Graphene Oxide induces sperm release from oviductal cells by modifying sperm membrane fluidity and binding proteins

Marina Ramal Sánchez

PhD Student

PhD supervisors: Pascal Mermillod

Physiology of Reproduction and Behaviours, INRA-Centre Val de Loire (France)

PhD supervisor: Nicola Bernabò

Unit of Basic and Applied Biosciences, University of Teramo (Italy)

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Bilbao (Spain)

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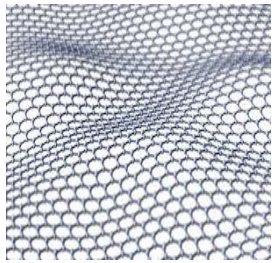
Marie Sklodowska-Curie ITN-2015-EJD REP-BIOTECH 675526

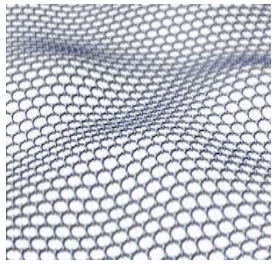
675526-REPBIOTECH-ITN MSCA H2020



WHY GRAPHENE?

WHY SPERMATOZOA?

Graphene Oxides induces sperm release from bovine oviductal epitelial cells by modifying sperm membrane fluidity and binding proteins



WHY GRAPHENE?

WHY SPERMATOZOA?

Properties:

- ✓ Strenght and elasticity
- ✓ Electrical and termal conductivity
- ✓ Mechanical stiffness
- ✓ Brightness
- ✓ Flexibility
- ✓ Thinness
- ✓ Strong
- ✓ Transparent conductor
- ✓ Cheap to produce

Applications:

- ❖ Rapid DNA sequencing, targeted delivery, tissue regeneration, bionic implants
- ❖ Batteries, touchscreens for mobile phones, tablets, etc.
- ❖ Electronic components
- ❖ **Biomedical applications**

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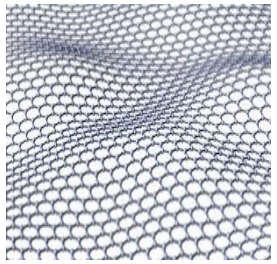

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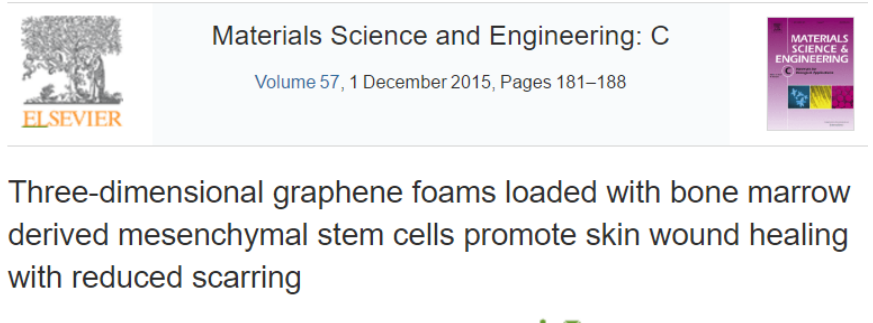

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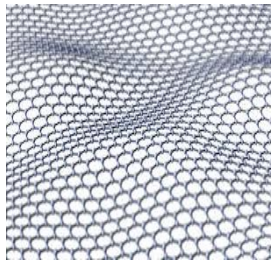

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Lack of works evaluating real toxicity on spermatozoa, controversials and uncertain

WHY SPERMATOZOA?

SCIENTIFIC REPORTS

OPEN

Toxicology Study of Single-walled Carbon Nanotubes and Reduced Graphene Oxide in Human Sperm

Received: 29 January 2016
Accepted: 24 June 2016
Published: 19 August 2016

Waseem Asghar[1,2], Hadi Shafiee[3], Vanessa Velasco[3,4], Vasu R. Sah[3], Shirui Guo[5], Rami El Assal[1], Fatih Inci[1], Adhithi Rajagopalan[3], Muntasir Jahangir[3], Raymond M. Anchan[6], George L. Mutter[7], Mihrimah Ozkan[5], Cengiz S. Ozkan[8] & Utkan Demirci[1,3]

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Contents lists available at ScienceDirect
Materials Science and Engineering C
journal homepage: www.elsevier.com/locate/msec

Probing suitable therapeutic nanoparticles for controlled drug delivery and diagnostic reproductive health biomarker development

Rakhi Jha[a,b], Pradeep K. Jha[a,*], Santosh Gupta[a], S.P. Bhuvaneshwaran[a], Maidul Hossain[c], Sujoy K. Guha[a]

Materials Science and Engineering: C
Volume 57, 1 December 2015, Pages 181–188

Three-dimensional graphene foams loaded with bone marrow derived mesenchymal stem cells promote skin wound healing with reduced scarring

Zhonghua Li[a, b], Haiqin Wang[c], Bo Yang[b], Yukai Sun[b], Ran Huo[a,]

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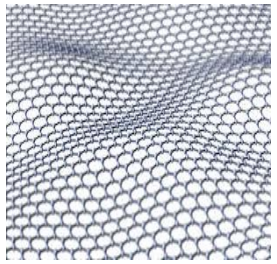

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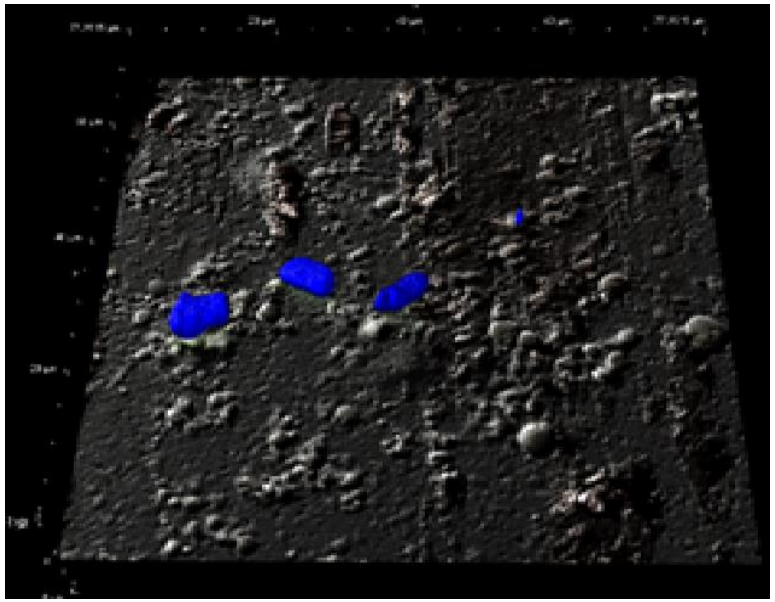
Evaluation of toxicity



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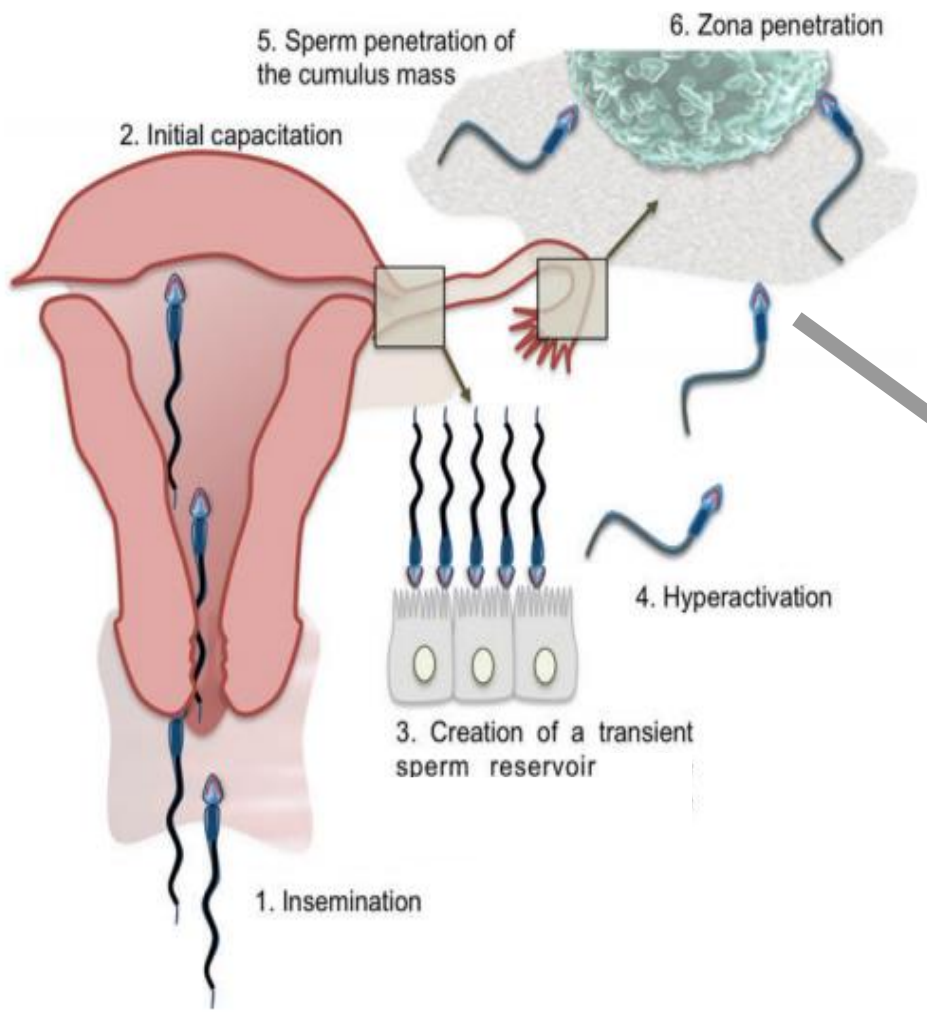
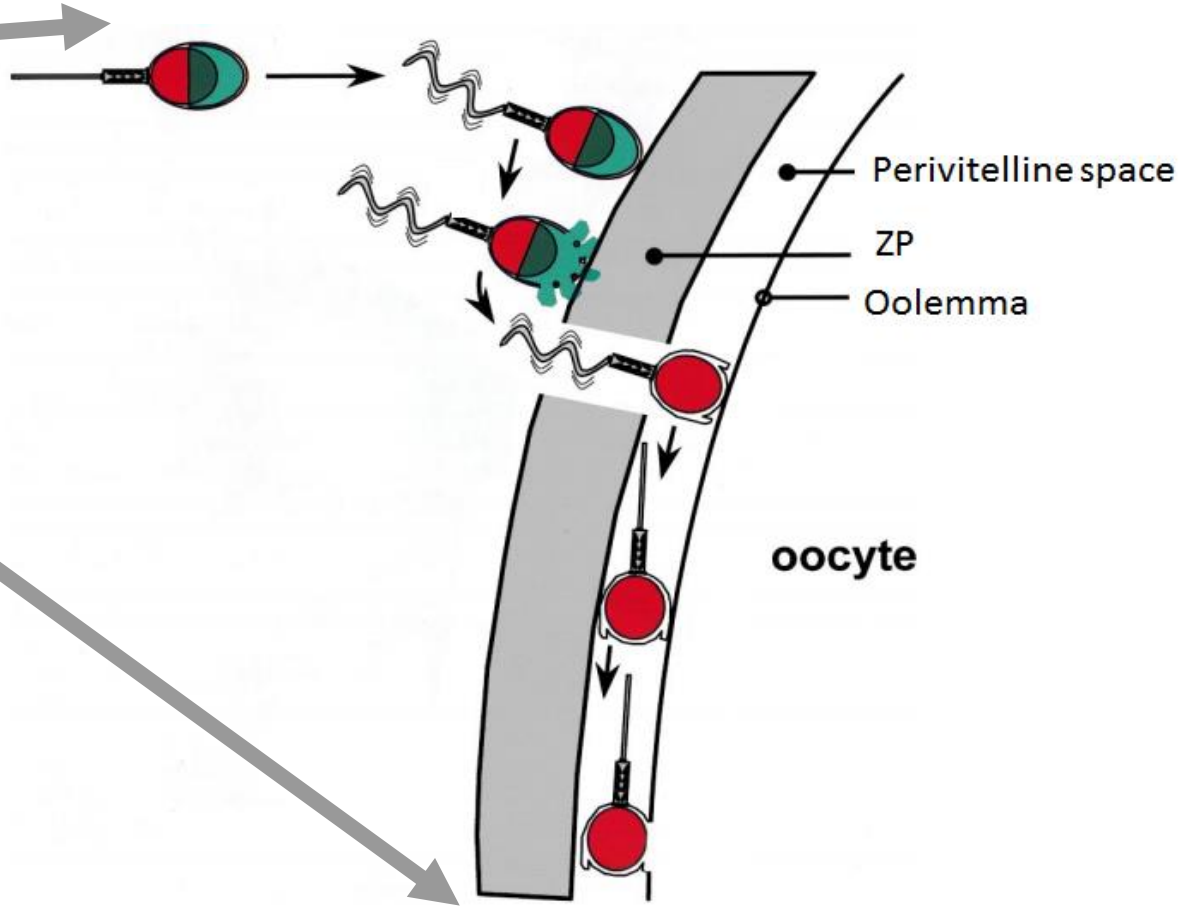
Towards the meeting with the oocyte



Modified from Aitken and Nixon, Mol.Hum.Reprod., 2013



Modified from Flesch et al. Bioch. And Bioph. 2000

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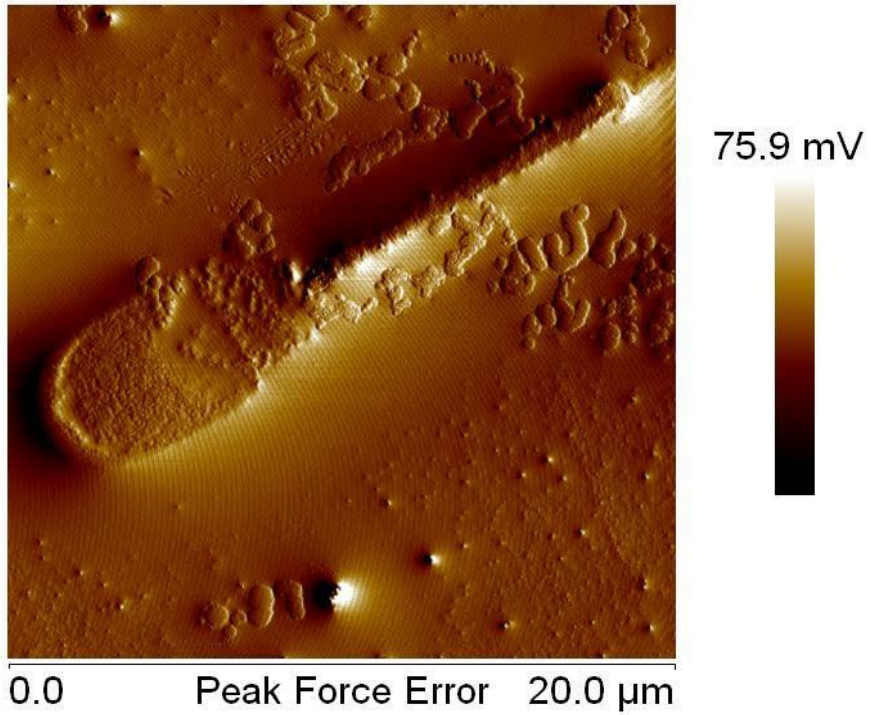
GRAPHENE OXIDE

- Modified GO 4mg/mL GRAPHENEA (Spain)
- Promtly dispersive in water
- Micrometer sized GO
- Single-layered or double layered sheets of GO
- Size: 670nm
- Polydispersity: 0,318
- Z potential: -26 ± 1



Evaluation of toxicity

Bernabò et al, Carbon 2018

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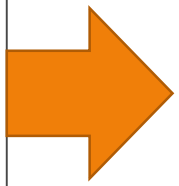
Evaluation of toxicity

Events of sperm capacitation

- Sperm reservoir formation
- Membrane fluidification
- Cholesterol release
- Loss of Binder of Sperm Proteins (BSP)
- Release from sperm reservoir
- Acrosome reaction
- …



FERTILIZATION

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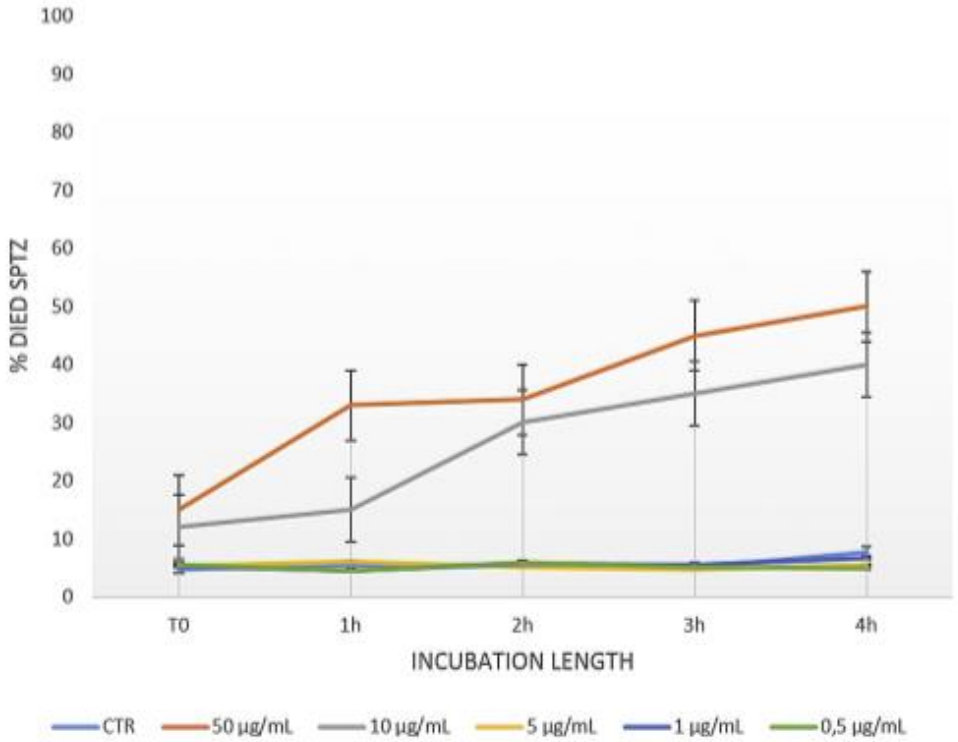
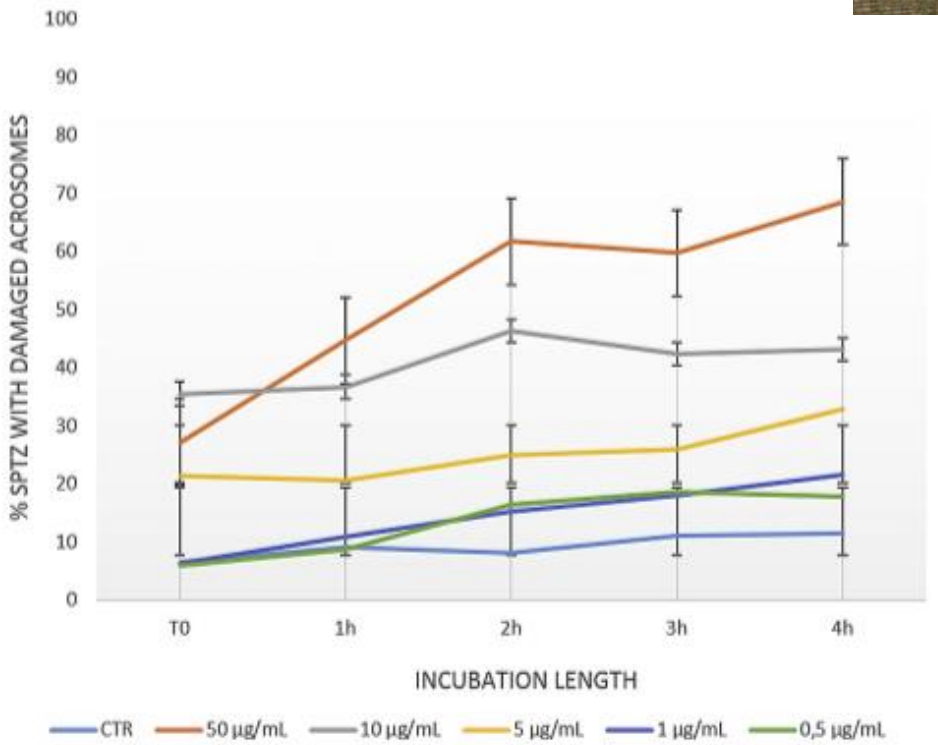
Evaluation of toxicity

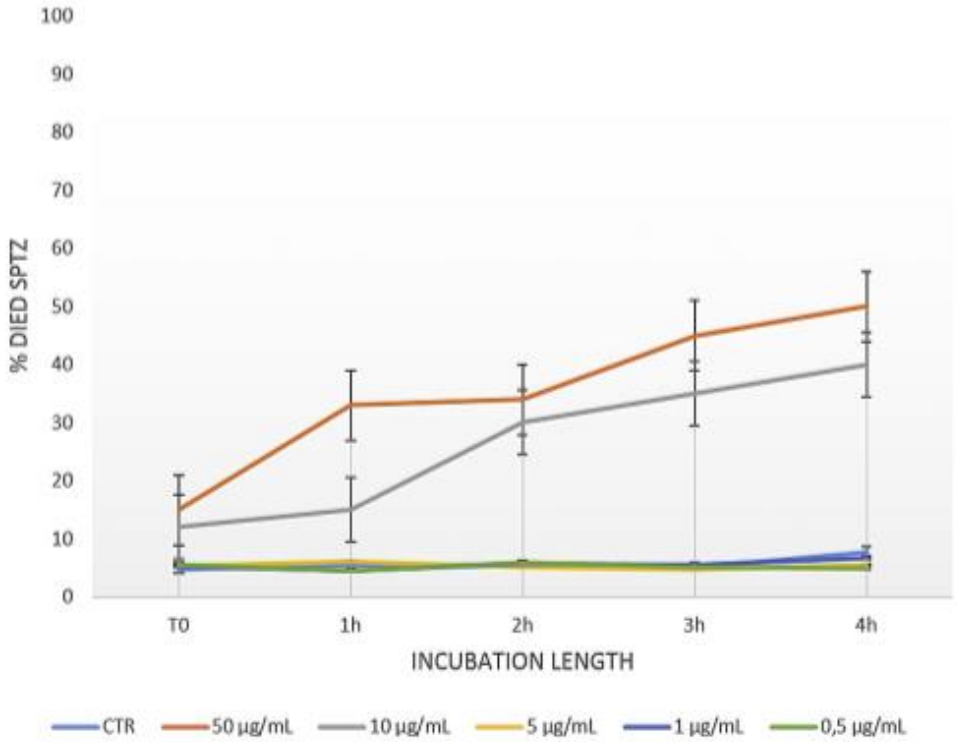


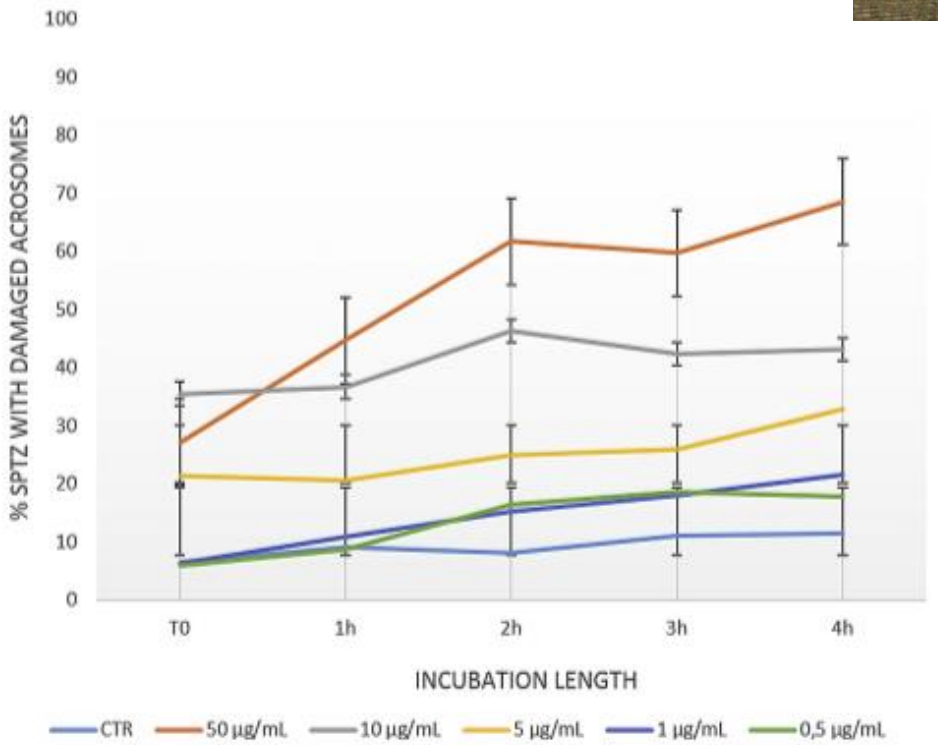


- Porcine spermatozoa (*swine model)*
- *In vitro*
- *Capacitating* conditions
- Different GO concentrations: 0,5 ; 1 ; 5 ; 10 ; 50 µg/mL

Bernabò et al, Carbon 2018

Evaluation of toxicity





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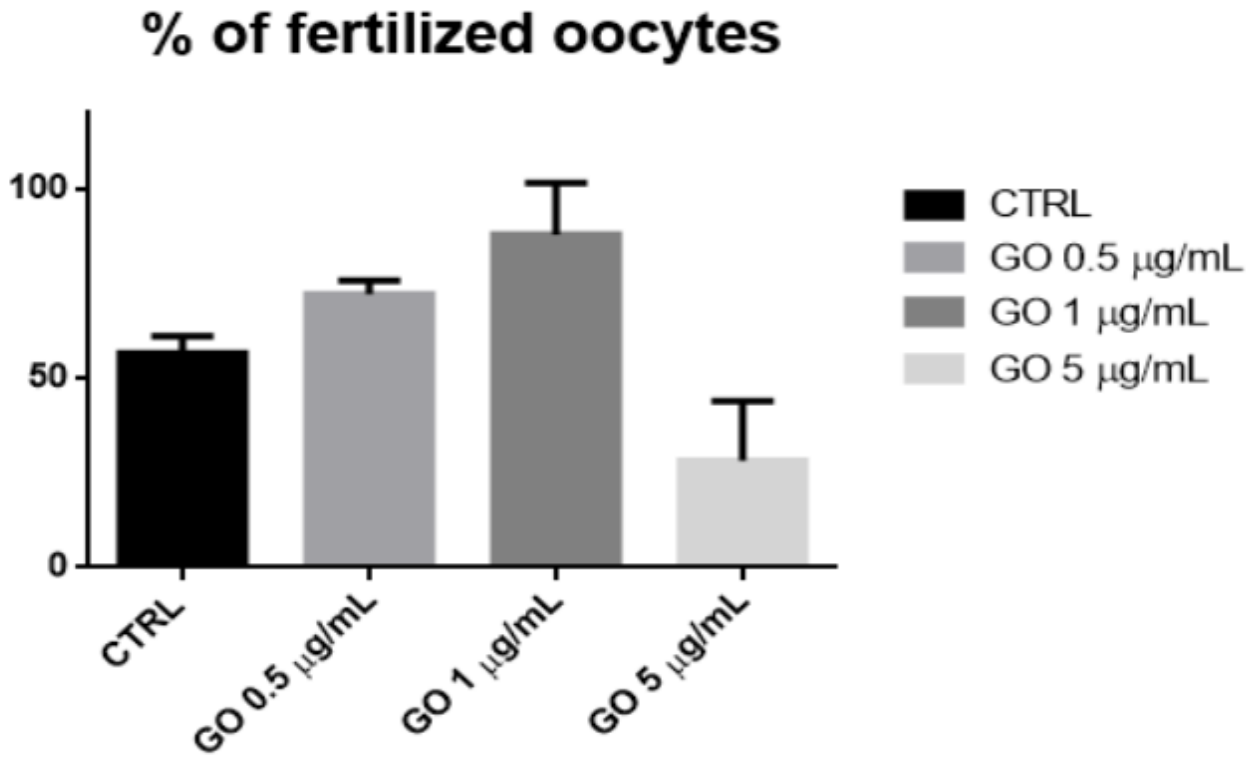
Does GO affect the fertilization ability of sperm?



Surprisingly, GO **0,5** and **1 μg/mL** has a **positive effect** in the *in vitro* fertilization (IVF)

(p<0.05, GO concentrations Vs. CTRL)

Bernabò et al, Carbon 2018

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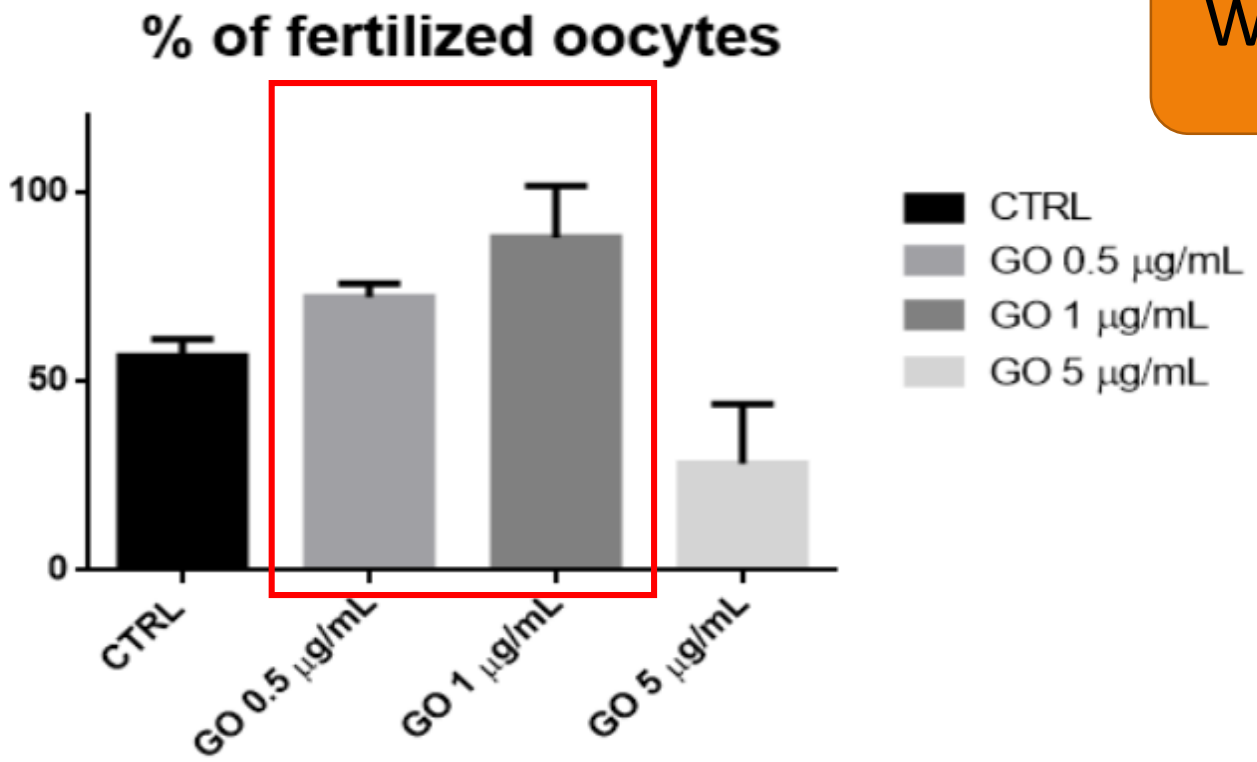
What is happening??!!



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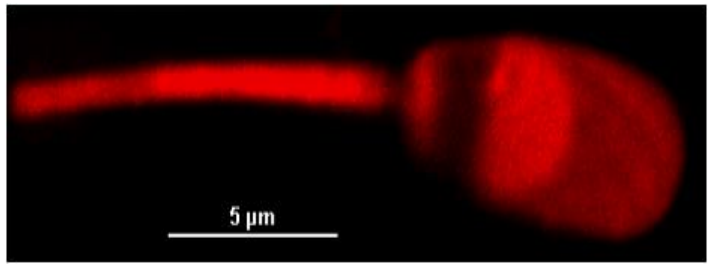
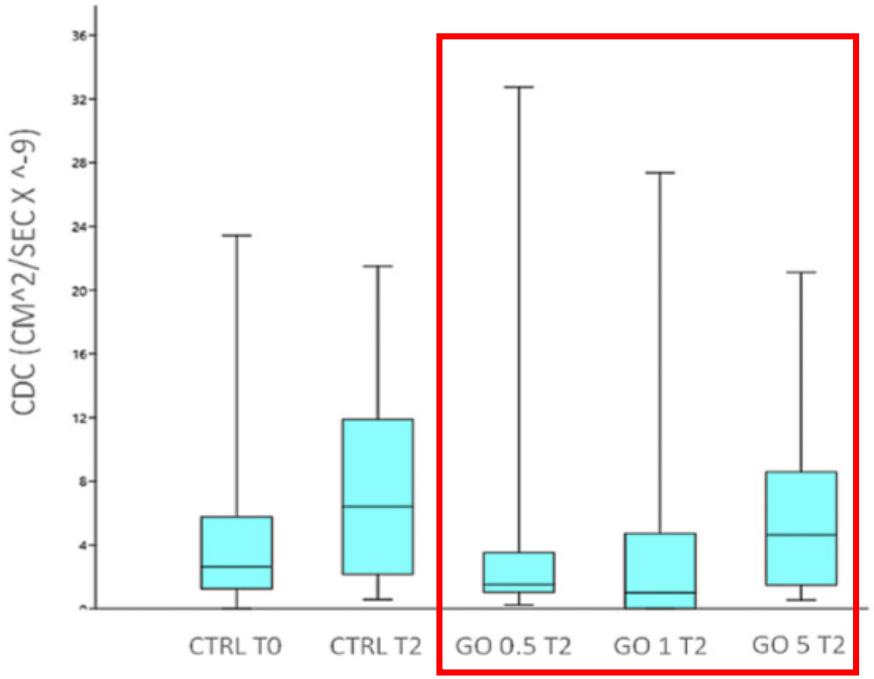
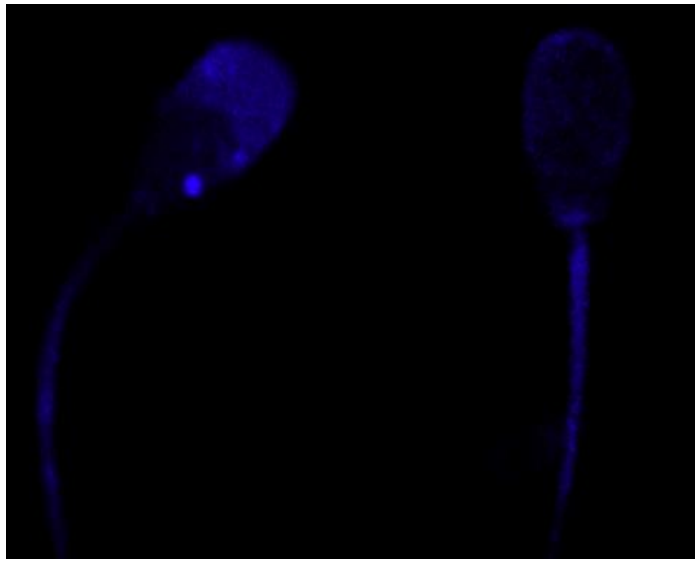
FRAP (Fluorescence Recovery After Photobleaching) analysis by confocal microscopy







Confocal microscopy with Filipin III staining

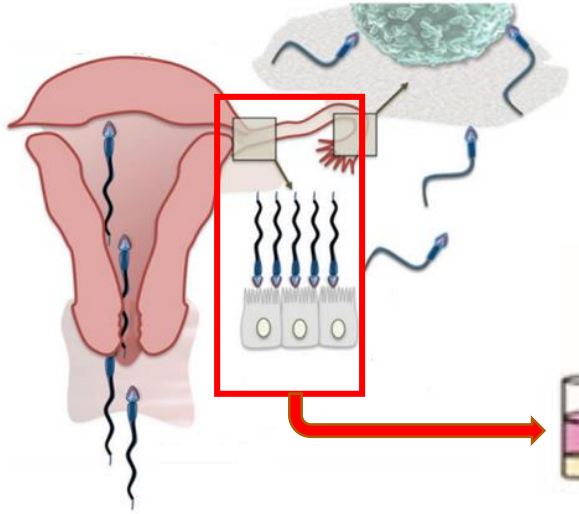
Capacitating conditions

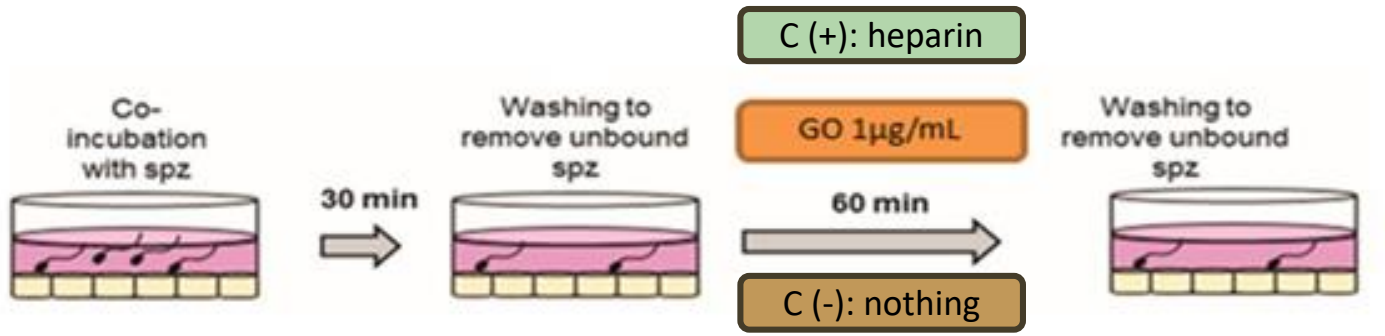
GO seems to act **stabilizing** the sperm membrane

Confocal microscopy (Filipin III) and Flow cytometry experiments confirm the **extraction of cholesterol** by GO

Bernabò et al, Carbon 2018

Different animal model: bovine (Bos Taurus)





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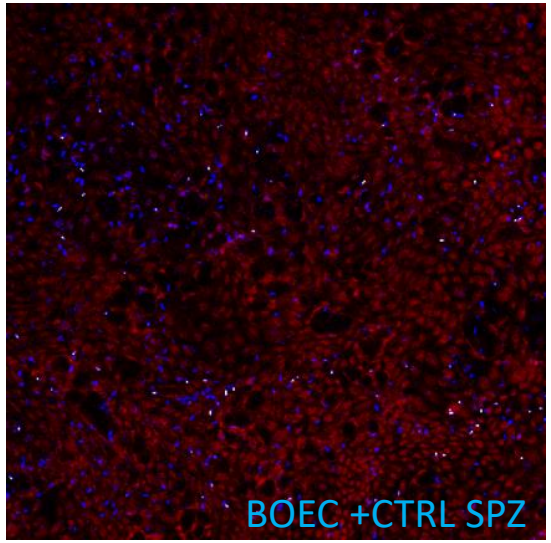
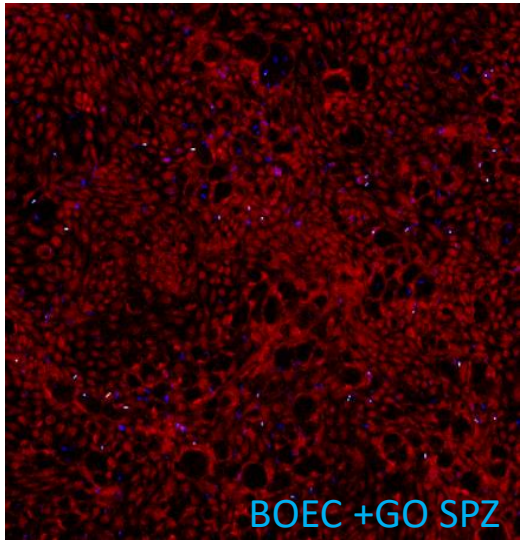
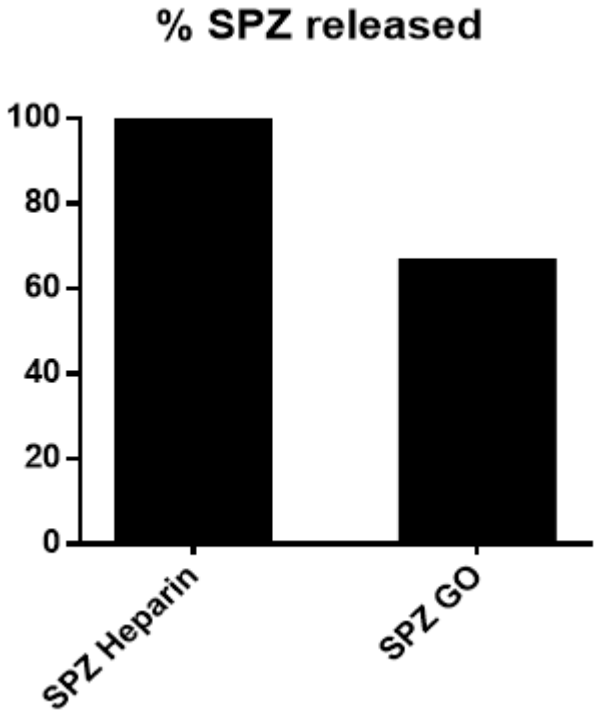
Sperm reservoir







GO induces the **SPZ release** from bovine oviductal epithelial cells

Manuscript in preparation

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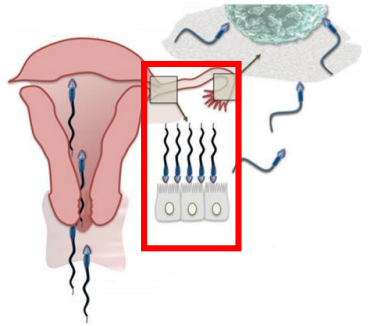
How does GO modify the sperm proteins?

Major protein components of bovine seminal plasma → **BSP**: Binder of Sperm Proteins

Sperm-membrane proteins involved in sperm-oviduct binding



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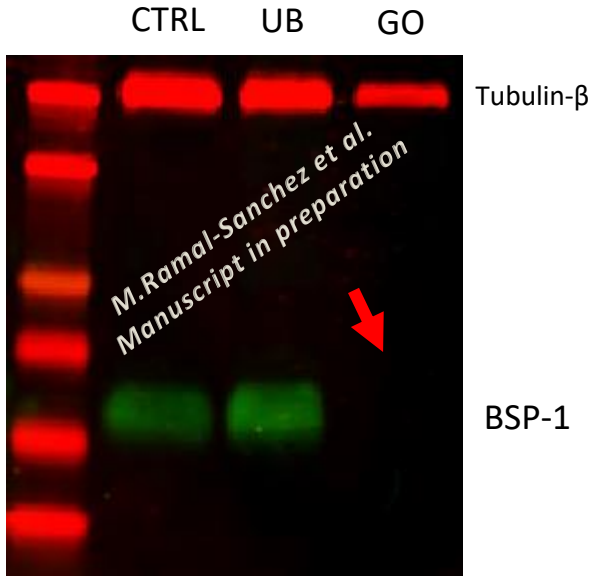
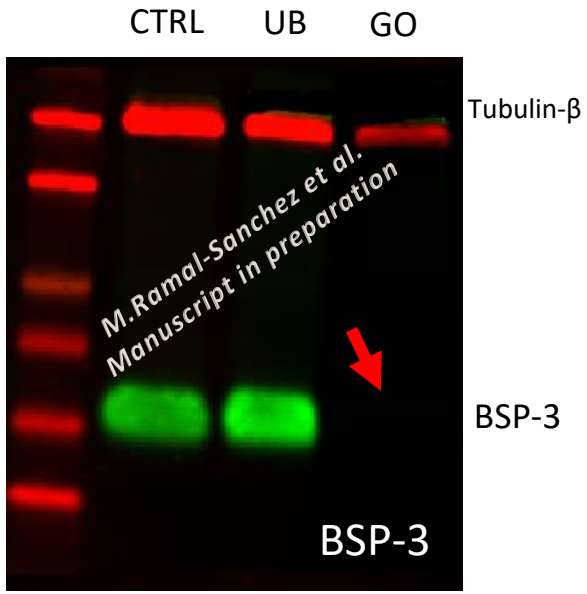
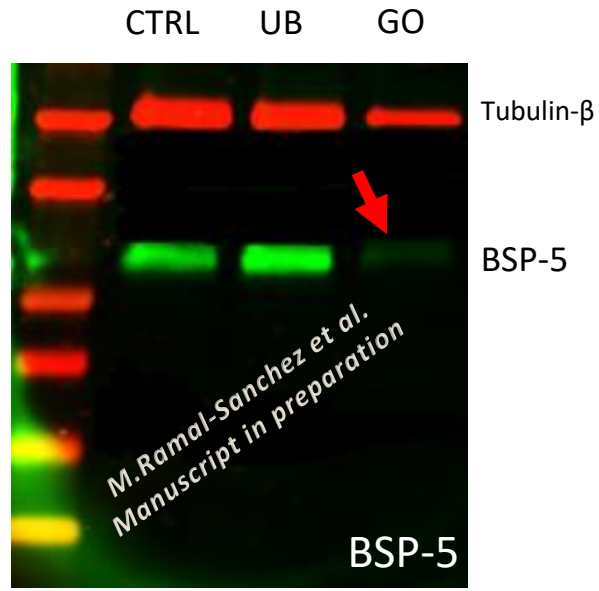
Spermatozoa are transcriptionally silent







CTRL: spz control
UB: spz unbound

GO induces the **loss of BSP-1,-3 and -5** after the release of spermatozoa from the oviductal cells

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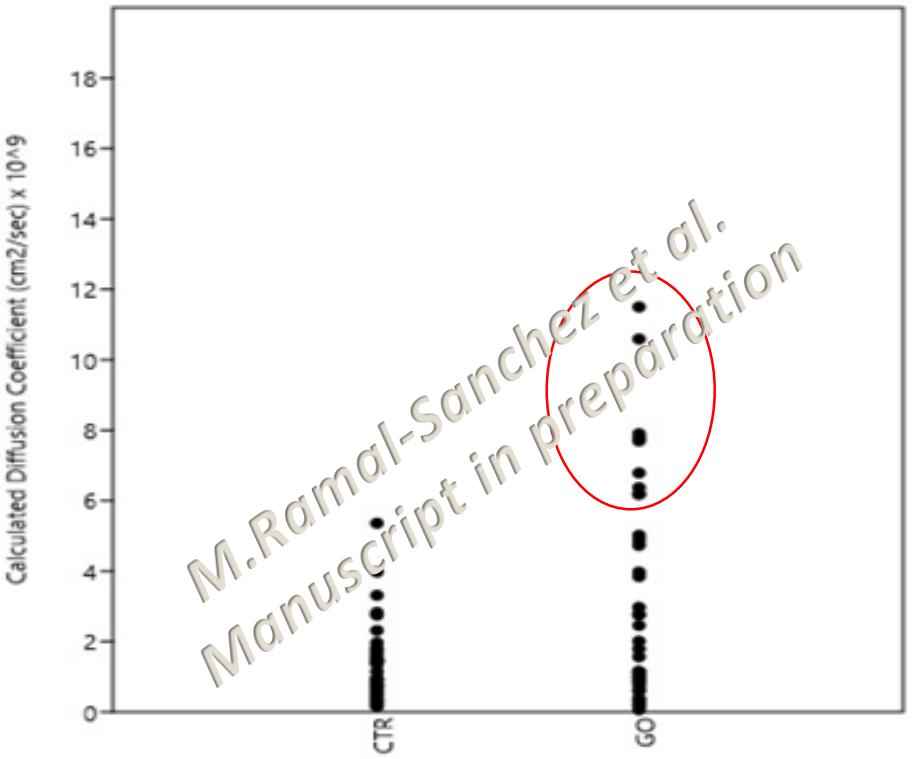
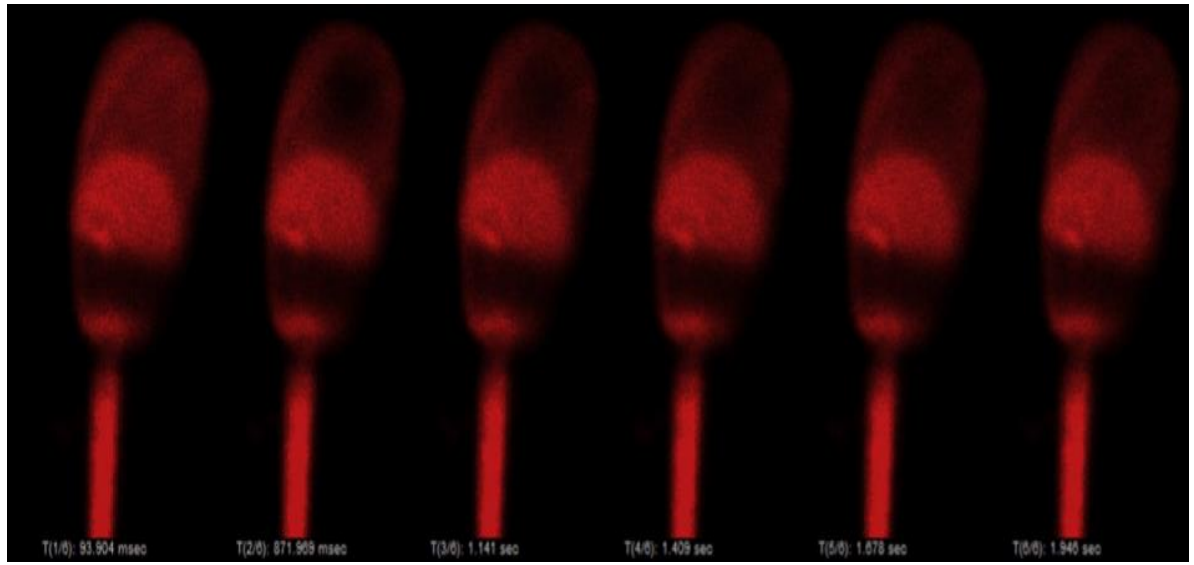
How does GO modify the bovine sperm membrane?



Kruskal-Wallis test for equal medians. (p=0,01443)



DilC-12 staining
Confocal microscopy
FRAP (Fluorescence Recovery After Photobleaching)

Populations with **higher fluidity** in spermatozoa released by GO action

Membrane fluidity → linked to capacitation

Manuscript in preparation

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Conclusions

- ✓ Graphene Oxide induces the release of bovine spermatozoa from oviductal cells (sperm reservoir)
- ✓ After inducing the release, bovine spermatozoa lose the presence of BSP proteins
- ✓ Graphene Oxide increases bovine sperm membrane fluidity, i.e modifies the sperm lipid composition
- ✓ Graphene Oxide has shown an interesting positive effect in IVF in swine and mice

and bovine?

What is next?

Many unsolved question

- Does GO affect the fertilizing ability of **bovine** spermatozoa?
- Does GO affect the in vitro bovine embryo development?
- How does GO interact with spermatozoa?
- Which other proteins/lipids are modified by GO?
- The need to find a marker of capacitation →diagnostic nanodevices

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Graphene?

To be continued…

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Acknowledgments

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