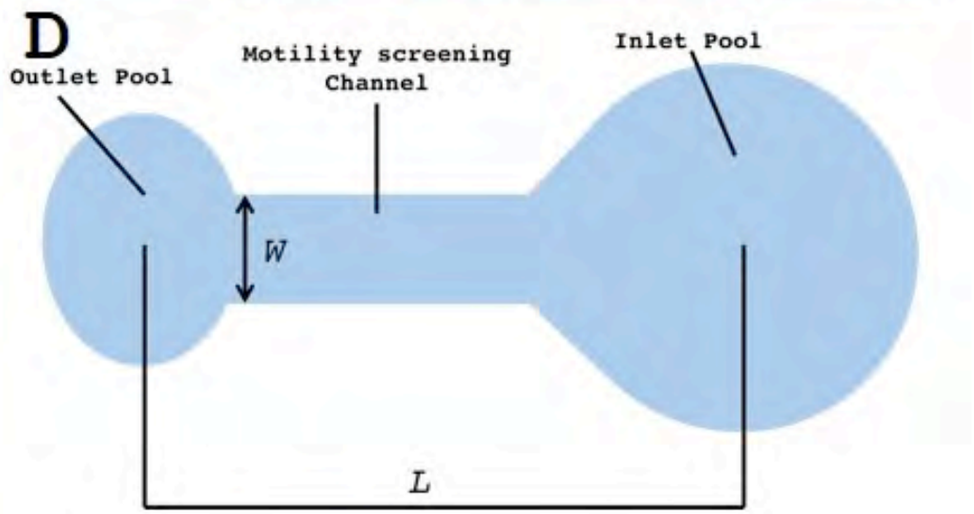
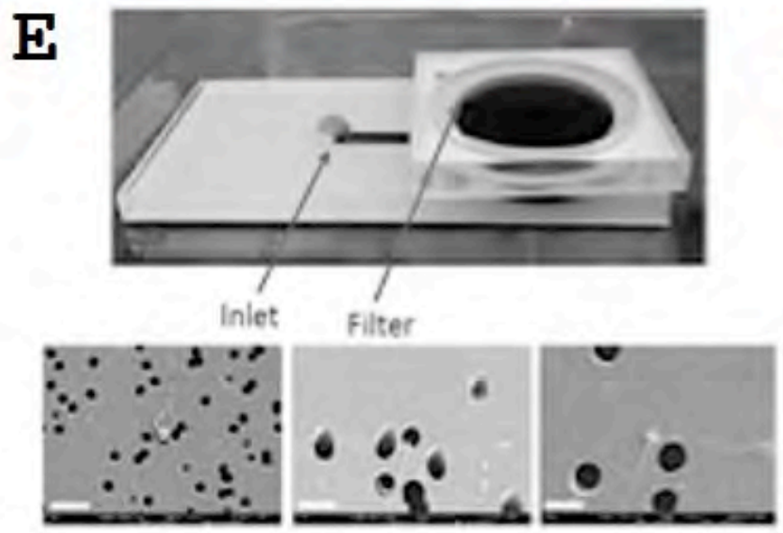




https://atlasofscience.org/can-we-select-best-sperm/

# Colloid selection

**Jane Morrell,**
**SLU, Sweden**

**Serafín Pérez-Cerezales,**
**INIA, Spain**

## Thermotaxis

## Photostimulation

**Marc Yeste,**
**UdG, Spain**

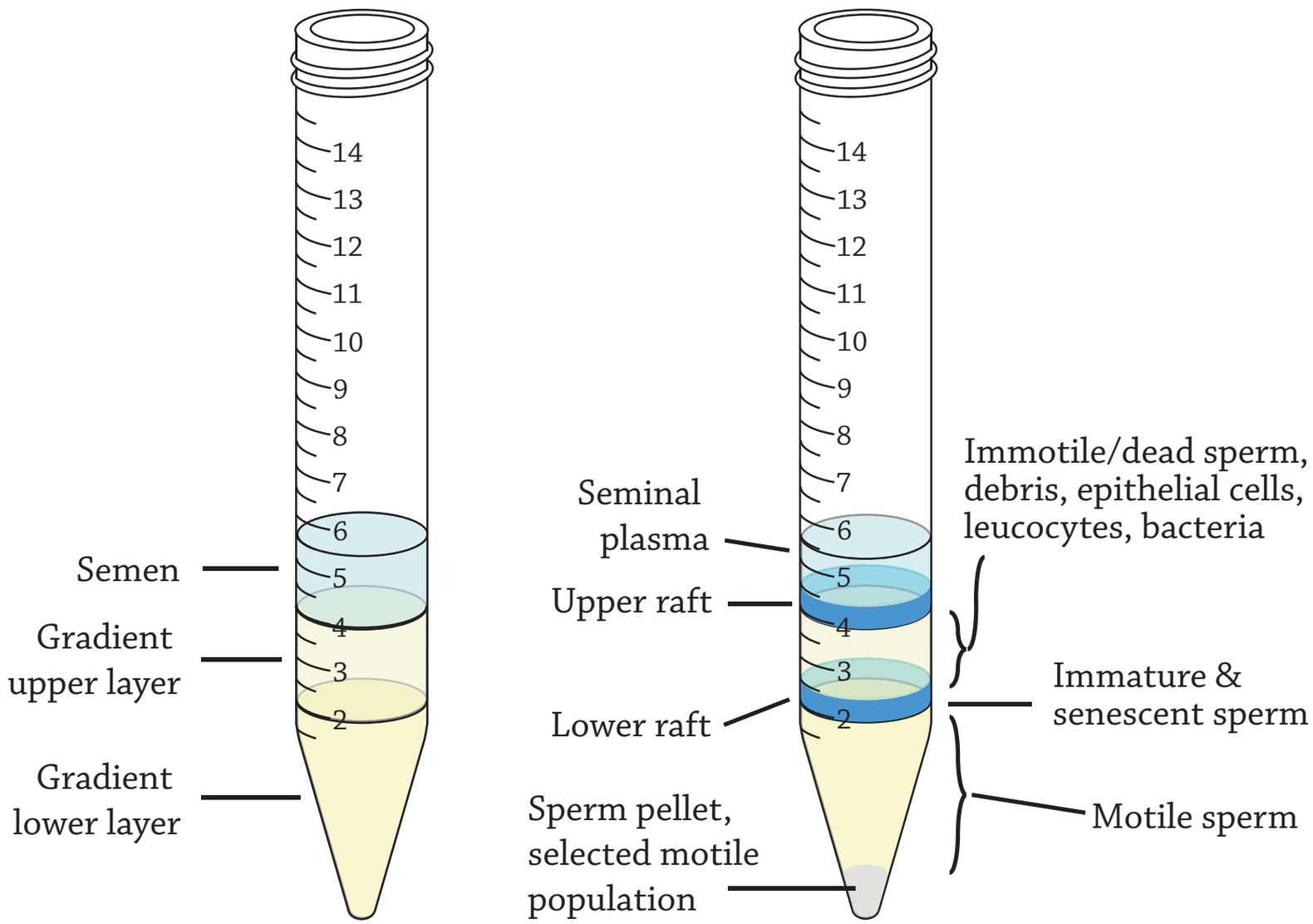
Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

# Sperm selection and microorganism removal by colloid centrifugation

**Jane M. Morrell**
Professor in Veterinary Reproductive Biotechnologies,
Clinical Sciences, Swedish University of Agricultural Sciences
Uppsala, Sweden

# Colloid centrifugation

## Density gradient centrifugation
## Double layer centrifugation (DLC)




### Advantages

Used in human fertility treatments

Select the best quality spermatozoa

### Disadvantages

Suitable only for small volumes of semen

Not user-friendly

# Single Layer Centrifugation (SLC)

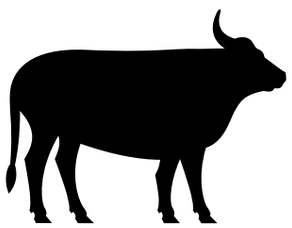
Scalable: Suitable for small or large volumes of semen

User-friendly

*Morrell, J.M., 2019. Effect of colloid centrifugation on boar sperm quality during storage and function in in vitro fertilization. Theriogenology, Proceedings of the IX International Conference of Boar Semen Preservation 137, 122–126.*

# Single Layer Centrifugation (SLC)

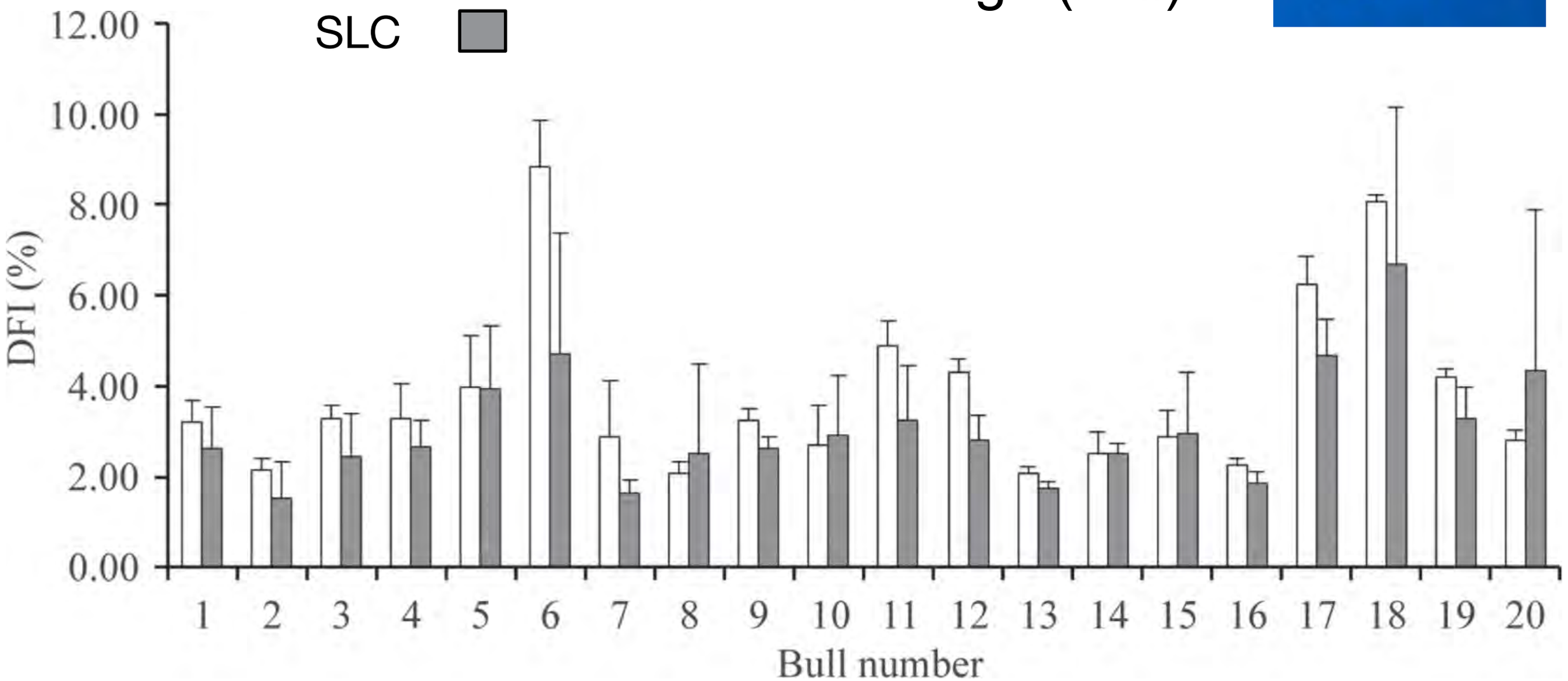
15-ml tubes, small-sized processing



Control

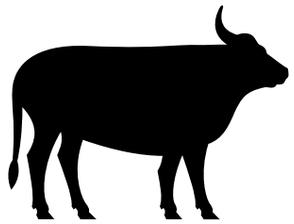
SLC

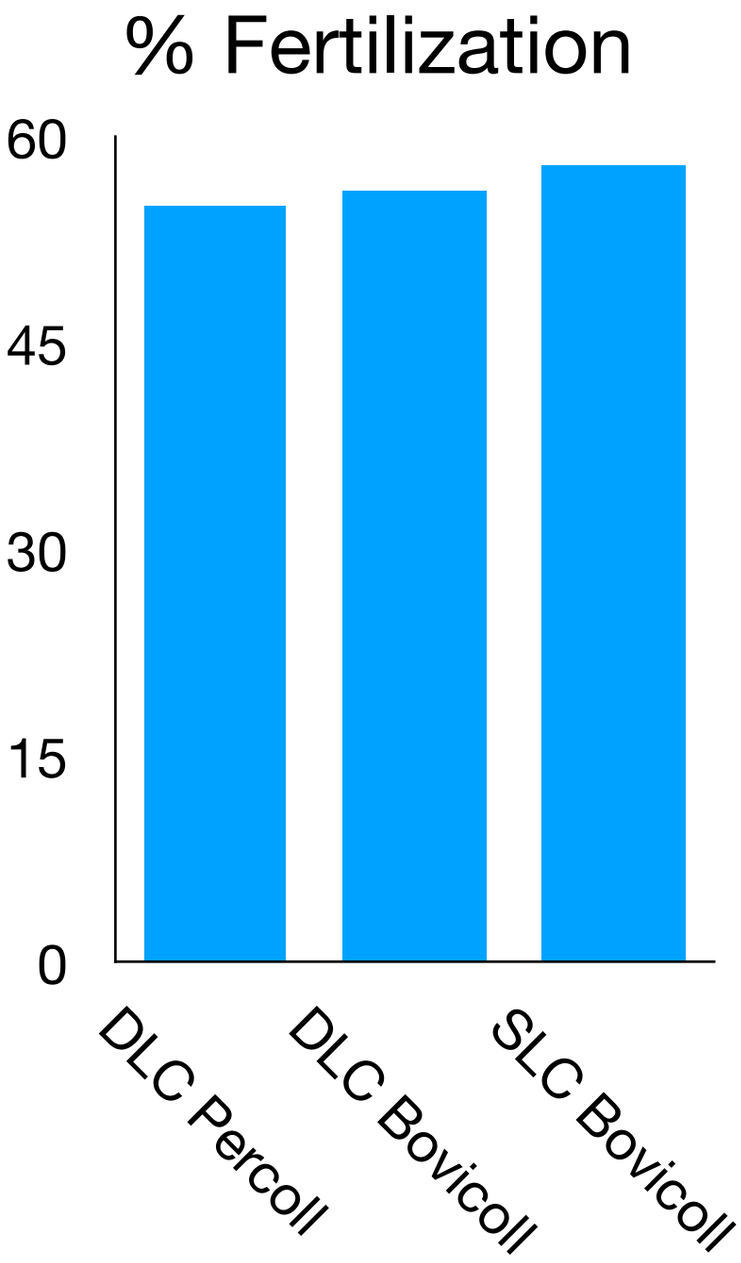
Chromatin damage (bull)

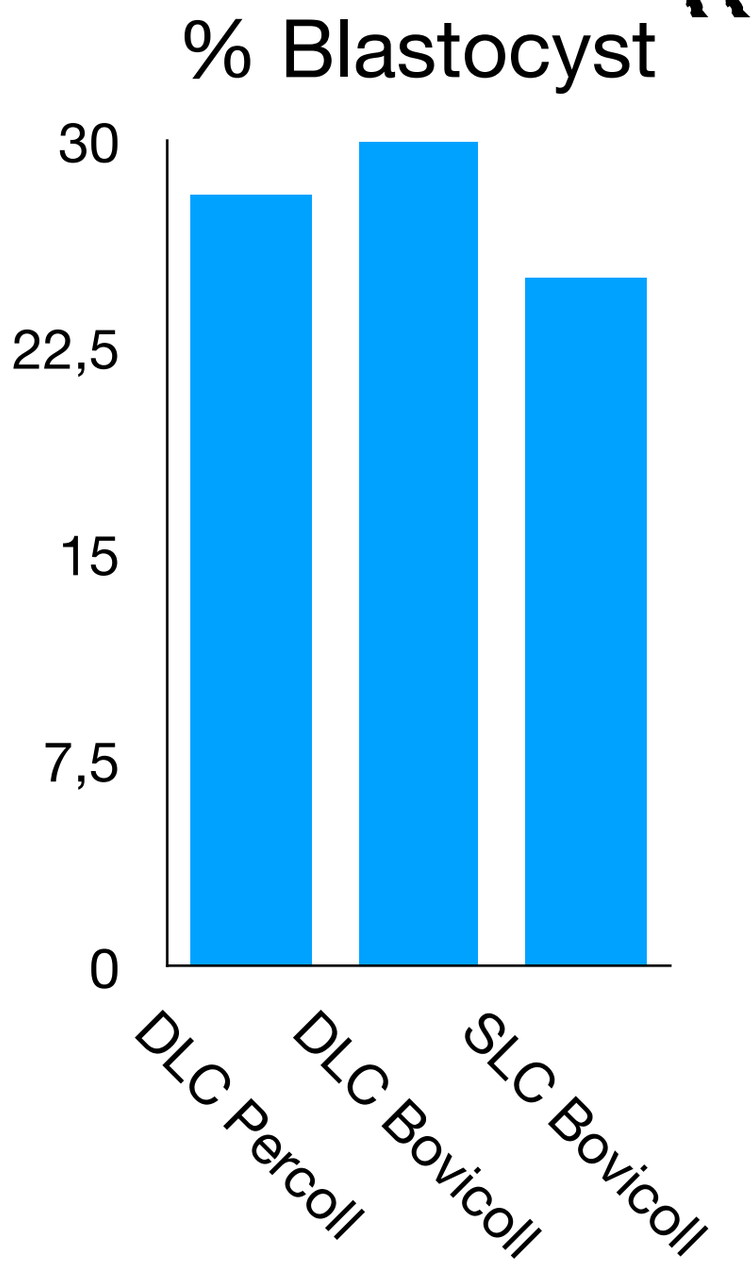



*Goodla et al. (2014) Journal Dairy Science 97, 2204-2212.*

# IVF with colloid-selected bull sperm



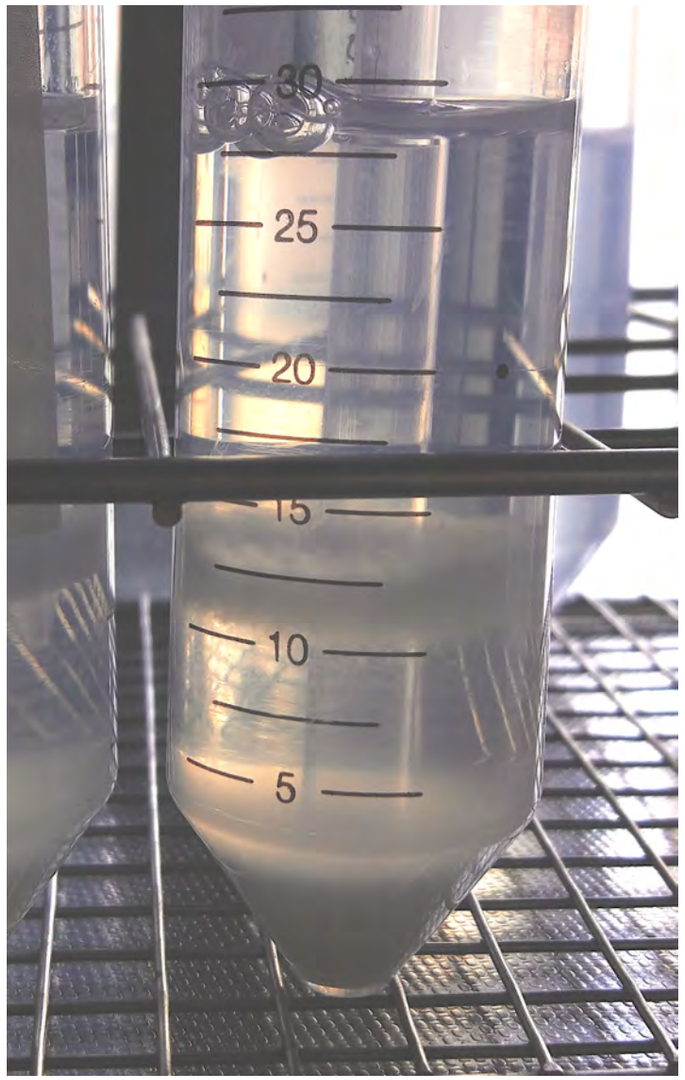


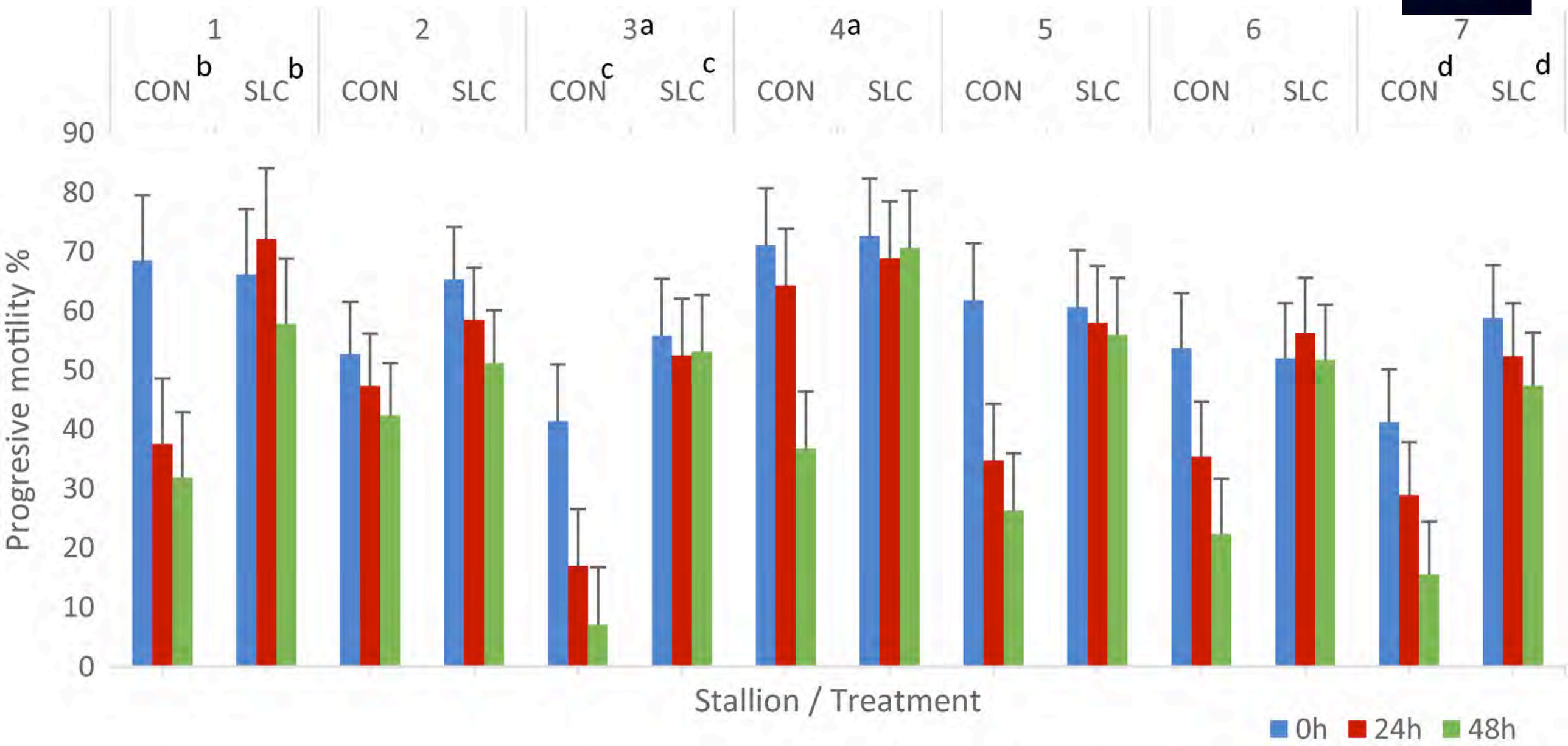

*Thys et al., 2009. In vitro fertilizing capacity of frozen-thawed bull spermatozoa selected by single-layer (glycidoxypropyltrimethoxysilane) silane-coated silica colloidal centrifugation. Reprod. Domest. Anim. 44, 390–394.*

# Single Layer Centrifugation (SLC)

## 50-ml tubes, medium-sized processing

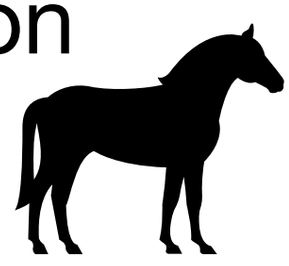


# Stallion sperm progressive motility

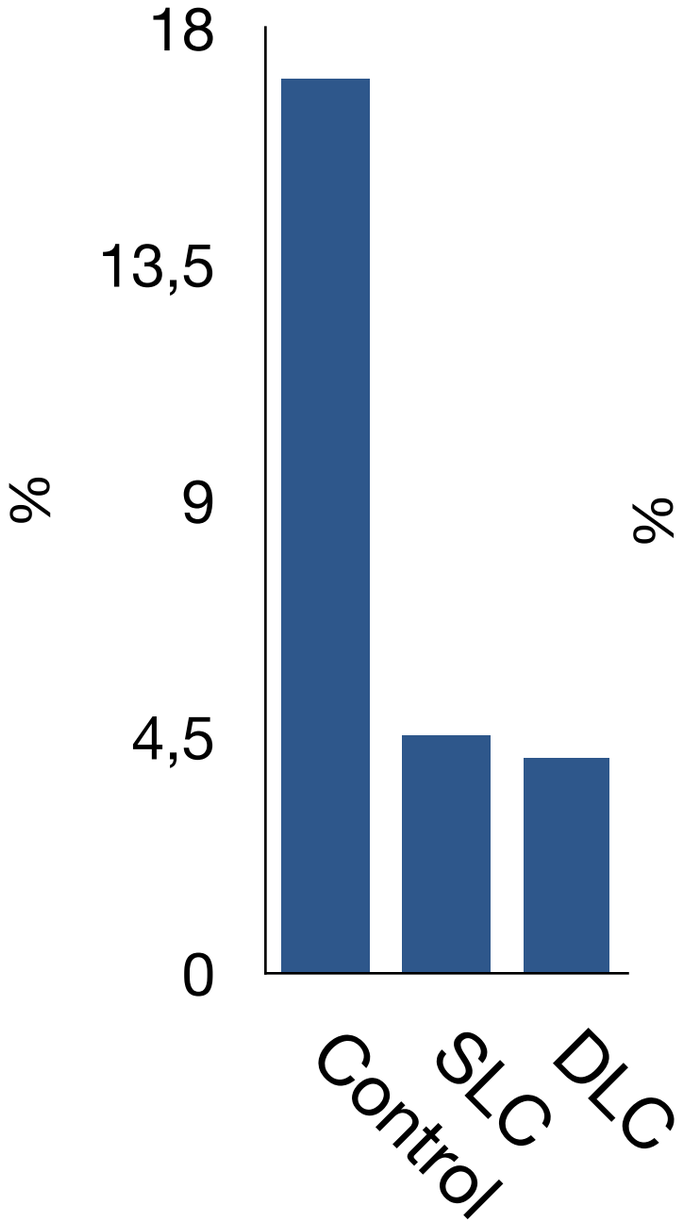



*Al-Kass Z et al. Reprod Domest Anim 2021;56:848–56.*

# Stallion semen and colloid centrifugation

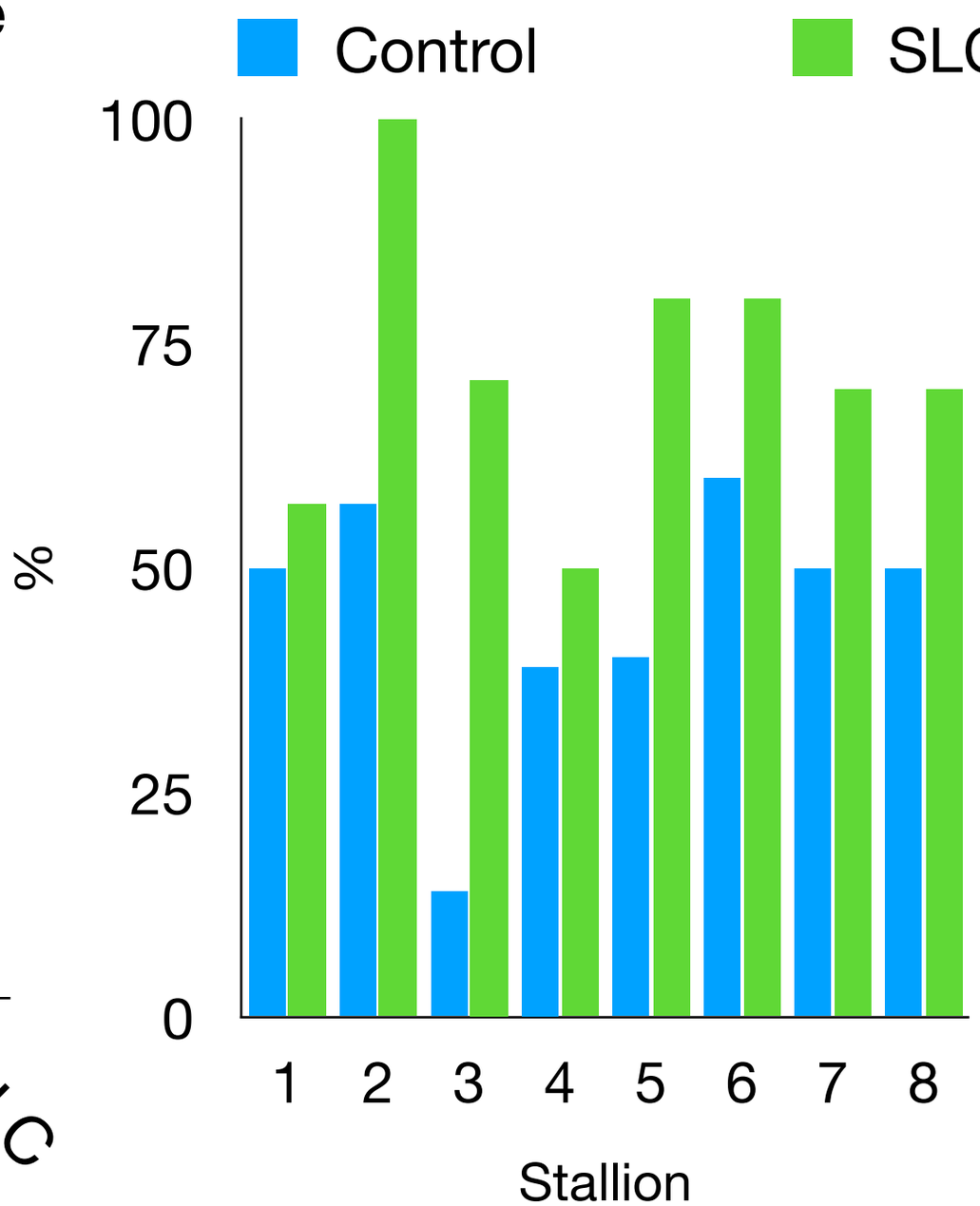


## Chromatin damage

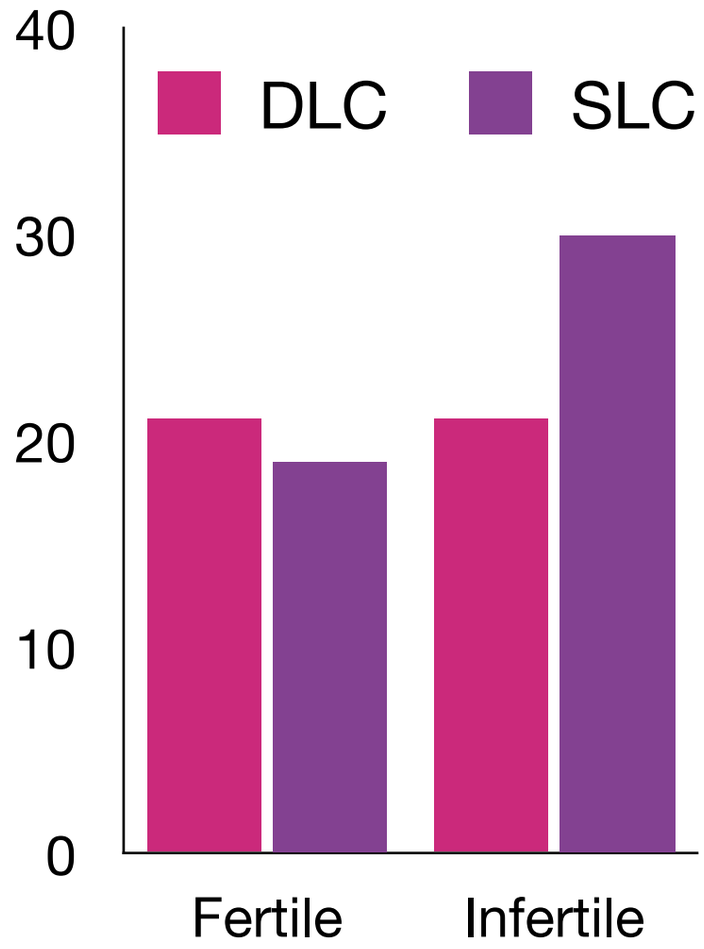



*Morrell et al. 2014. Theriogenology 82, 1102–1105.*

## Fertility



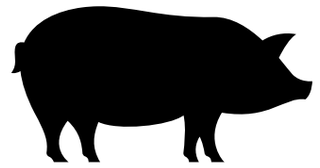

## % Blastocyst after ICSI

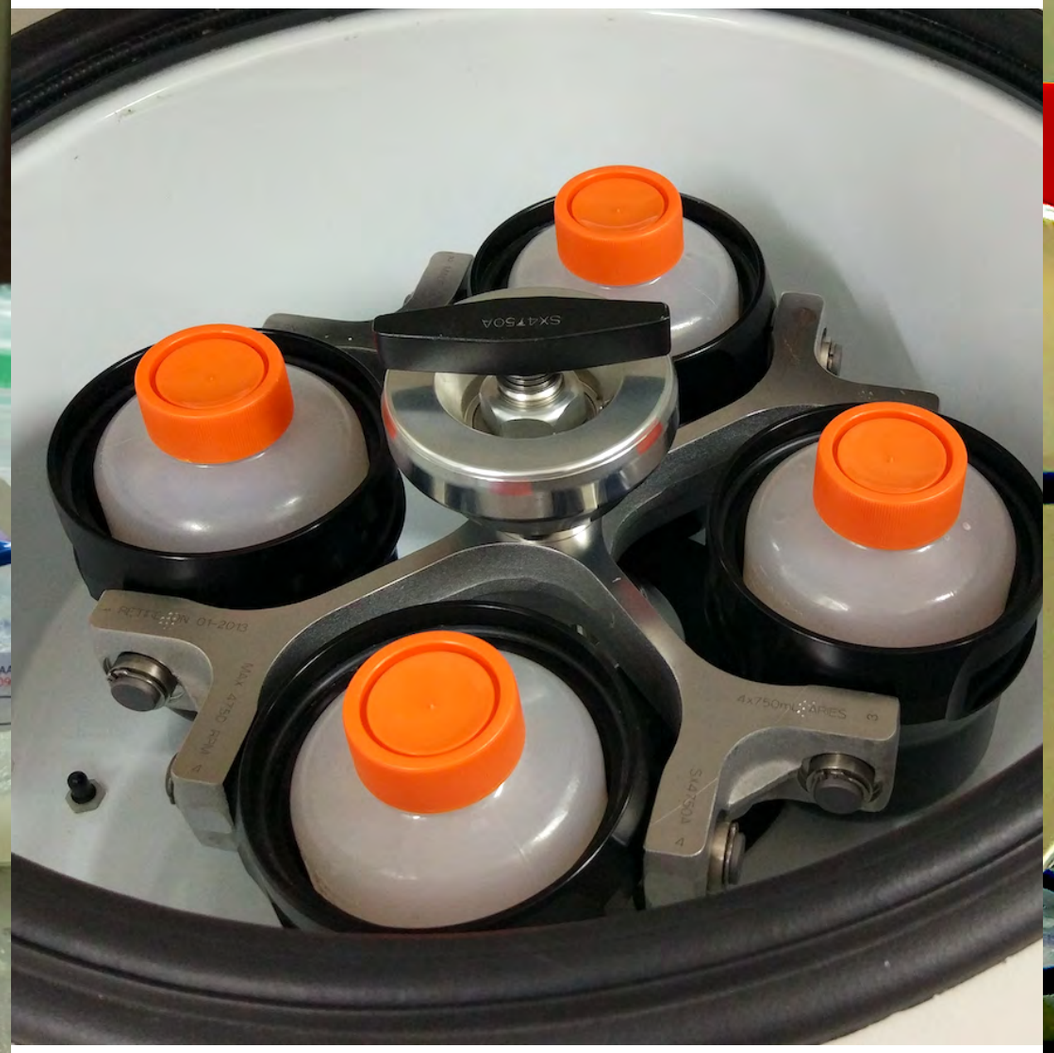



*Colleoni, et al., 2011. J. Equine Vet. Sci. 31, 536–541.*

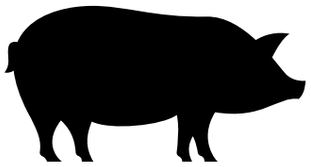
# Single Layer Centrifugation (SLC)

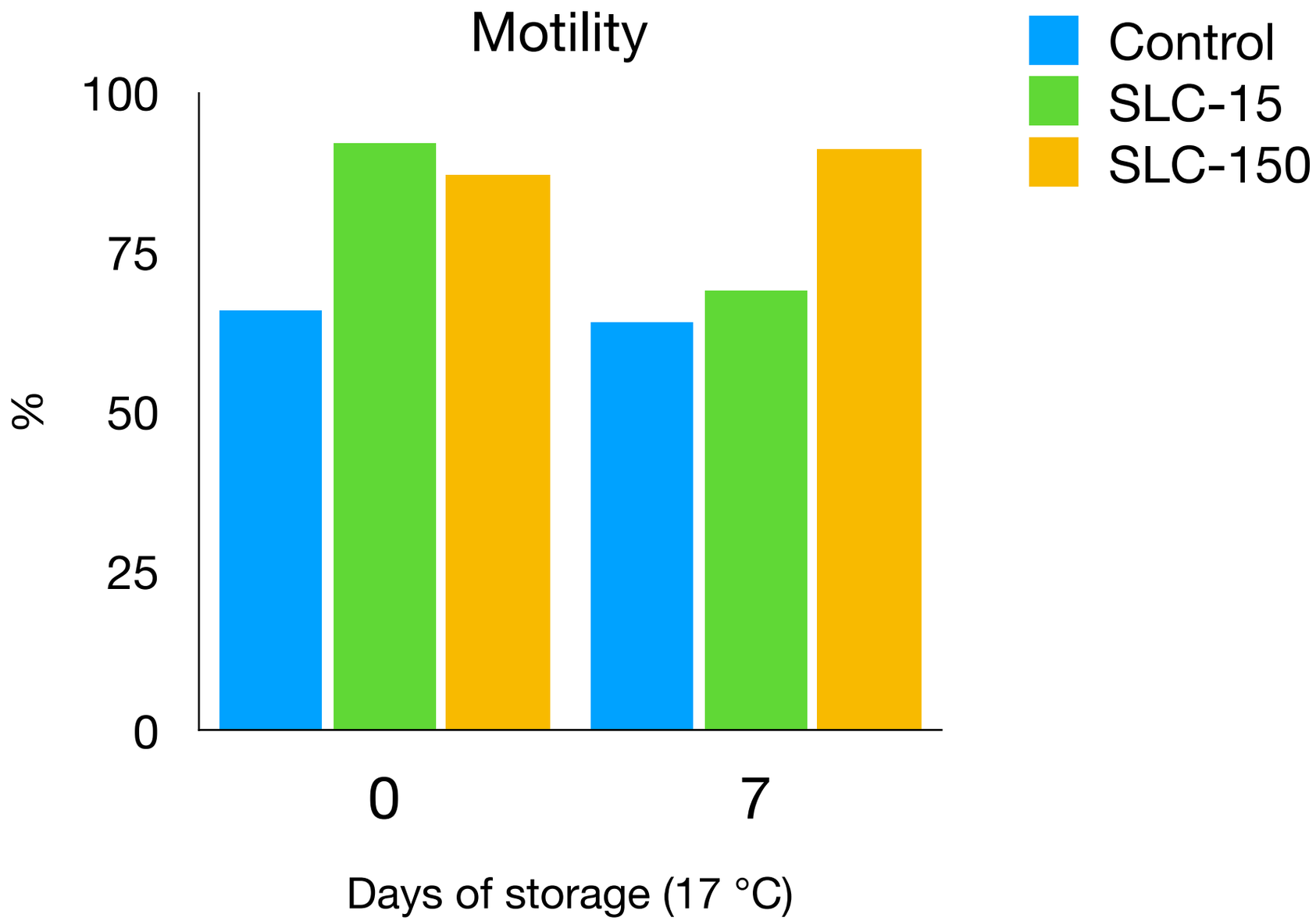
500-ml tubes, large-size processing





# Boar sperm in 500 ml bottles (150-200 ml semen)





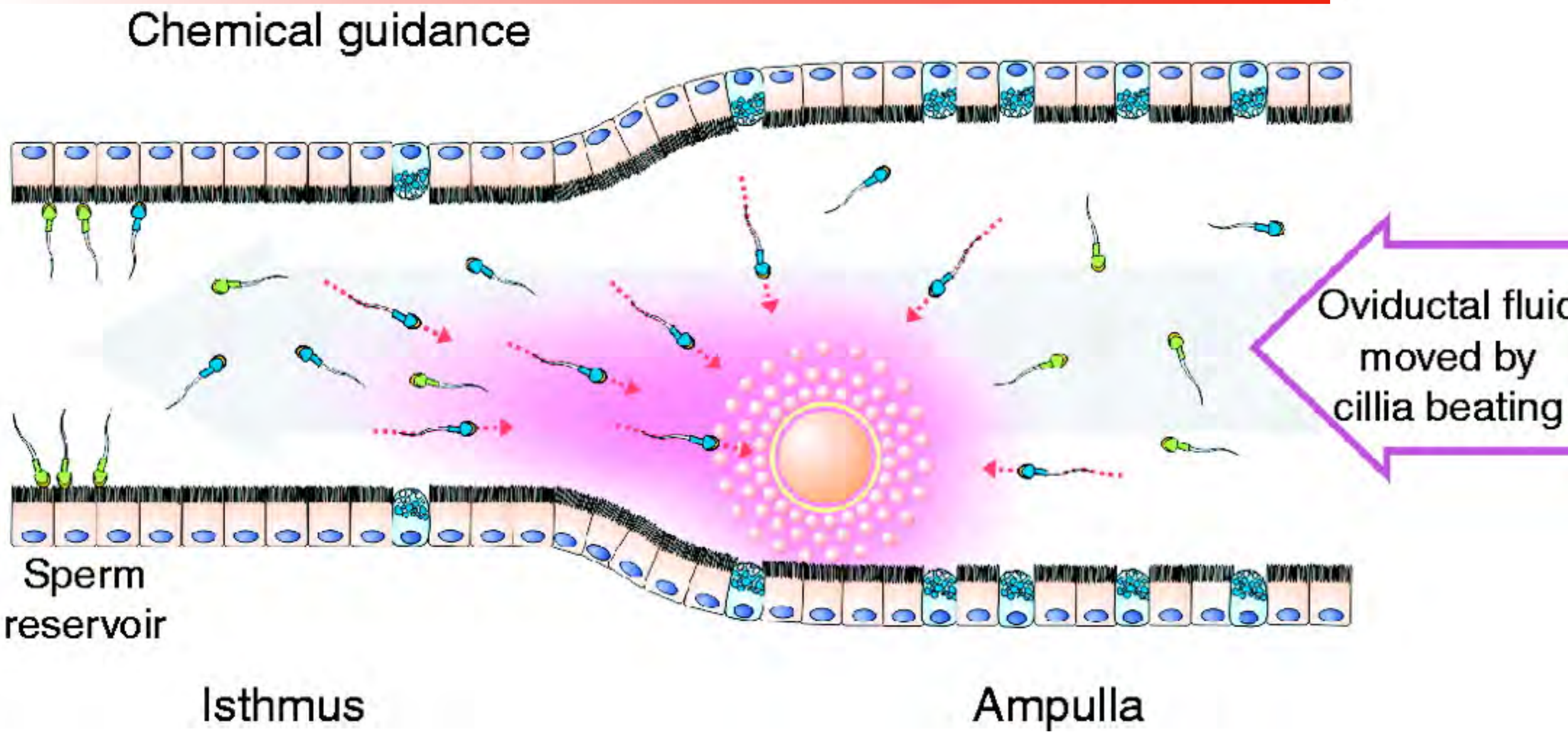

*Morrell et al. 2012. ISRN Vet Sci 2011.*

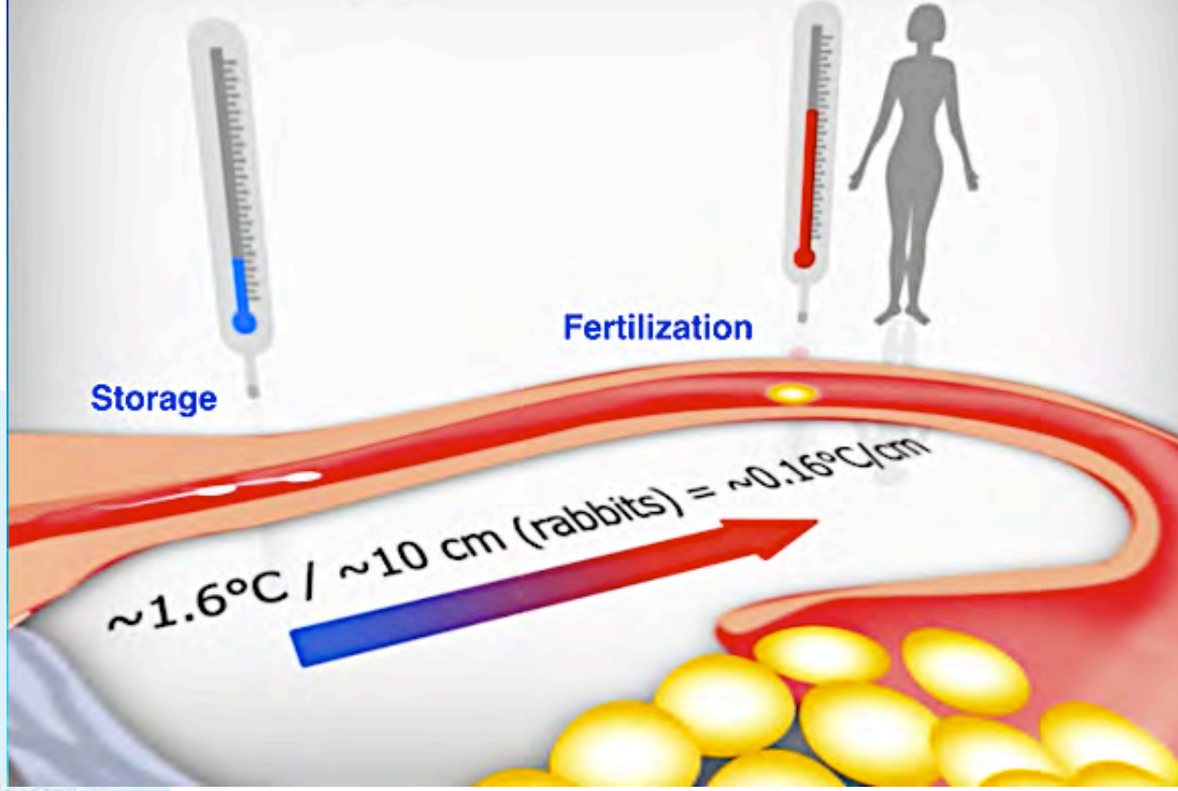
INIA
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria

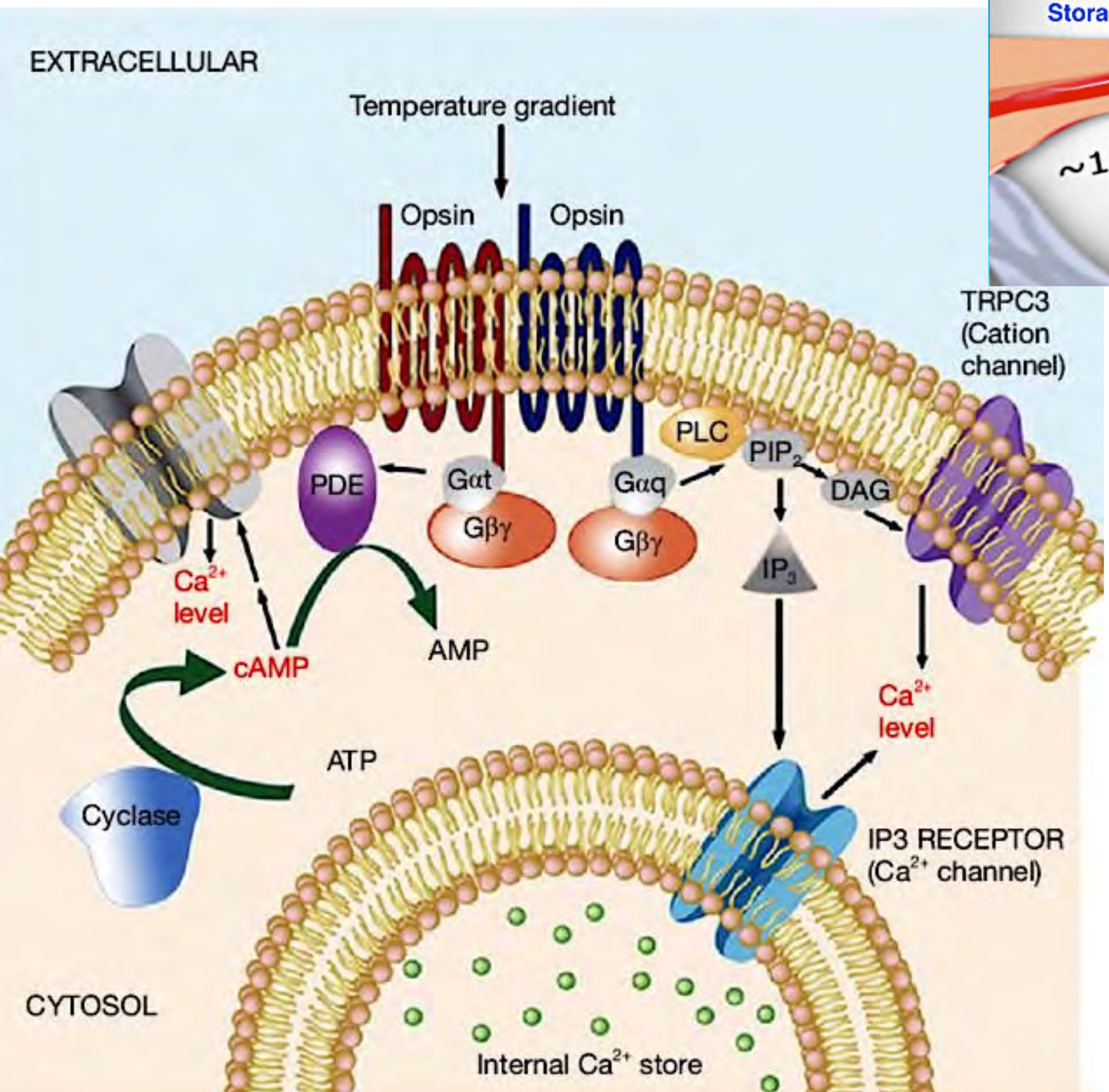
# Use of sperm thermotaxis for improving DNA status and embryo development

Serafín Pérez-Cerezales†

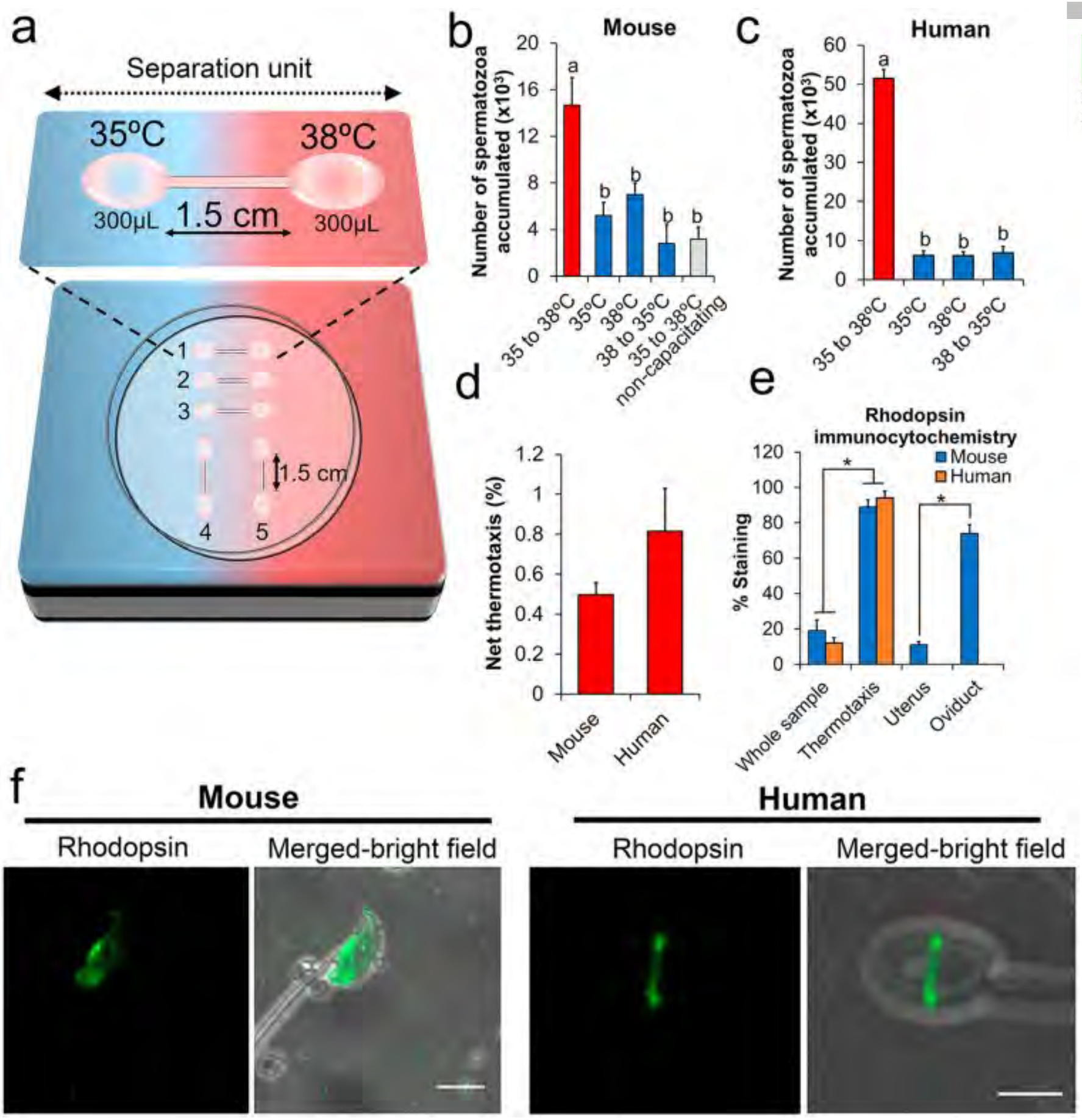
# Thermal gradient





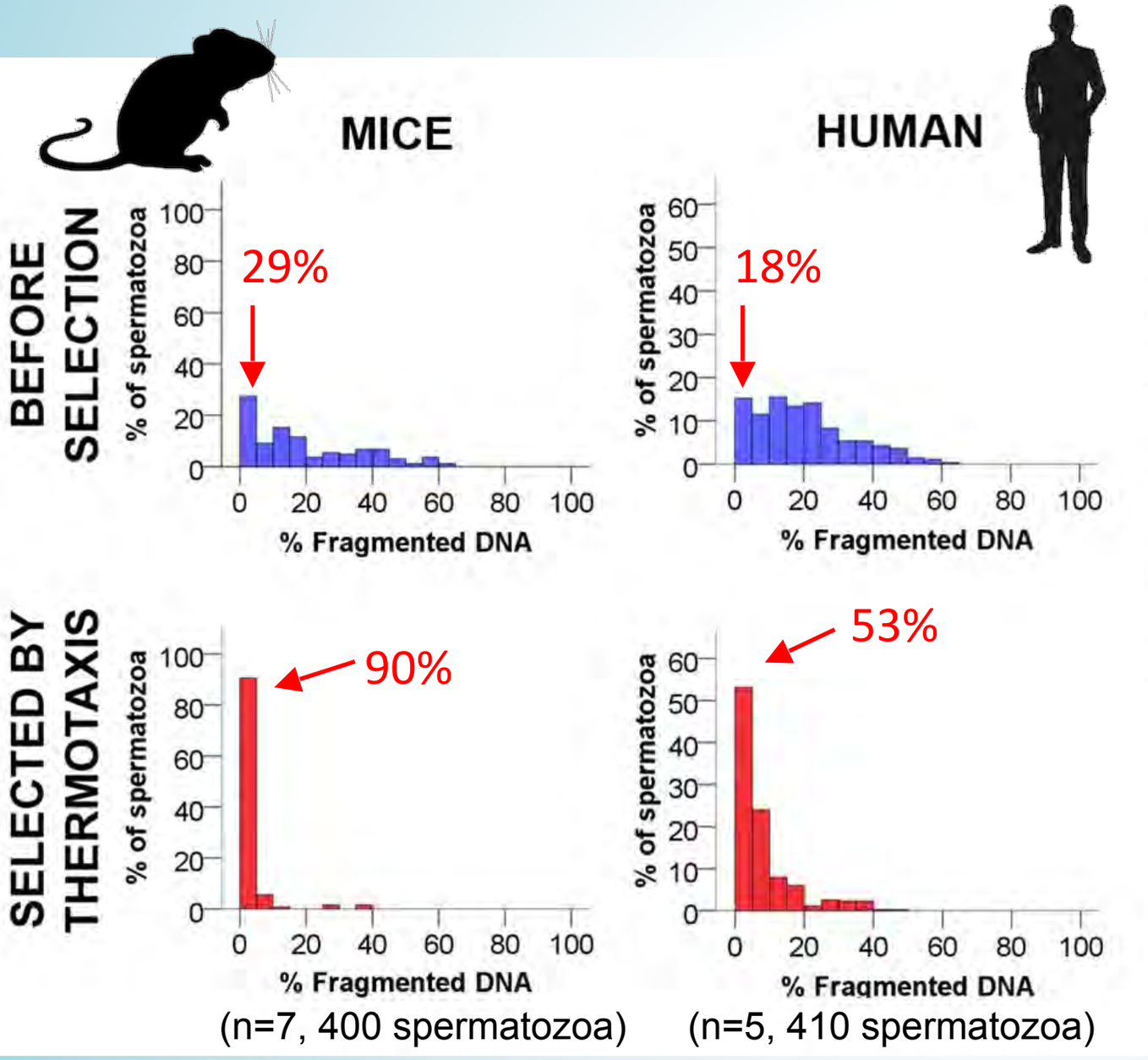



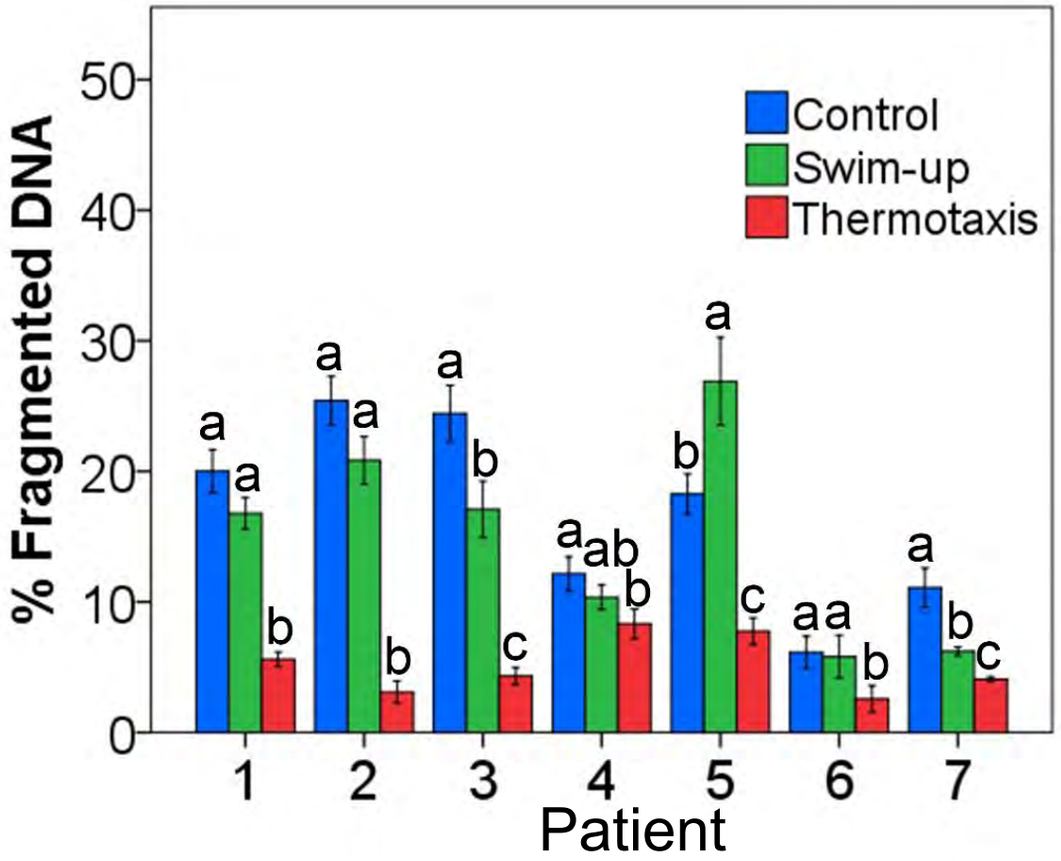

*Pérez-Cerezales et al., 2015. Involvement of opsins in mammalian sperm thermotaxis. Sci Rep 5, 16146.*




*Pérez-Cerezales et al. Sci. Rep. 2018, 8:2902*

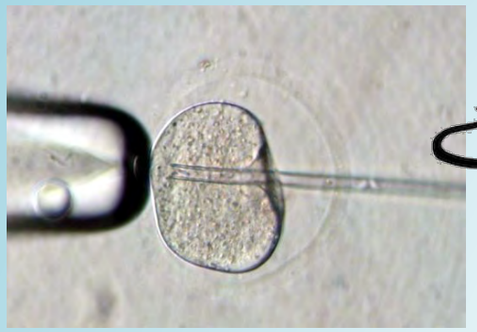
# Thermotaxis sperm selection: DNA integrity

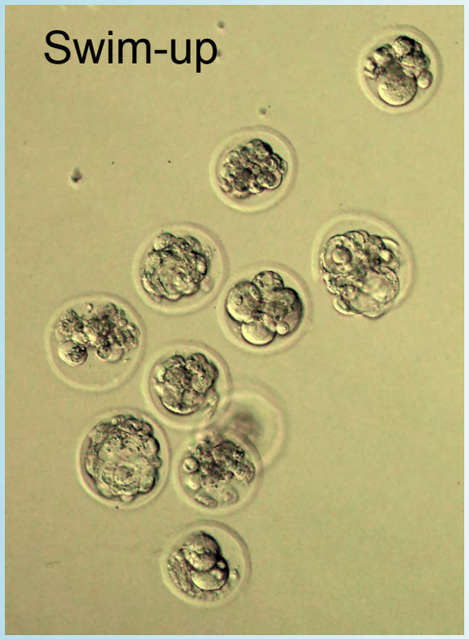


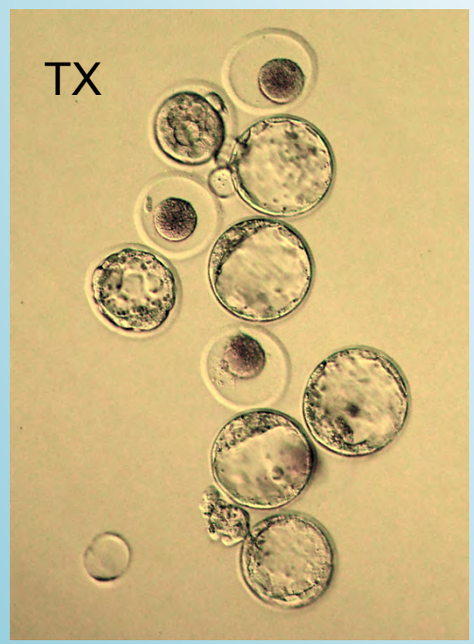



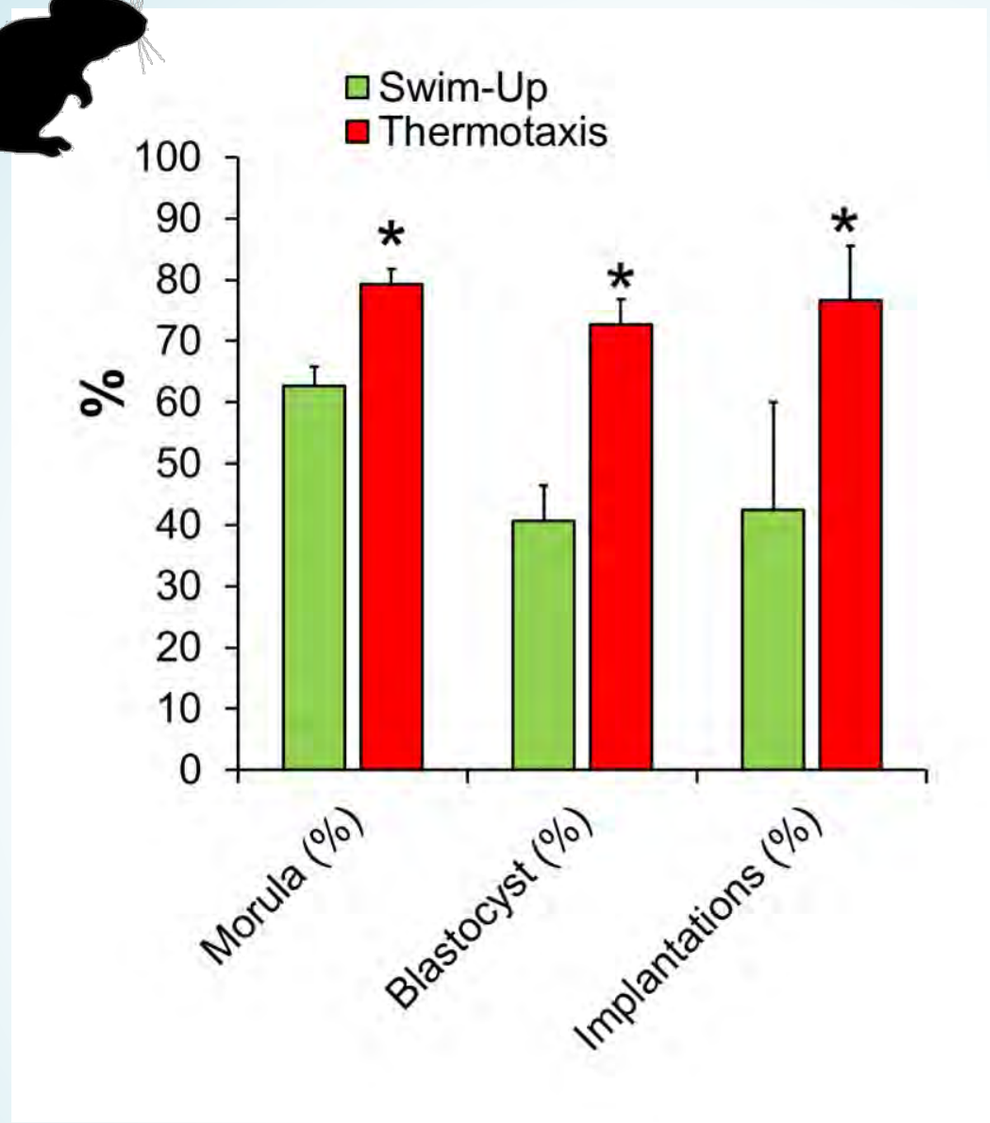

*Pérez-Cerezales et al. Scientific Reports 2018, 1:2902.*

# TRANSFERENCE OF MOUSE EMBRYOS PRODUCED BY ICSI

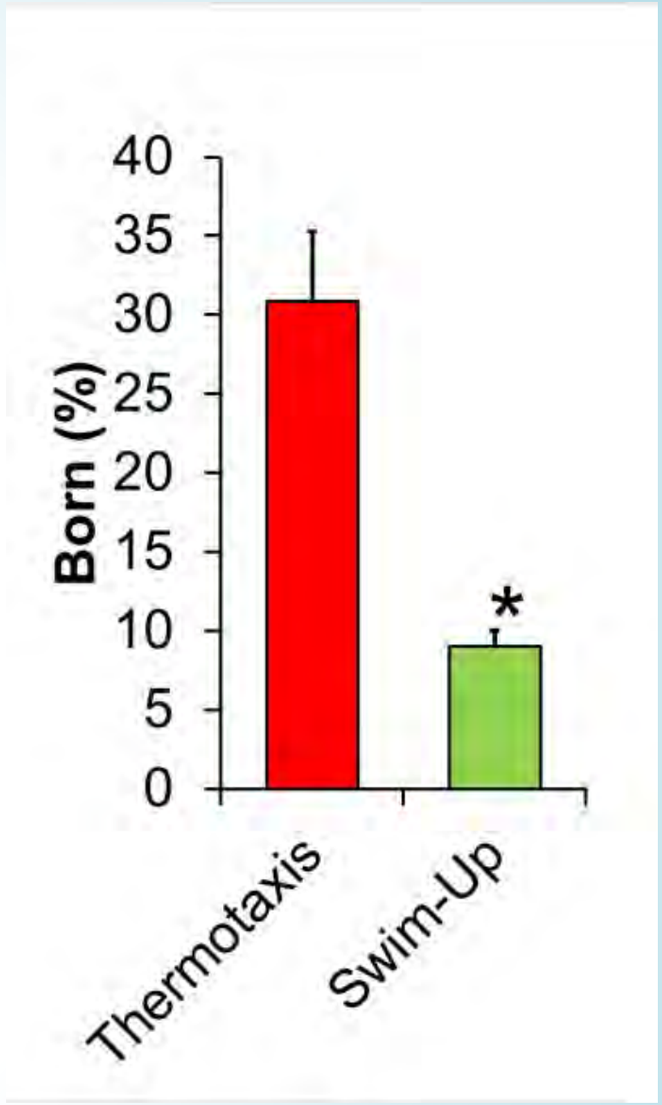





n=8, ~122 and 126 divided embryos
**P* < 0.05 (Student's t-test) between Swim-up and hermotaxis

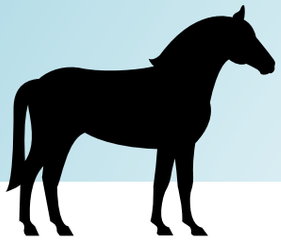


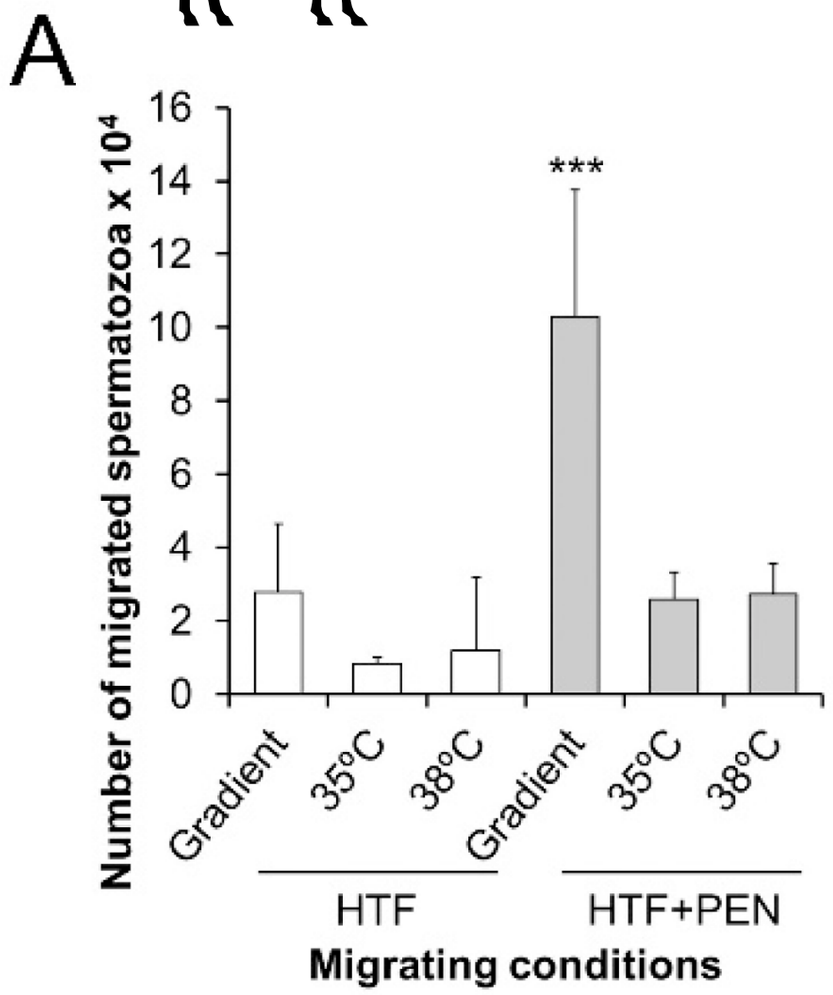

(n=15, 150 and 202 divided embryos)

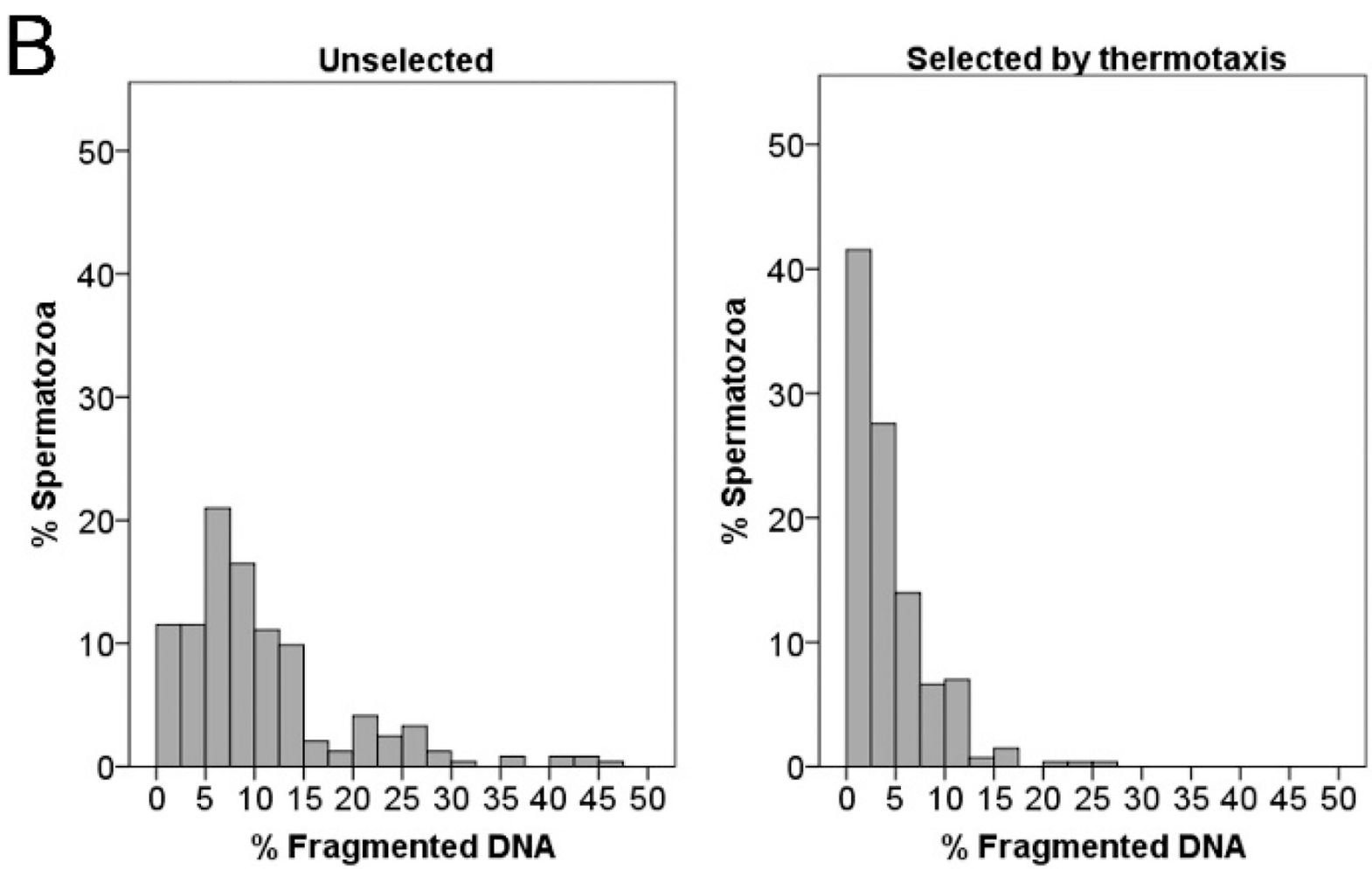
**P* < 0.01 (Student's t-test) between Swim-Up and thermotaxis

*Pérez-Cerezales et al. Sci. Rep. 2018, 1:2902.*

# Thermotaxis with stallion spermatozoa

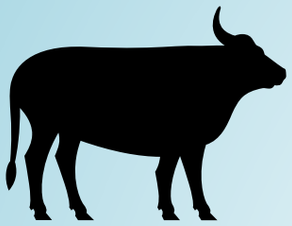


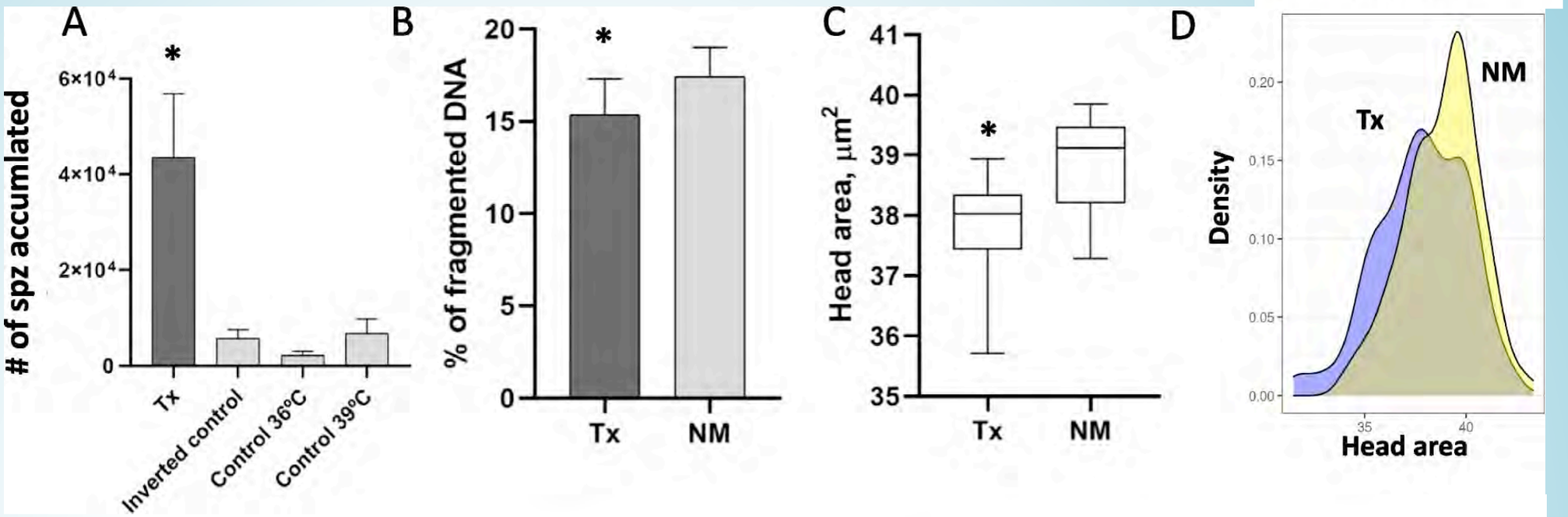


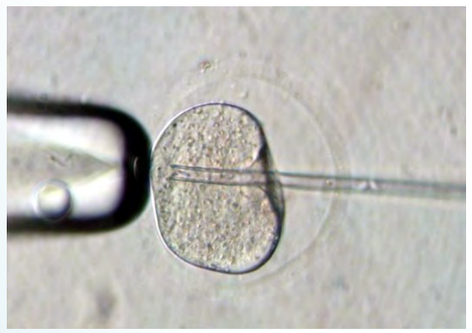

*Ruiz-Díaz, S. 2020. Animals 10, 1467.*

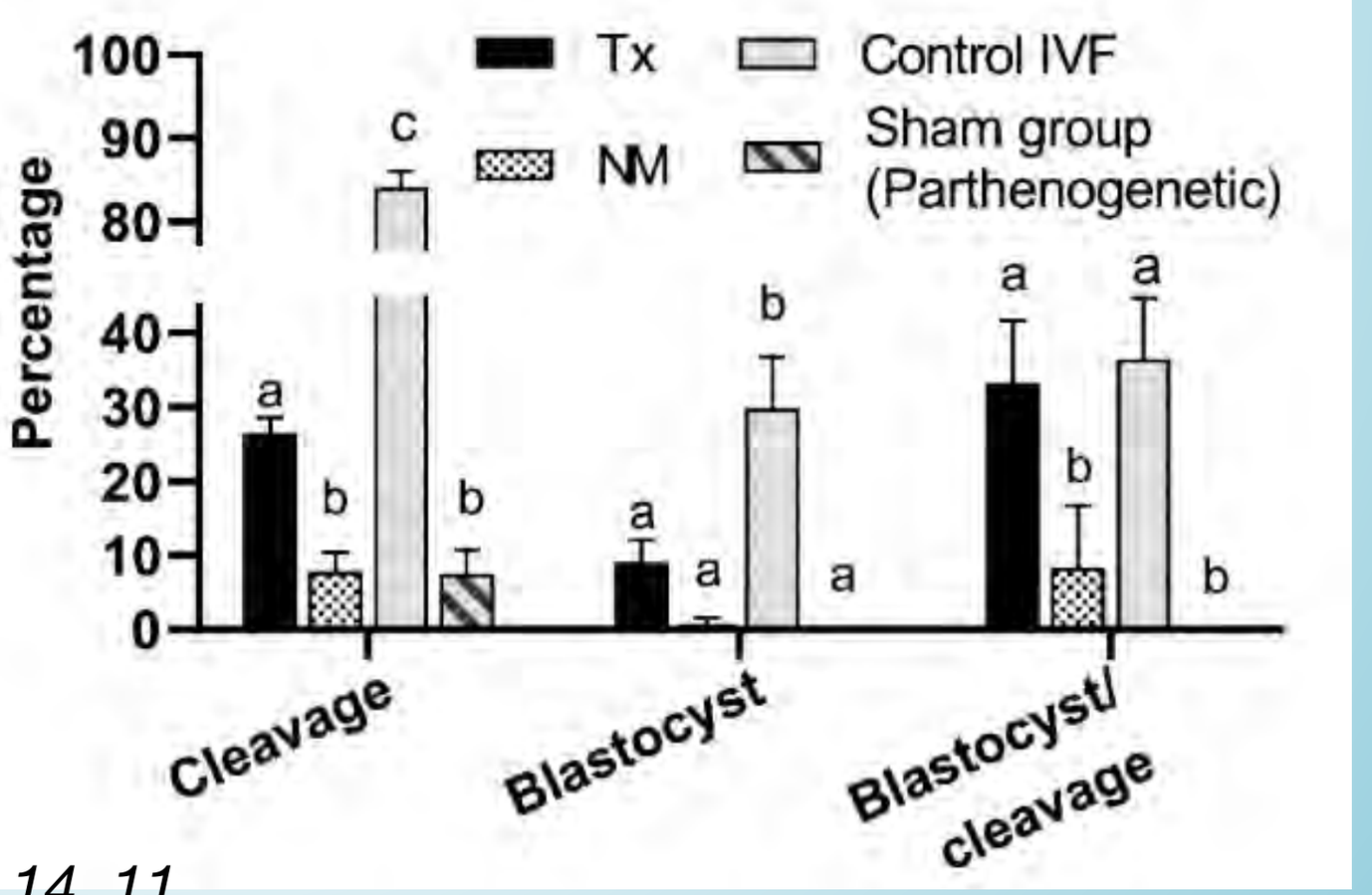
# Thermotaxis and ICSI with bull spermatozoa






ICSI in cattle is inefficient



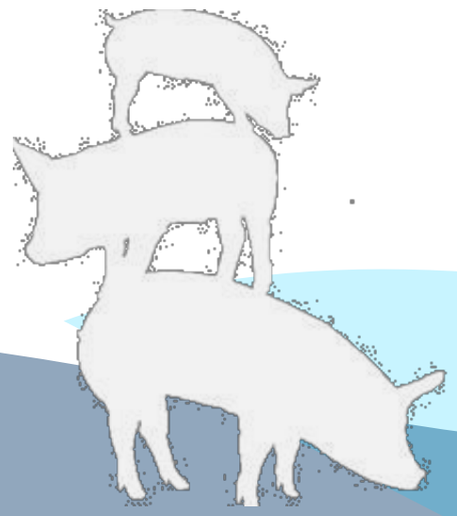



*Ruiz-Díaz, S. 2023. J Anim Sci Biotechnol 14, 11.*

# Impact of red-light stimulation upon preservation, function and fertilizing ability of boar and bull semen

**Marc Yeste**

marc.yeste@udg.edu



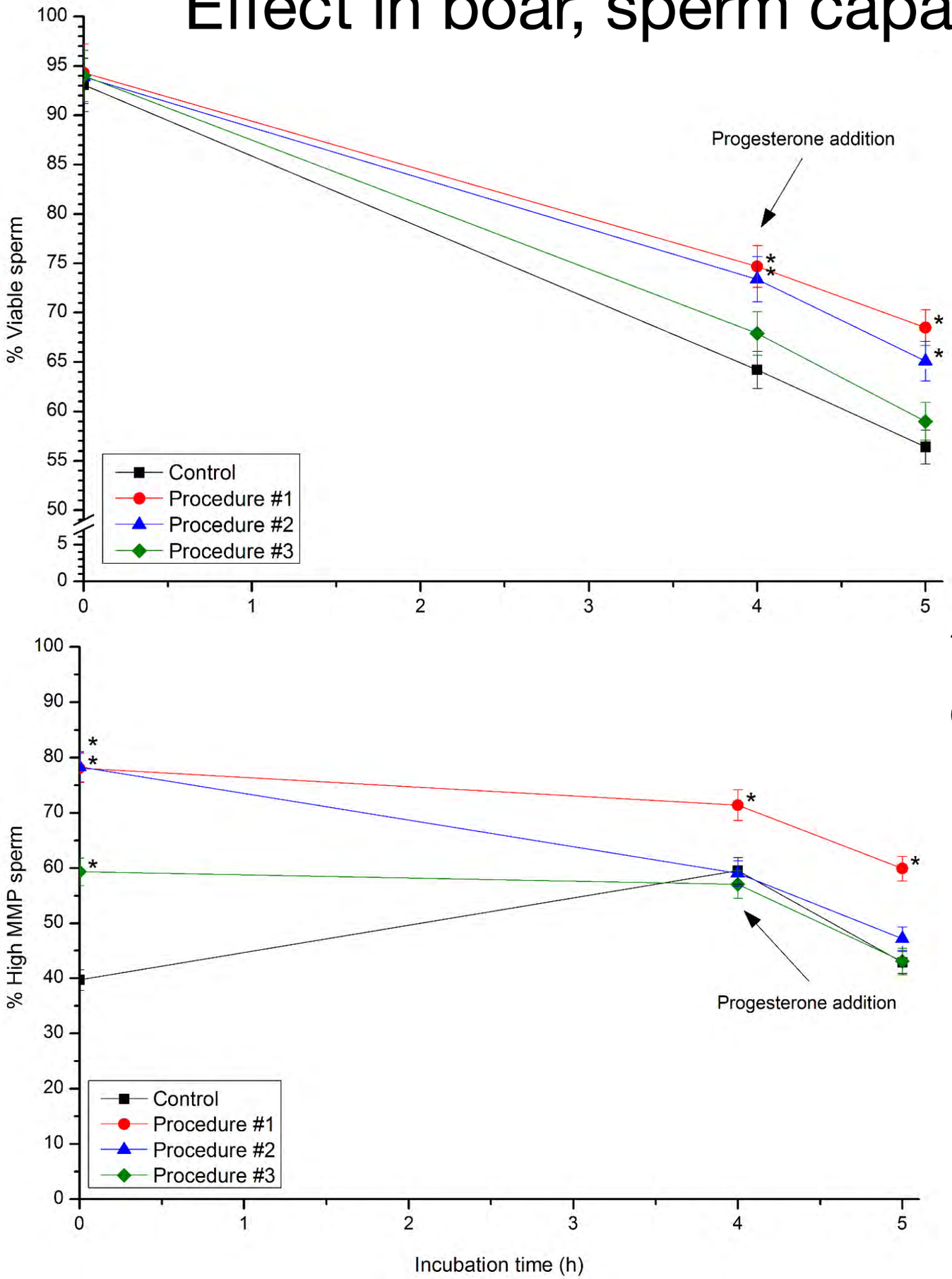
# Spermatozoa are affected by light

**Table 1**
Summary of the principal effects of light-stimulation on mammalian spermatozoa.

| Species | Type | Light source | Wavelength | Intensity of irradiation tested | Best treatment | Effects | Reference |
|---|---|---|---|---|---|---|---|
| Bovine | FT | Laser (He-Ne and Diode | 632 nm (He-Ne)<br>780 nm (Diode) | He-Ne (10 and 35 mW)<br>Diode laser (13 and 40 mW)<br>Energy density ranging from 2 to 30 J $cm^{-2}$ | 6-18 J $cm^{-2}$ (He-Ne)<br>3 J $cm^{-2}$ (Diode laser) | Increase of $Ca^{2+}$ transport | Lubart et al. (1992) |
| Bovine | FT | Laser (He-Ne) | 633 nm | 0.3 mW and 10 mW | 0.3 mW He-Ne laser with energy doses between 0.06 and 0.2 J | Increase of $Ca^{2+}$ transport | Breitbart et al. (1996 |
| Bovine | FT | Laser (Diode) | 780 nm | Power: 4, 9 and 24 mw | 9-24 mW | Increase binding of $Ca^{2+}$ to plasma membrane | Lubart et al. (1997) |
| Bovine | Fresh | Lamp | 400-800 nm | Power = 40–80 mW $cm^{-2}$ | N/A | Increase in the production of ROS species by mitochondria | Lavi et al. (2010) |
| Bovine | Fresh | Laser (Diode) | 660 nm | 4 J (80 sec)<br>6 J (120 sec) | 4 J (80 sec) | Increase in sperm cryotolerance before cryopreservation | Fernandes et al. (2015) |
| Bovine | FT | Laser (He-Ne) | 633 nm | Output powers: 5, 7.5 and 10 mW<br>Irradiation times: 5 and 10 min) | Irradiation for 10 min, regardless of output power | Transient increase of VSL, VCL and proportions of sperm with intermediate membrane potential. Increase of ALH after 30 min of beginning light-stimulation | Siqueira et al. (2016). |
| Buffalo | Fresh | Laser (Diode) | 532 nm | Energy fluencies: 0.076 J $cm^{-2}$, 0.15 J $cm^{-2}$, 0.23 J $cm^{-2}$, 0.31 J $cm^{-2}$ and 0.38 J $cm^{-2}$ | 0.31 J $cm^{-2}$ | Increase in several sperm motility parameters | Abdel-Salam et al. (2011). |
| Dog | Fresh | Laser (Diode) | 655 nm | Energy densities: 0, 4, 6 and 10 J $cm^{-2}$ | 4, 6 and 10 J $cm^{-2}$ | Increase of VAP, LIN and BCF<br>No effect on total sperm motility | Corral-Baqués et al. (2005) |
| Dog | Fresh | Laser (Diode) | 655 nm | Powers: 6.8 mW, 15.4 mW, 33.1 mW and 49.7 mW | 49.7 mW | Increase of VSL, VAP, LIN, STR, WOB and BCF<br>Decrease of ALH | Corral-Baqués et al. (2009). |
| Human | Fresh | Laser (Krypton) | 647 | Energy densities: 4 J $cm^{-2}$, 8 J $cm^{-2}$ and 32 J $cm^{-2}$ | 32 J $cm^{-2}$ | Increase of total sperm motility | Sato et al. (1984) |
| Human | Fresh | Laser (infrared) | N/A | Power/Frequency: 5 mW/2 Hz; 5 mW/2200 Hz; 30 mW/2 Hz; 30 mW/2200 Hz, | 30 mW and 2200 Hz | Increase of progressive sperm motility, LIN and ALH and ATP consumption<br>Decrease of BCF | Lenzi et al. (1989) |
| Human | Fresh | Laser (Gallium-Aluminum-Arsenide) | 830 nm | Energy densities: 0, 2, 4, 6 and 10 J $cm^{-2}$ | 4 and 6 J $cm^{-2}$ | Better maintains progressive sperm motility<br>No impact on DNA integrity | Salman Yazdi et al. (2014) |
| Human | Fresh | LED | 470-850 nm | 850 nm (2.16 mW $cm^{-2}$); 625, 660 and 850 nm (3.92 mW $cm^{-2}$); 470 nm (5.06 mW $cm^{-2}$); 625, 660 and 470 nm (8.23 mW $cm^{-2}$), | 470 nm (5.06 mW $cm^{-2}$) | Increase of fast and progressive sperm<br>Decrease of non-motile spermatozoa | Ban Frangez et al. (2015) |

*Yeste et al. 2018. Anim Reprod Sci 194, 19–32.*

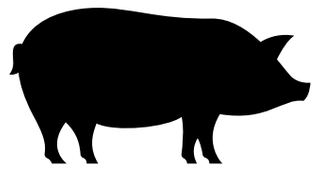
# Effect in boar, sperm capacitation experiments



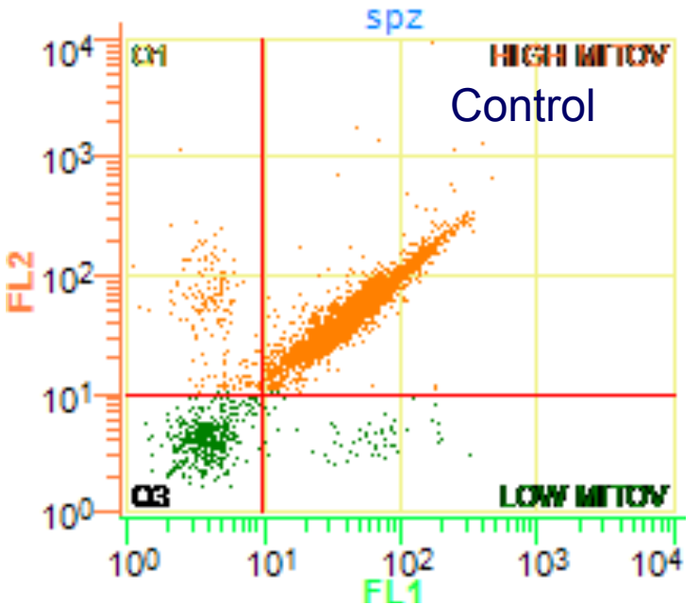

P#1: 10-10-10

P#2: 15-10-15

P#3: 20-10-20

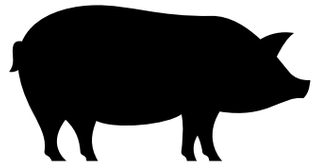


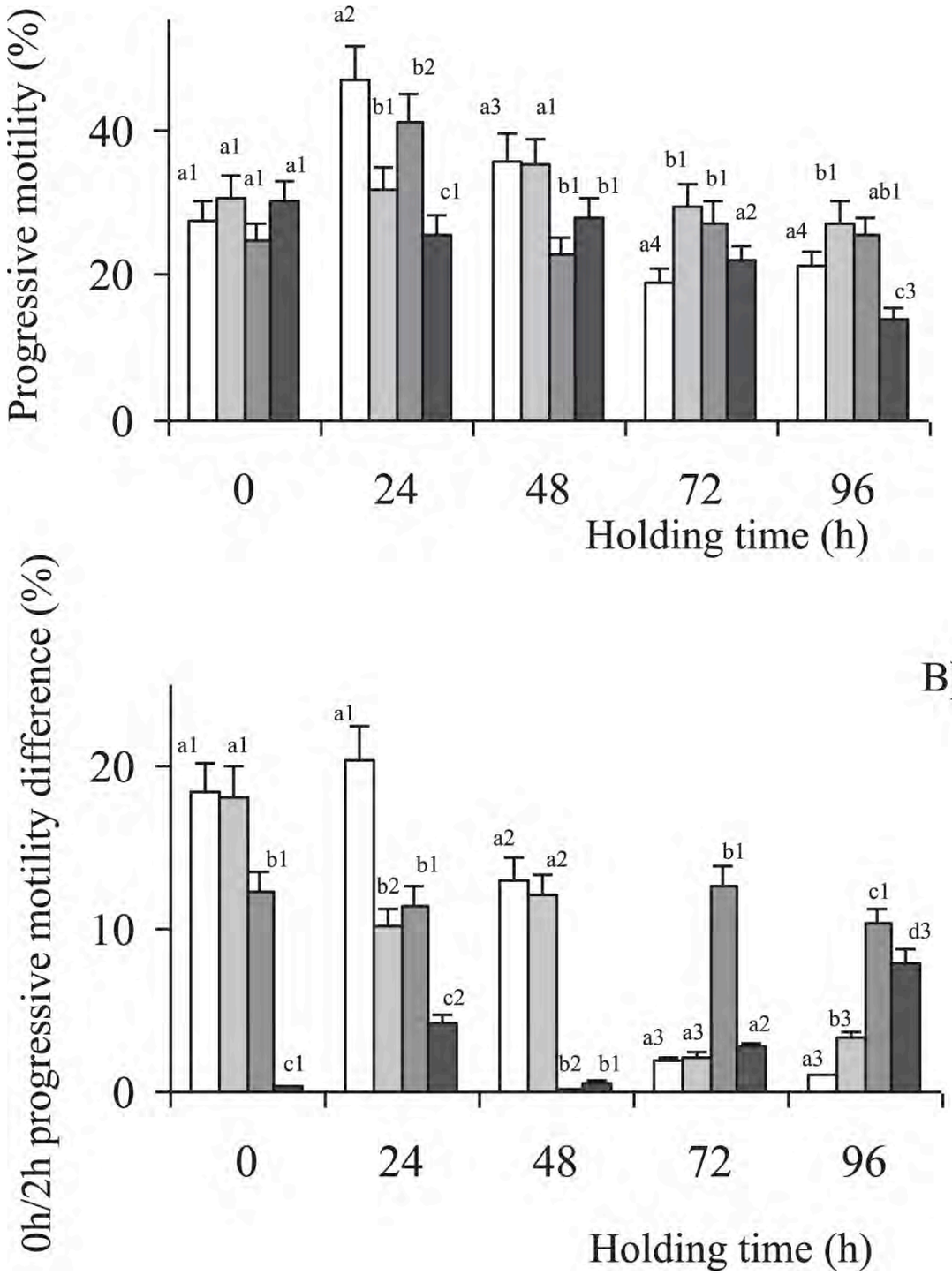
*$P<0.05$ *vs.* control

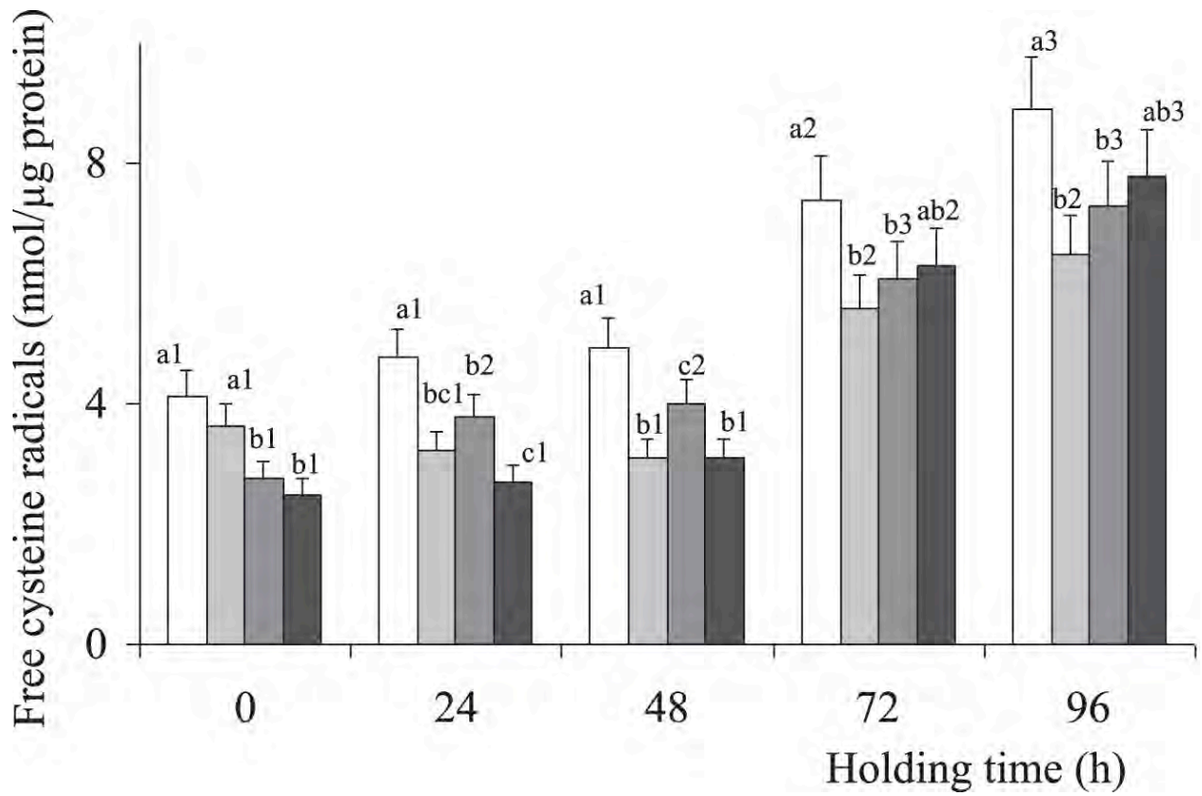



*Yeste et al., 2016. Sci Rep 6, 22569.*

# Effect in boar, storage

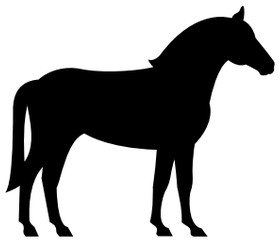




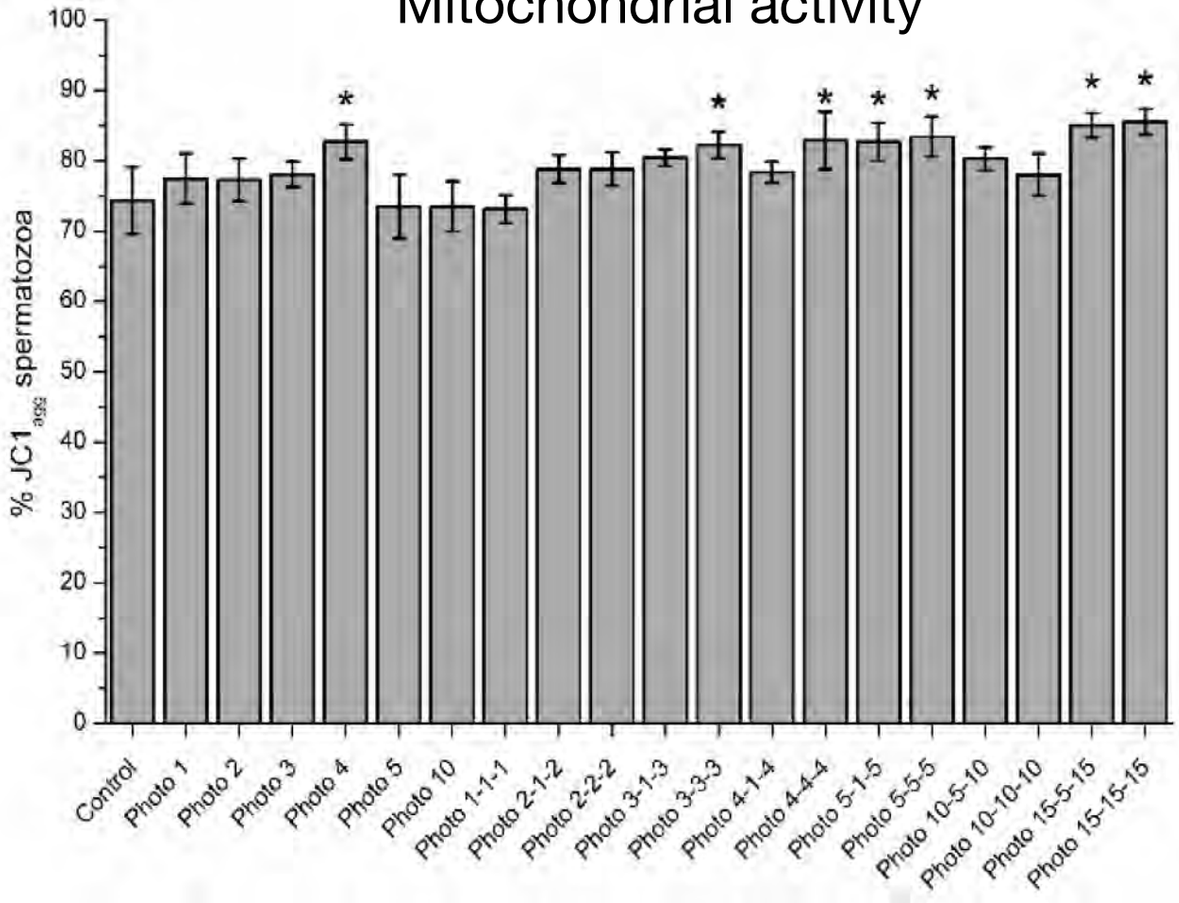




*Blanco-Prieto et al. 2020. Theriogenology 157, 388–398.*

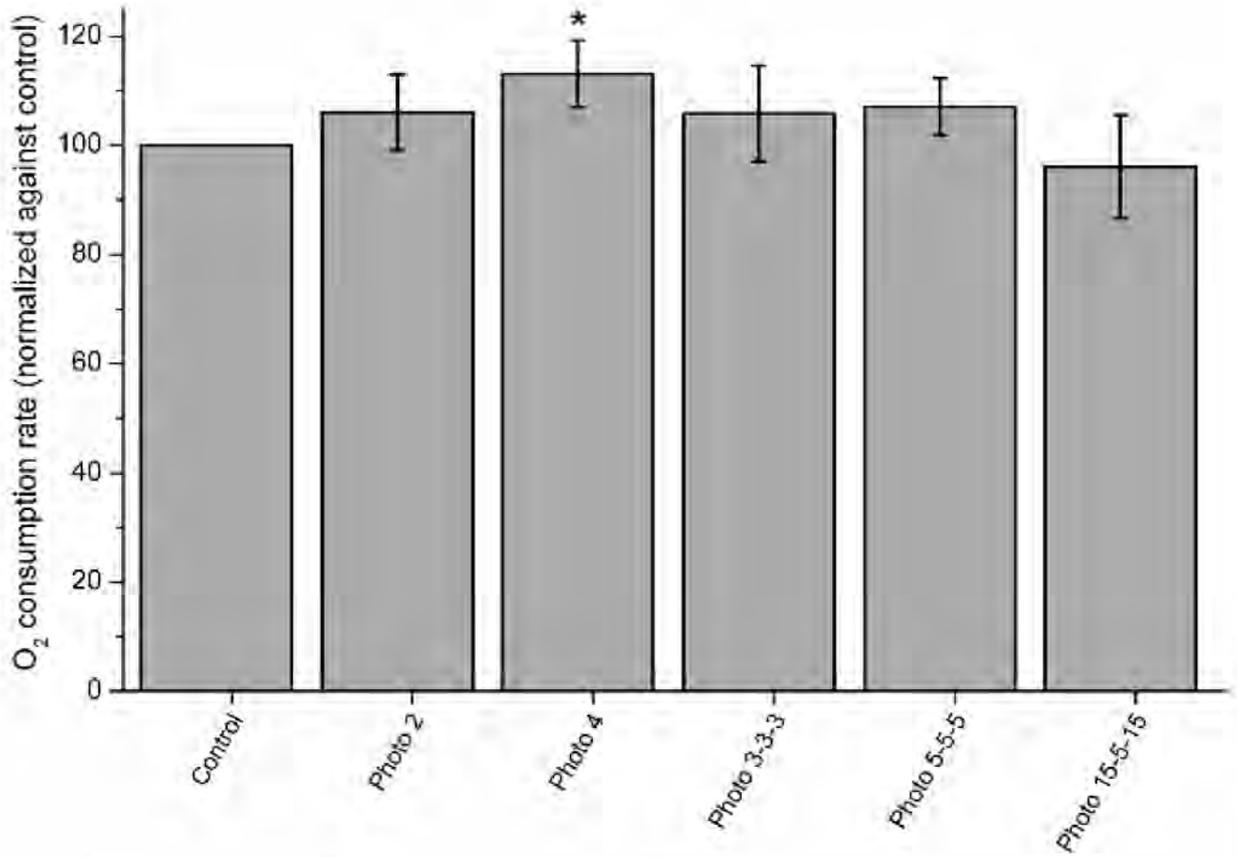
# Stallion



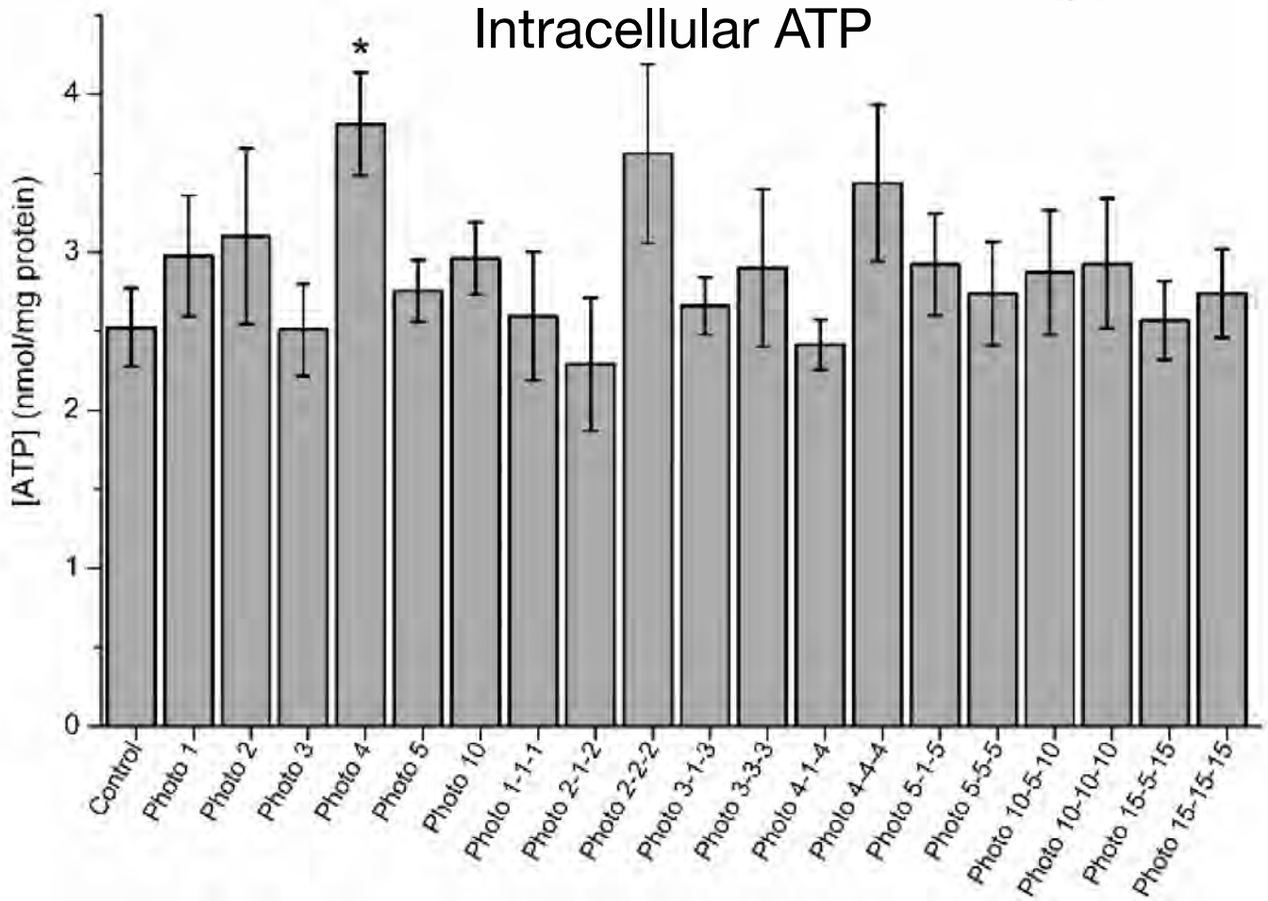
## Mitochondrial activity



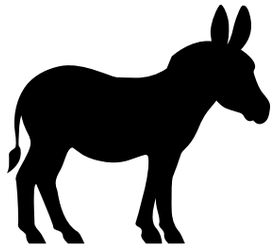

## $O_2$ consumption rate




## Intracellular ATP

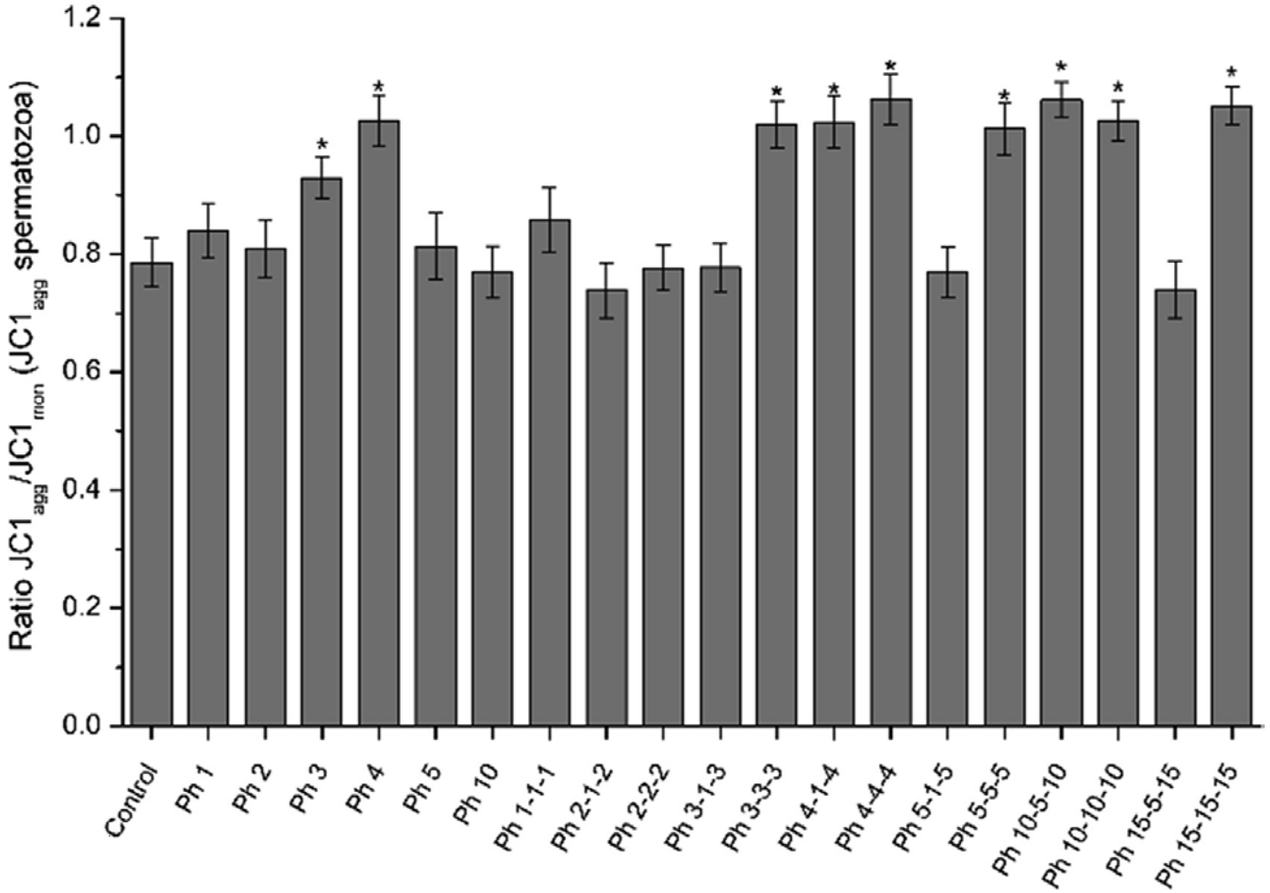



*Catalán et al. 2020. Biology (Basel) 9:254.*

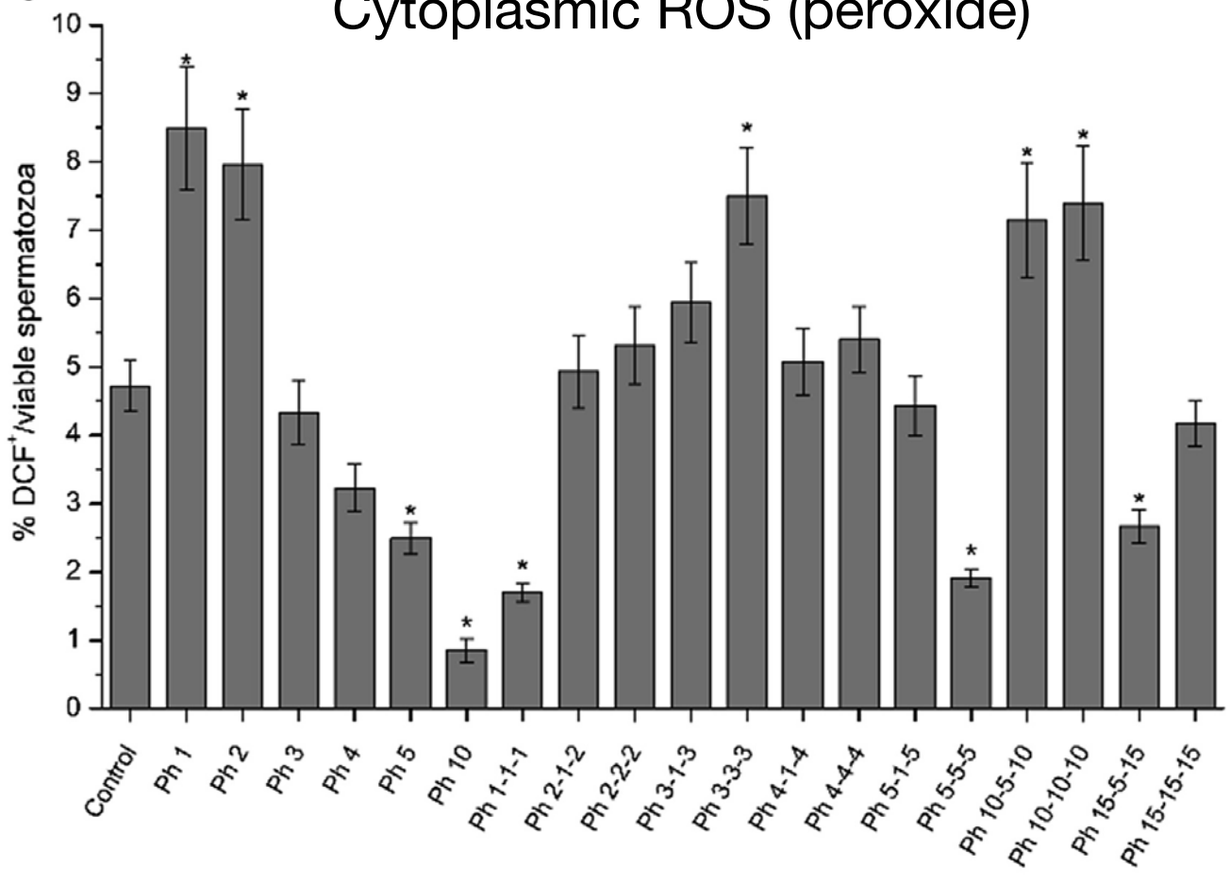


# Donkey

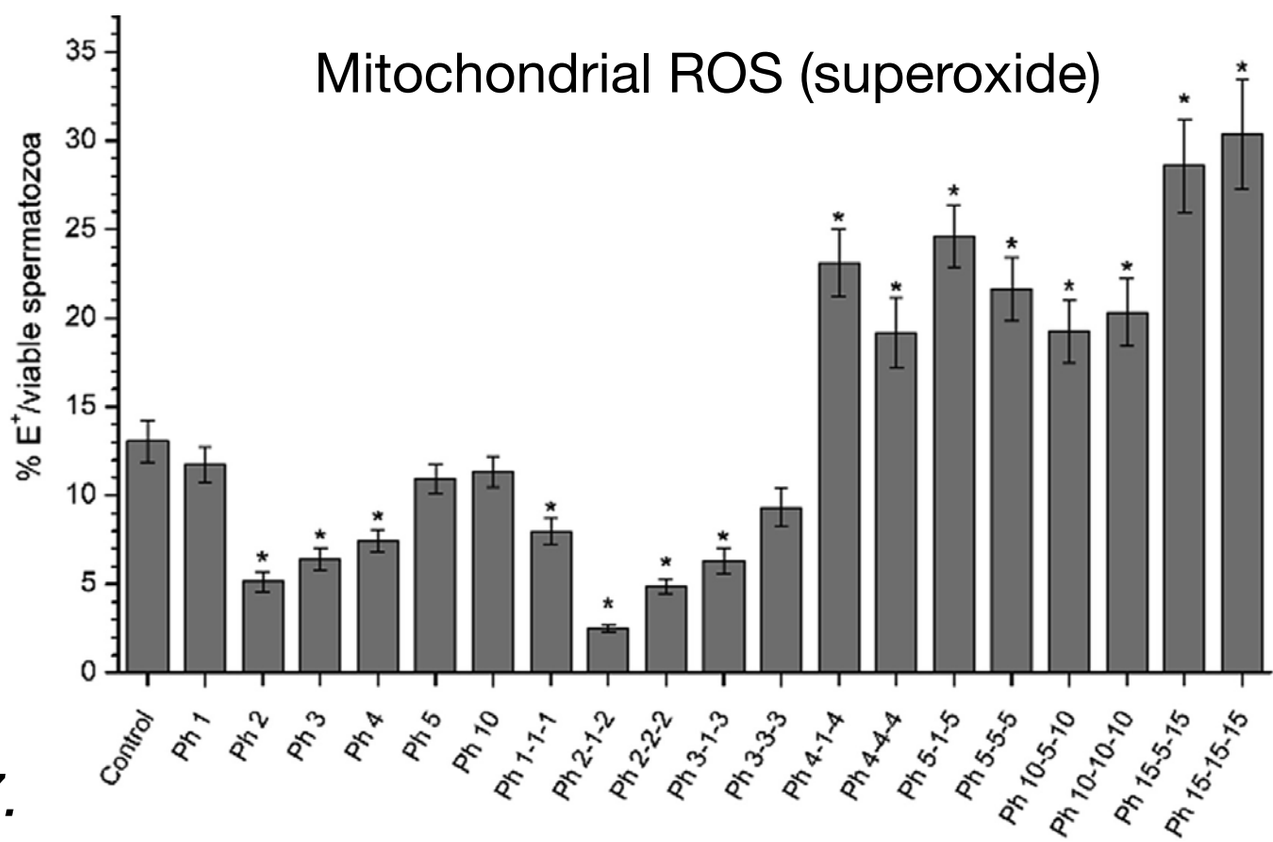
## Mitochondrial activity




## Cytoplasmic ROS (peroxide)




## Mitochondrial ROS (superoxide)



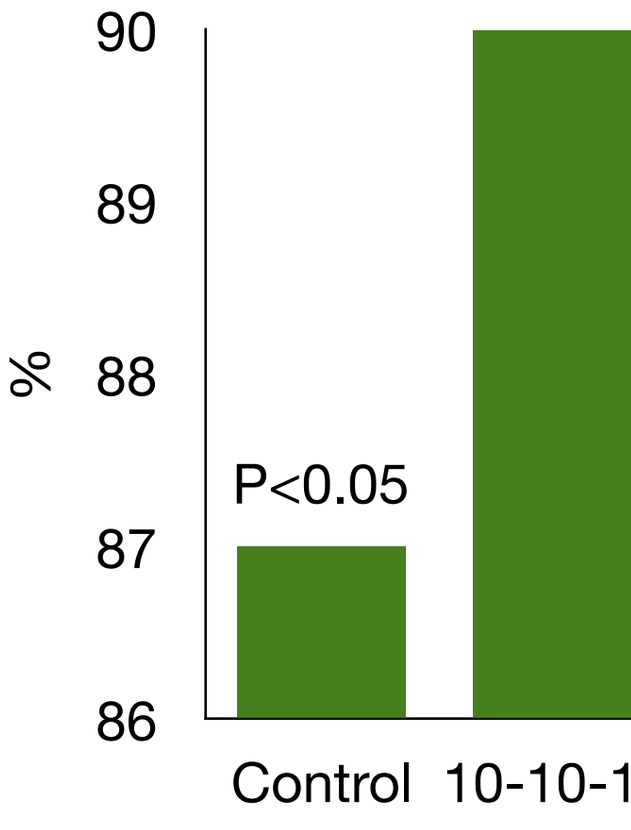

*Catalán et al. 2020. Theriogenology 149, 88–97.*

# AI trials: Worldwide survey

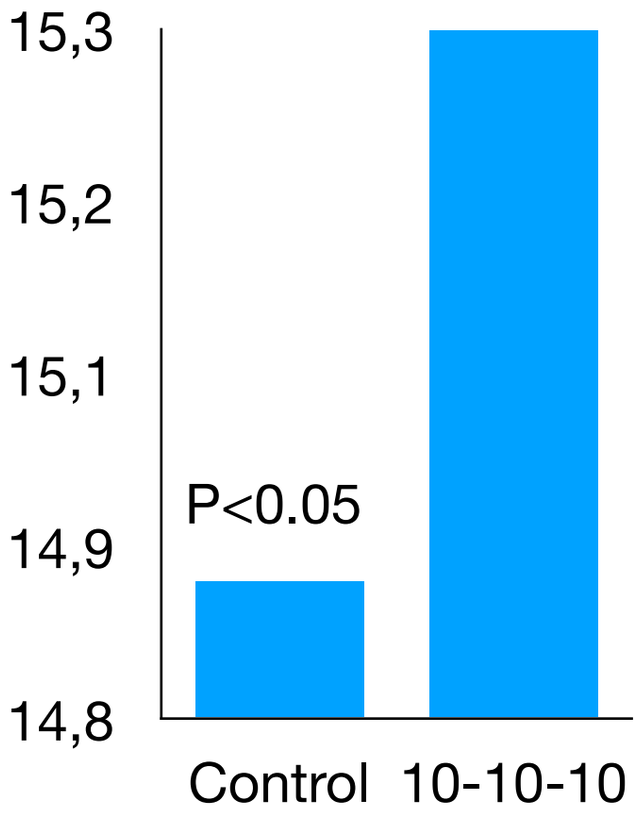
## Refrigerated pig semen

post-cervical/IUI insemination

### Farrowing

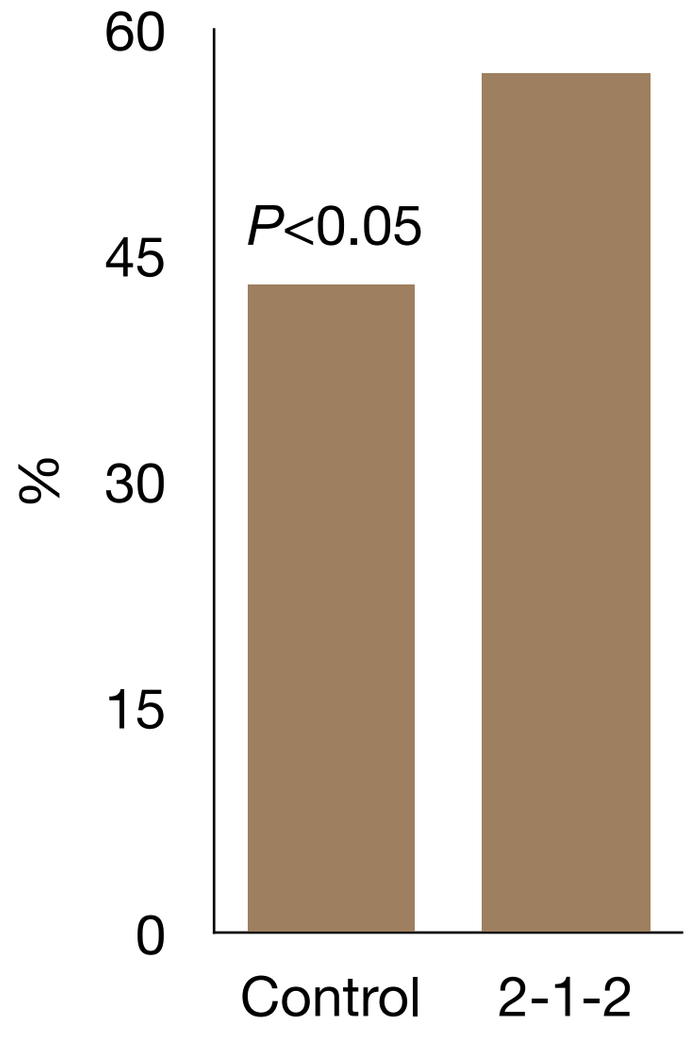



### Piglets




## Frozen-thawed bull semen

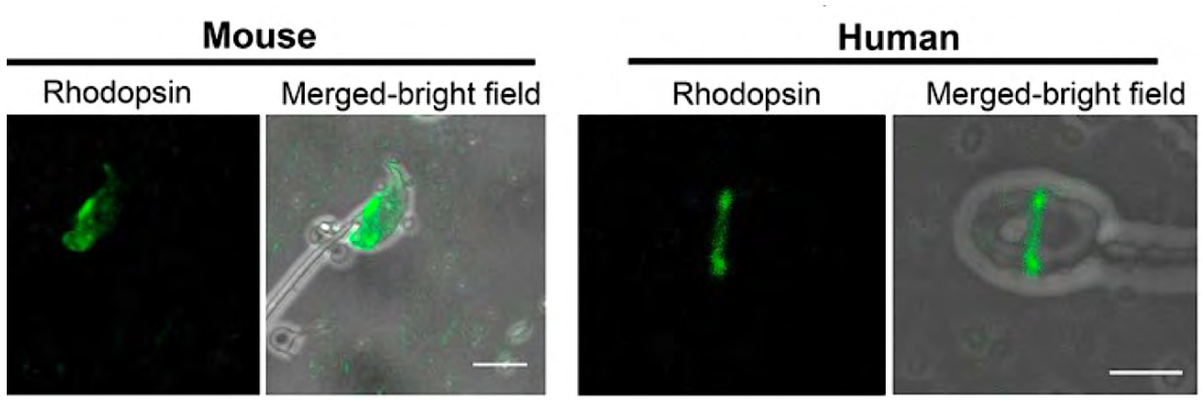
### Fertility



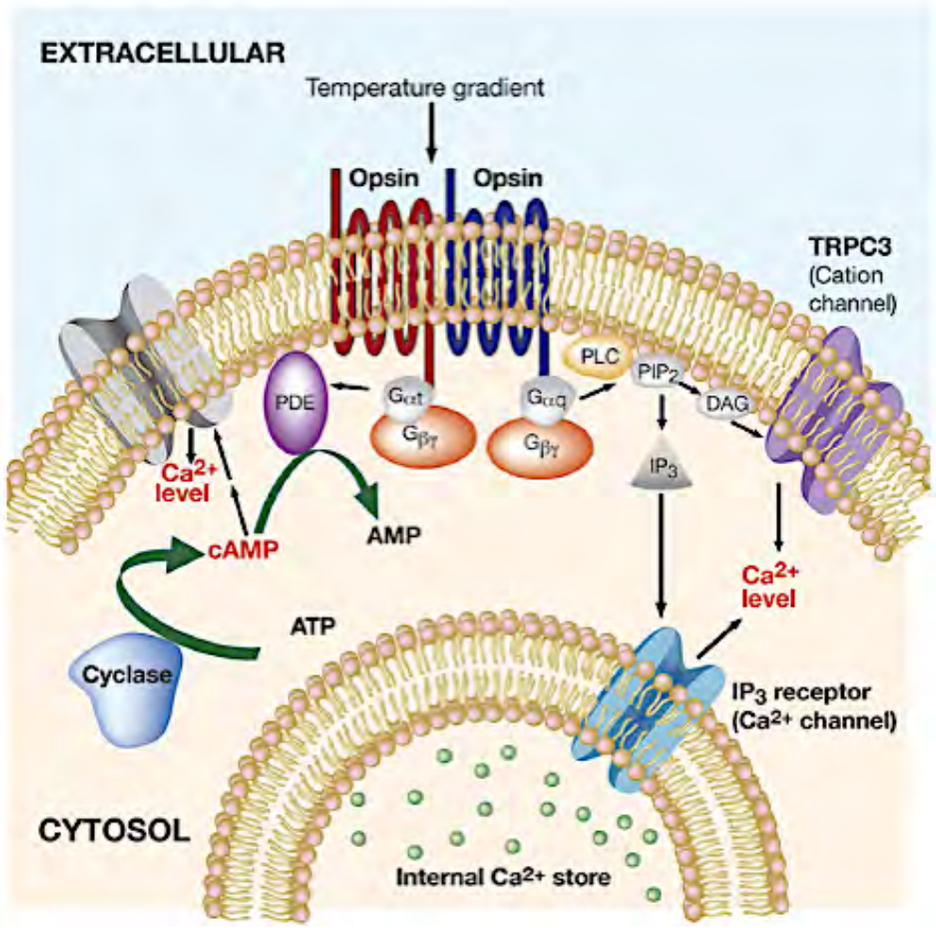

*Blanco Prieto et al. 2019. Reprod Domest Anim 54, 1145–1148.*

# Can we explain the light effects?

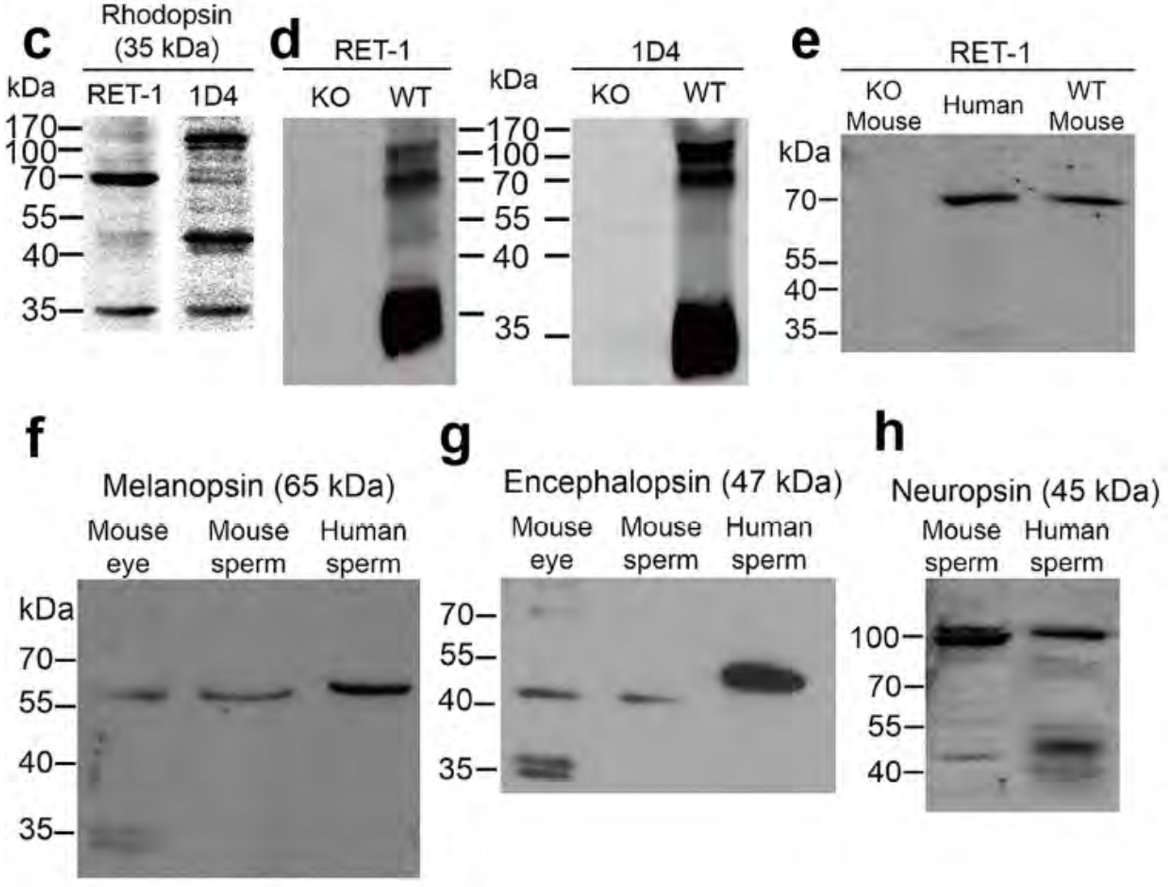
## Via Opsins – Transducin (Protein G signalling cascade)



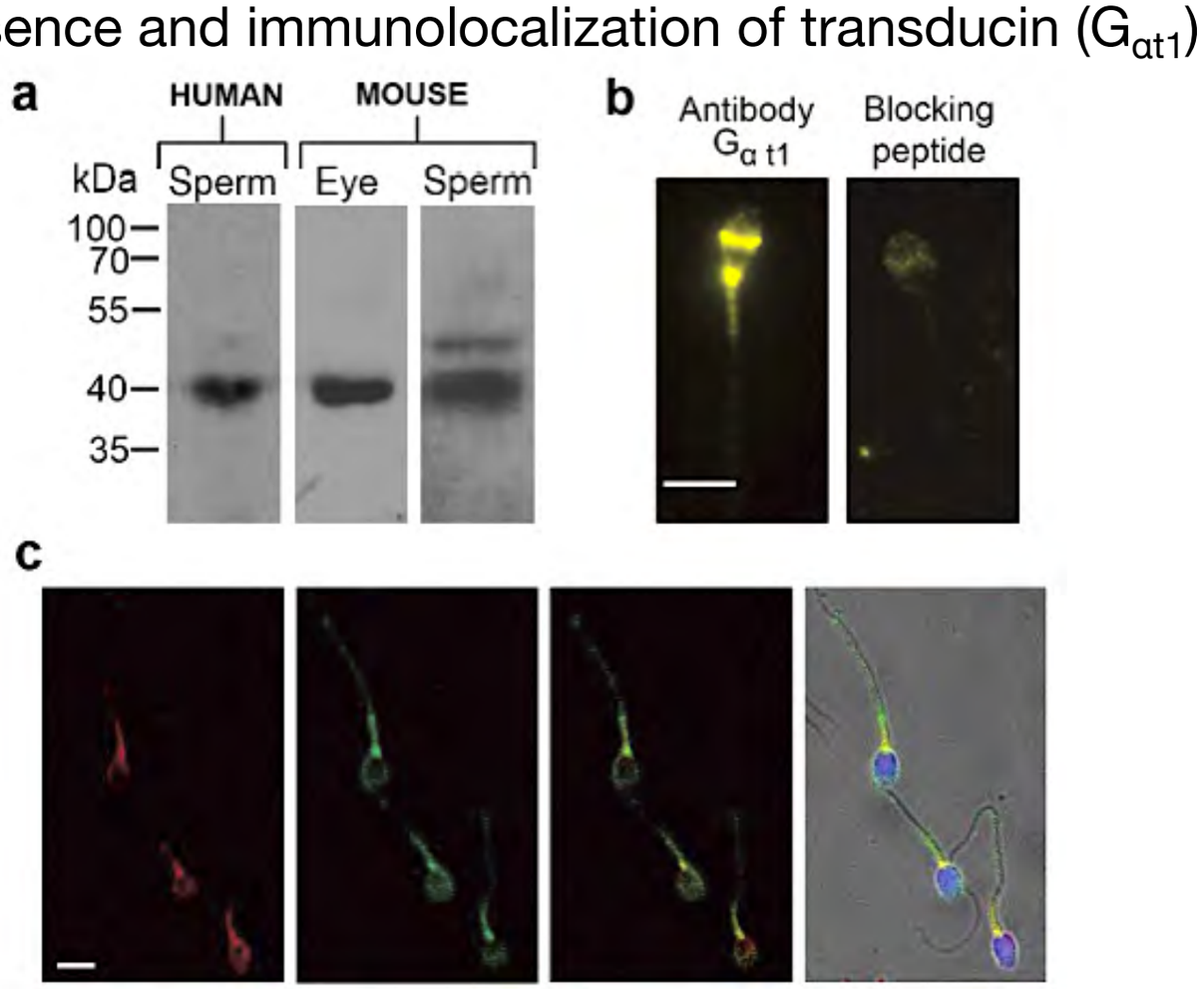

*Pérez-Cerezales et al. Sci. Rep. 2018, 8:2902*




Presence and immunolocalization of transducin ($G_{\alpha t1}$)

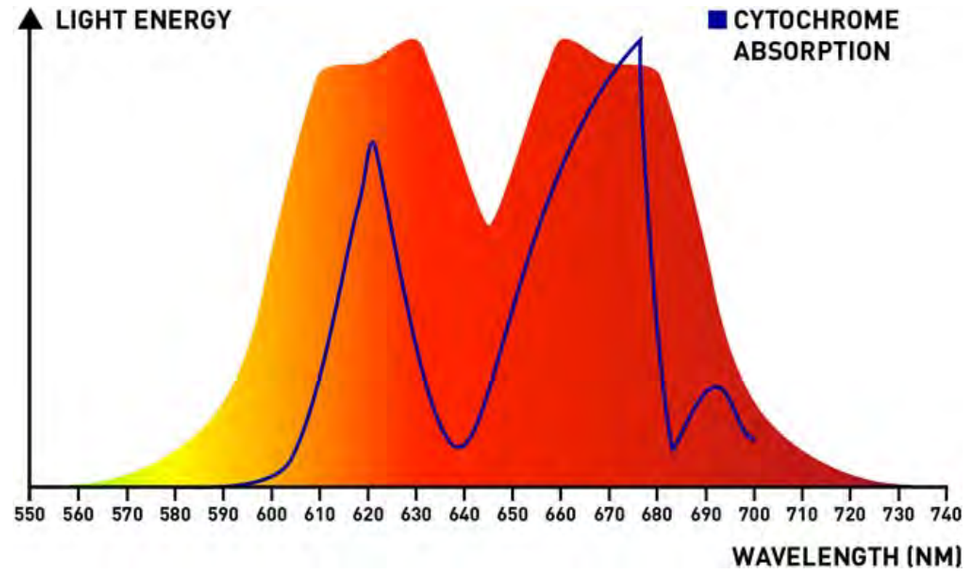


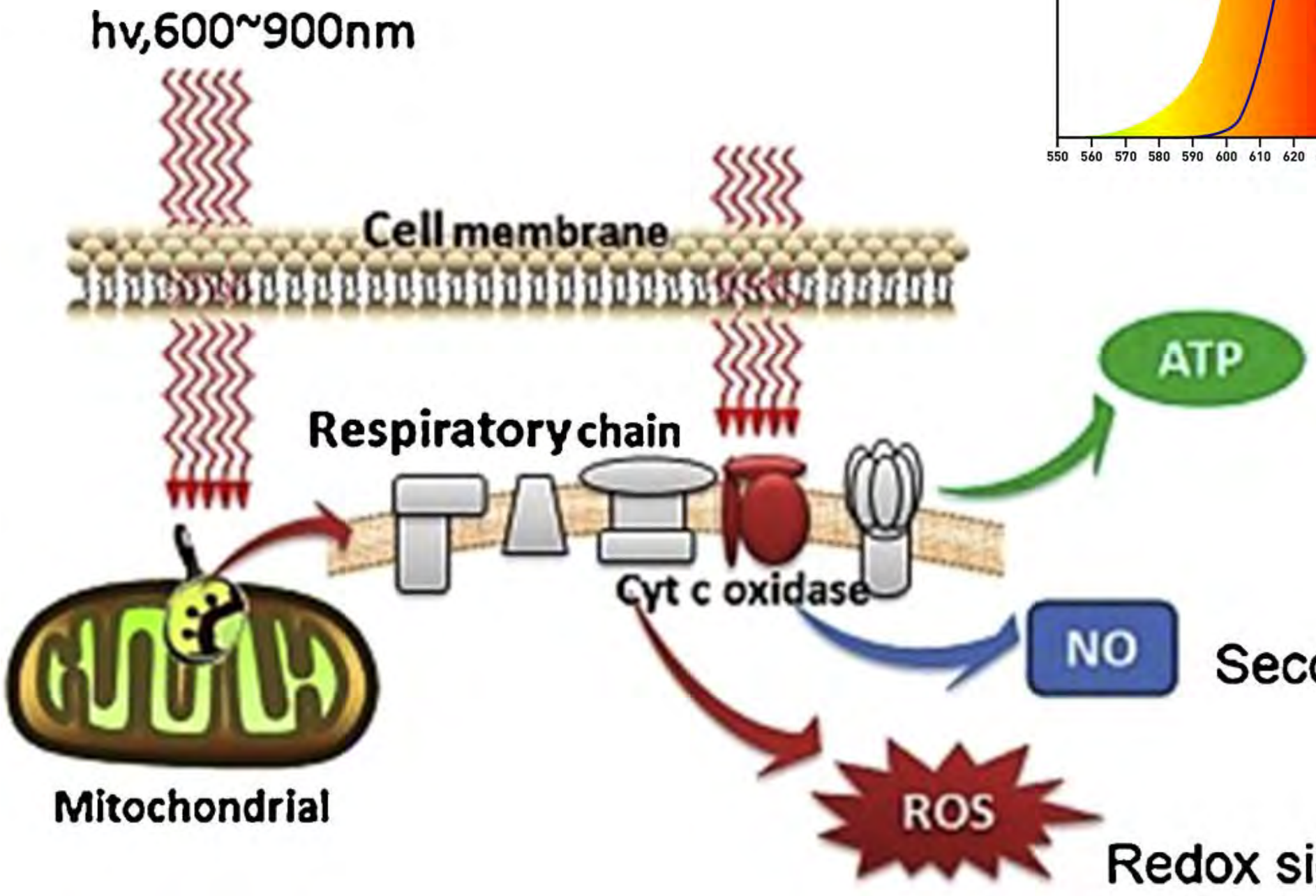

*Pérez-Cerezales et al. 2015. Sci Rep 5: 16146*



# Can we explain the light effects?

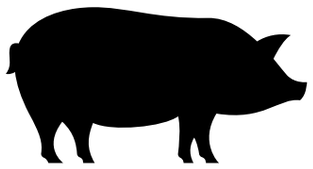
Via Cytochrome c - electron transport chain

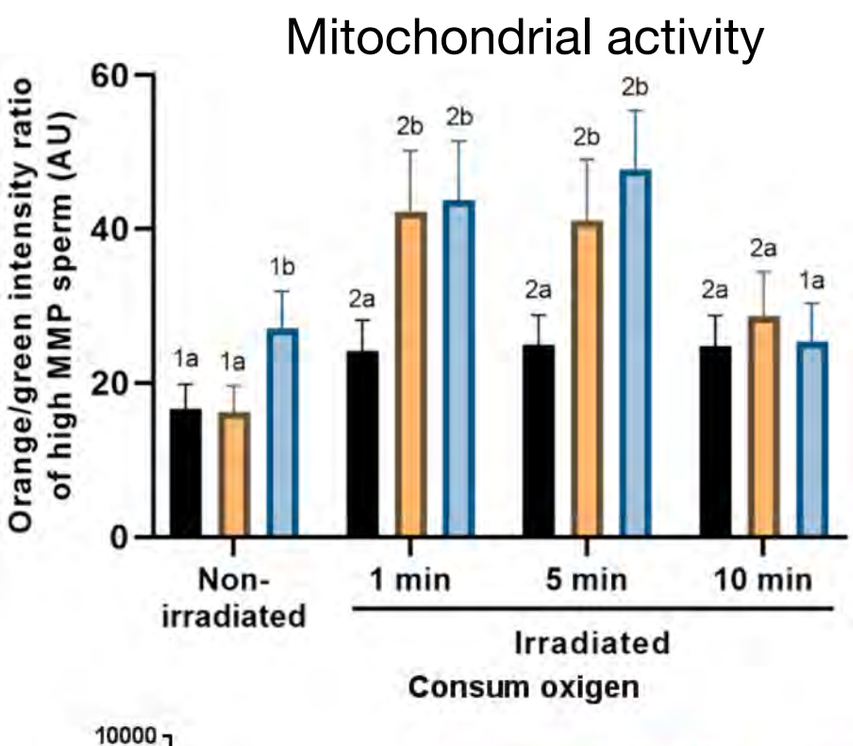


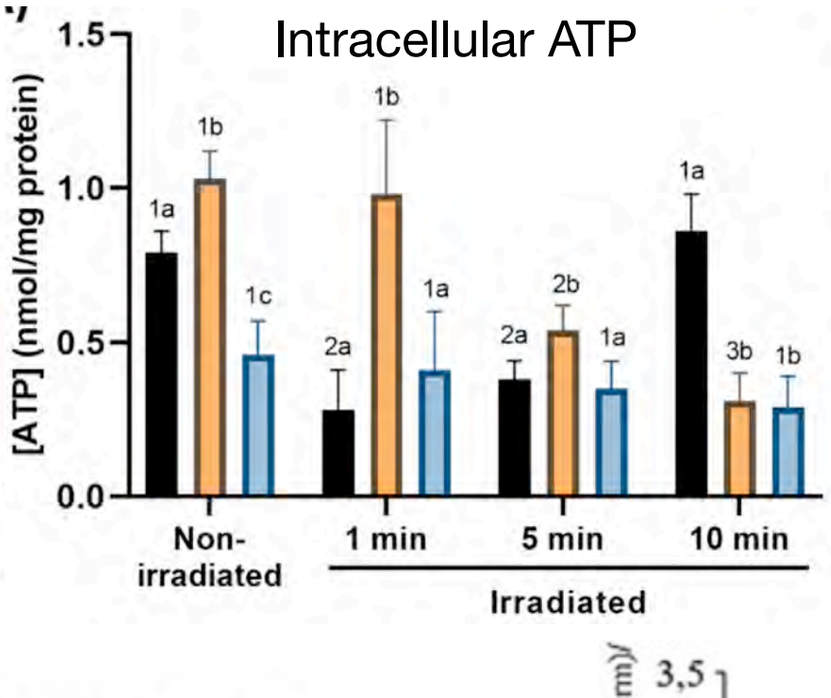



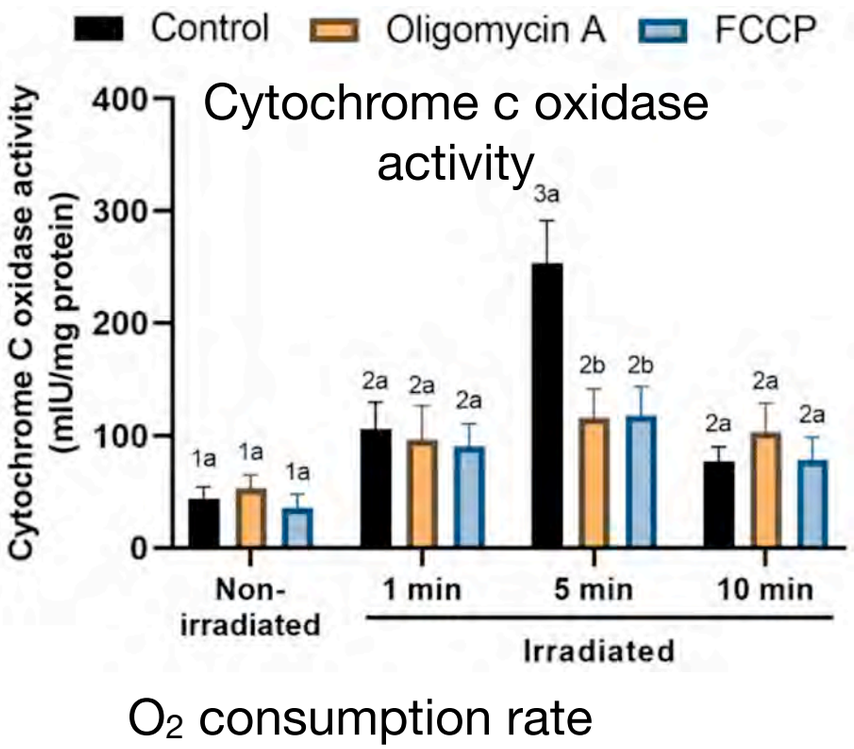
# Can we explain the light effects?

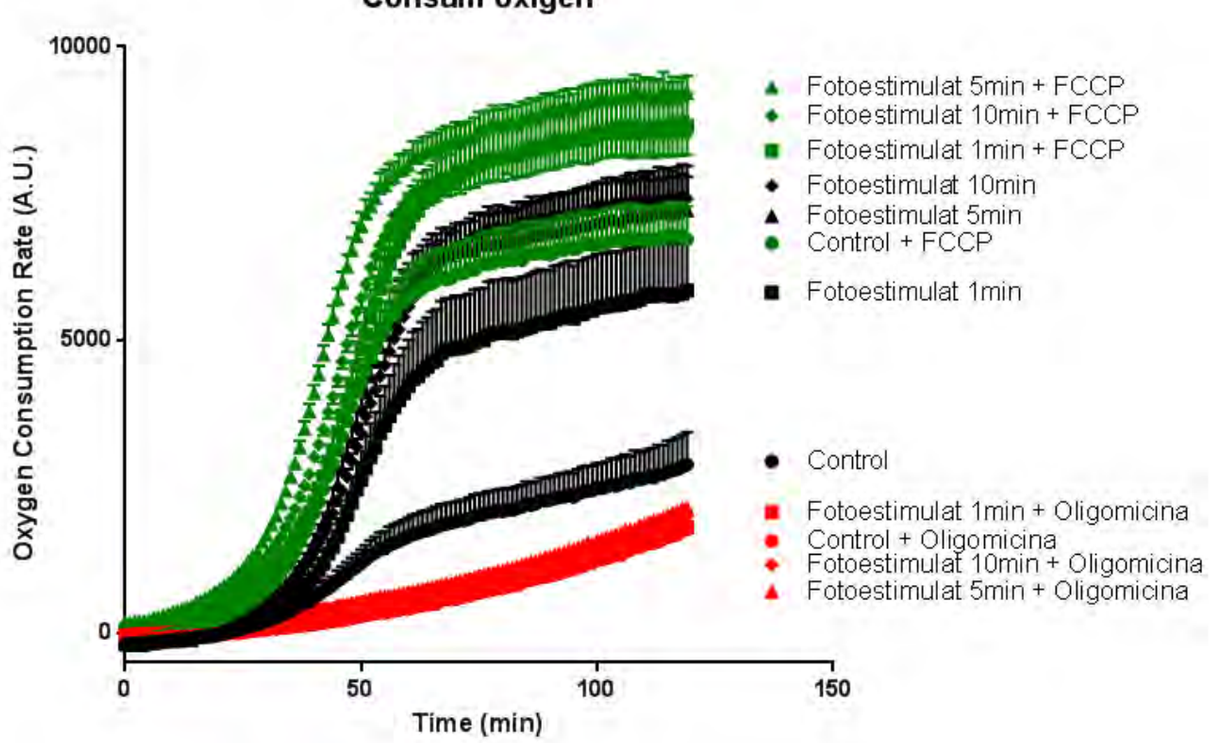
FCCP: Mitochondrial ETC decoupler.
Oligomycin: ATP synthase inhibitor.
Antimycin A: Blocks Complex III in the ETC.

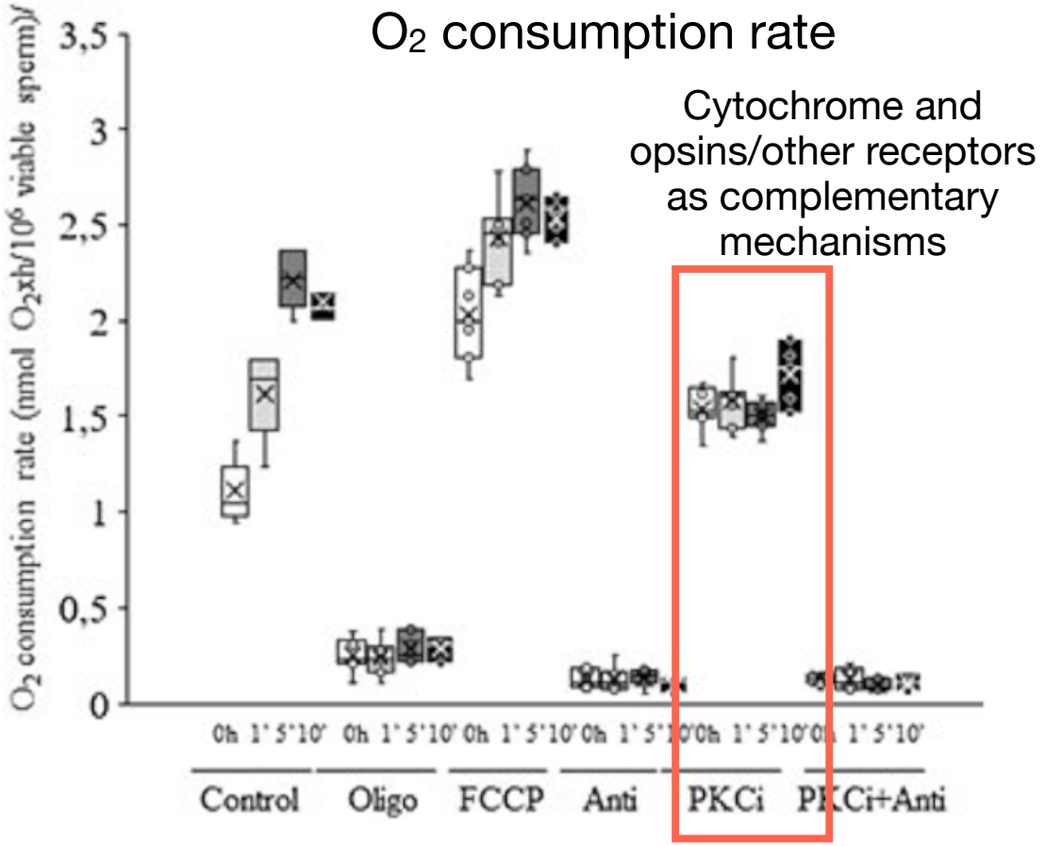





*Blanco-Prieto et al. 2020. Cells 9, E2546.*

*Blanco-Prieto et al. 2022. Front Cell Dev Biol 10, 930855.*

# Some conclusions

Red-light photostimulation improves the sperm ability to reach the full capacitation status.

Light-stimulation **immediately before AI** improves the reproductive performance.

Although red-light photostimulation exerts its effects via a yet-unidentified mechanism, it could involve opsins and electron chain proteins.

Different factors (including season, male, semen origin, etc.) may influence the effects.

# Děkuji!

Colloid selection

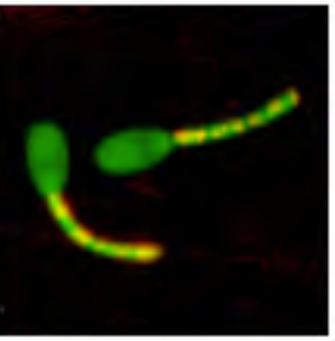
**Jane Morrell,**
**SLU, Sweden**

**Serafín Pérez-Cerezales,**
**INIA, Spain**

Thermotaxis

Photostimulation

**Marc Yeste,**
**UdG, Spain**



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reprobio.unileon.es

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