BIOLOGICAL EFFECTS OF IN VITRO NANOPLASTICS INTERNALIZATION IN MAMMALIAN GAMETES

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Supervisor: Prof.ssa Marcella Spinaci

Co-supervisor: Prof. Salvatore Nesci

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ACTIVITIES 1ST YEAR of my PhD

- ✓ Focus on mammalian semen physiology, capacitation processes, and reproductive biotechnologies.

- ✓ **In vitro gamete manipulations** and **flow cytometry** assays to investigate the biological effects of **nanoplastics (NP) on mammalian gametes**

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INTRODUCTION – NANOPLASTICS (NP)

Disaggregation of plastic products from human activities

Microplastics
(MP, 100 nm - 5 mm)

Nanoplastics
(NP, 1-100 nm)

Contamination of all ecosystems

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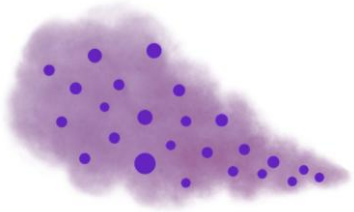
Petersen & Hubbart, 2020; Yang et al., 2023; Pujol et al., 2024

INTRODUCTION – NANOPLASTICS (NP)



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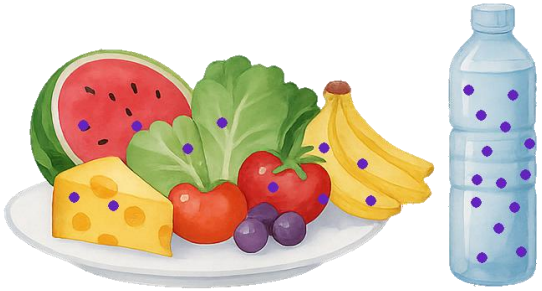
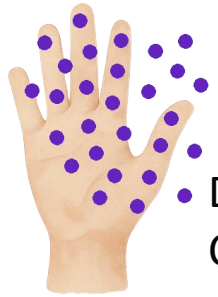
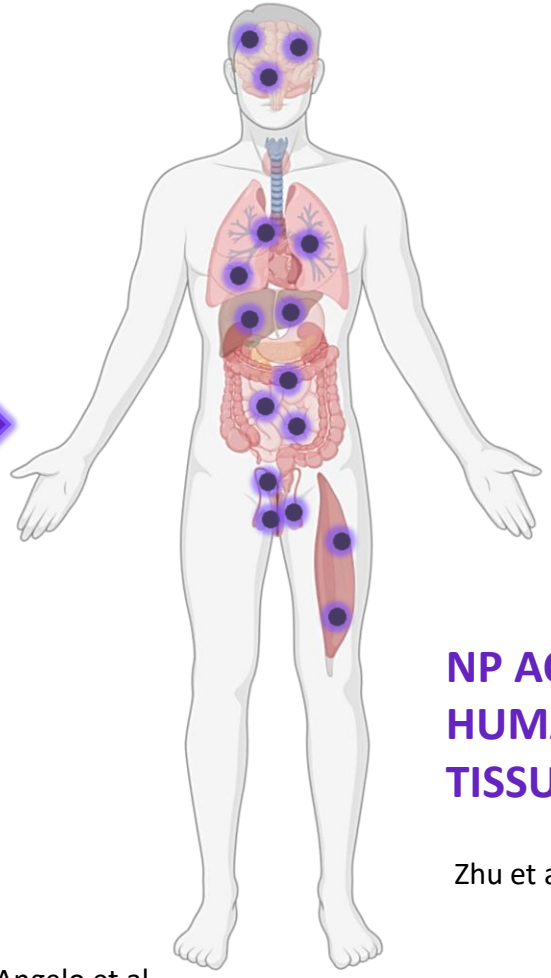


INGESTION



DERMAL CONTACT

Amereh et al., 2020; D'Angelo et al., 2021; Ali et al., 2024



NP ACCUMULATION IN HUMAN AND ANIMAL TISSUES

Zhu et al., 2024; Zhao et al., 2023

- ✓ oxidative stress
- ✓ inflammation
- ✓ immune dysfunction
- ✓ alterations in biochemical and energy metabolism
- ✓ impaired cell proliferation
- ✓ disruption of microbial metabolic pathways
- ✓ potential carcinogenicity

Ali et al., 2024; Zhang et al., 2022; Goodman et al., 2021; Xu et al., 2019; Dong et al., 2019; Xiao et al., 2022; Li et al., 2019; Huang et al., 2021

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INTRODUCTION – NANOPLASTICS (NP)

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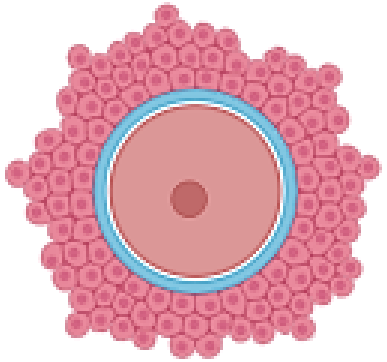
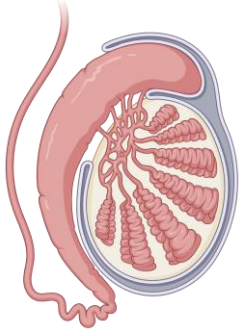


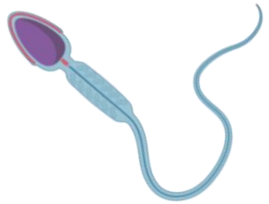
- ✓ **Impaired meiotic progression** and chromosome organization
- ✓ **Impaired mitochondrial activity**
- ✓ Increased **oxidative stress**, **apoptosis** and **DNA damage**
- ✓ **Negative** effect on **nuclear** and **cytoplasmic maturation**

He et al., 2024; Park et al., 2022; Xue et al., 2024; Merlo et al., 2025

♂



- ✓ Damages in testicular structure
- ✓ Disruption of blood–testis barrier (BTB)
- ✓ Testis inflammation → **spermatogenesis disorders** and **increased sperm abnormalities**



- ✓ Increased acrosomal damage
- ✓ **Oxidative stress** marked by ROS generation
- ✓ **DNA fragmentation**
- ✓ **Impaired mitochondrial activity**

Jin et al., 2021; Hou et al., 2021; Hong et al., 2023; Gao et al., 2023; Contino et al., 2023

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AIM of the STUDY

NP are supposed to be one of the causes of the worldwide increasing human subfertility

Could nanoplastics pose a threat to mammalian reproductive performance?

Evaluate the **effect of NP on mammalian gametes**

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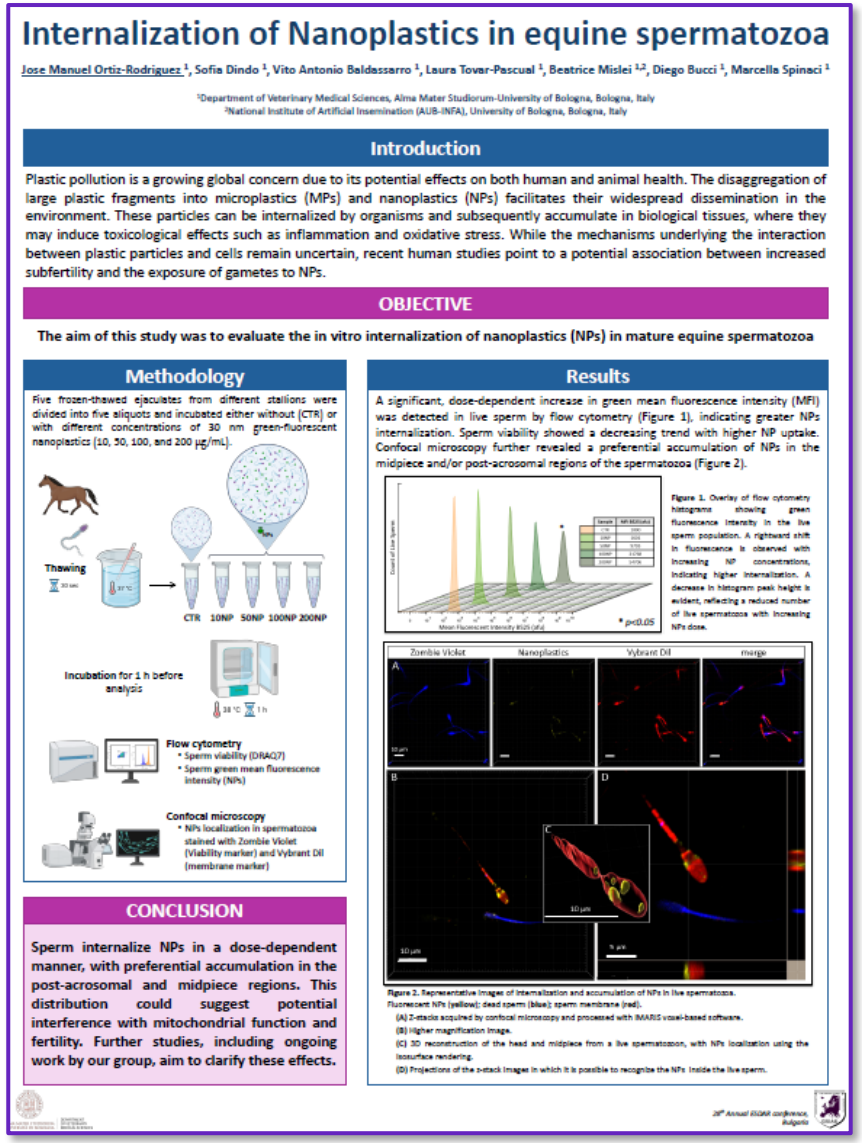
PROJECT 1

UNDER REVIEW

FOR DOMESTIC ANIMAL REPRODUCTION
EUROPEAN SOCIETY
ESDAR

OC 5.3 | Polystyrene nanoplastics impair stallion sperm function

S. Dindo[1]; L. Tovar-Pascual[1]; G. Mari[2]; M. Spinaci[1]; J. Ortiz-Rodriguez[1]



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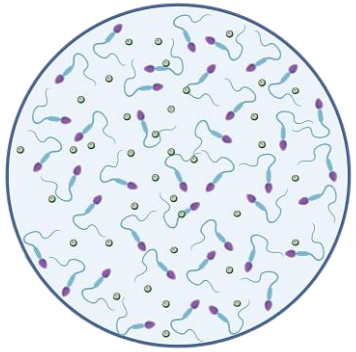
EXPERIMENT 1

15 frozen-thawed ejaculates
from **five stallions**

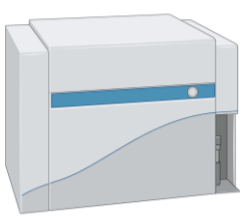


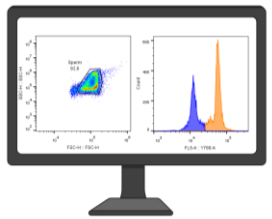
Incubated at **38ºC** with different concentrations of **30nm PS-NP**

CTR
10, 50, 100, 200 µg/mL

T0, T1, T3

Sperm kinetic parameters (CASA)
&
FLOW CYTOMETRY





Sperm marker (Hoechst 33342)
Sperm **viability** (PI)

Hydrogen Peroxide production (H_2DCFDA)
Superoxide ion production (DHE)
Mitochondrial activity (JC1)

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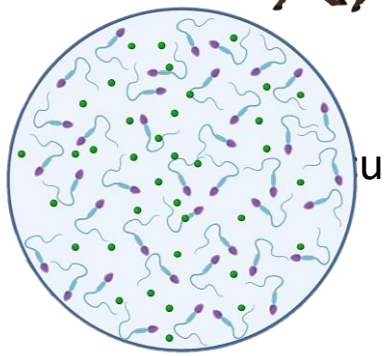
MATERIALS & METHODS

EXPERIMENT 2

5 frozen-thawed ejaculates from **five stallions**



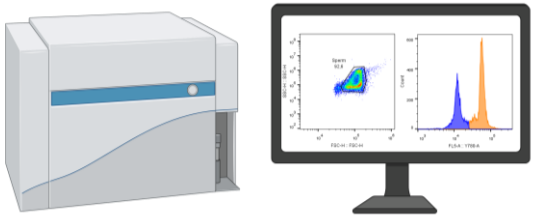
ubated at 38ºC with different concentrations of 30nm Fluorescent yellow-green PS-NP

CTR
10, 50, 100, 200 µg/mL

T1

FLOW CYTOMETRY

CONFOCAL MICROSCOPY

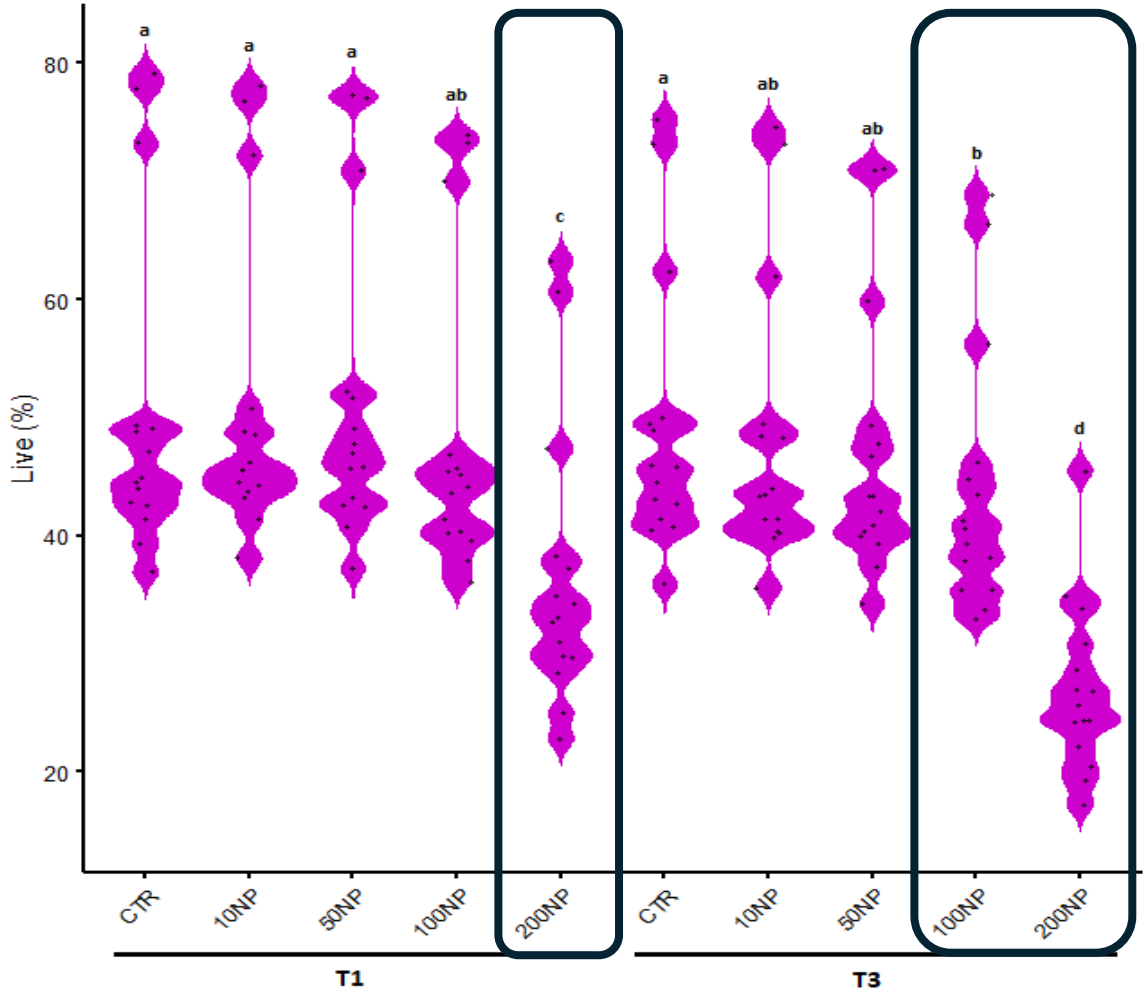


Green Mean Fluorescence Intensity (MFI) within the live sperm population to evaluate **intracellular NP levels**

Zombie Violet (viability)
Vybrant Dil (membrane marker)

MATERIALS & METHODS

VIABILITY & MOTILITY



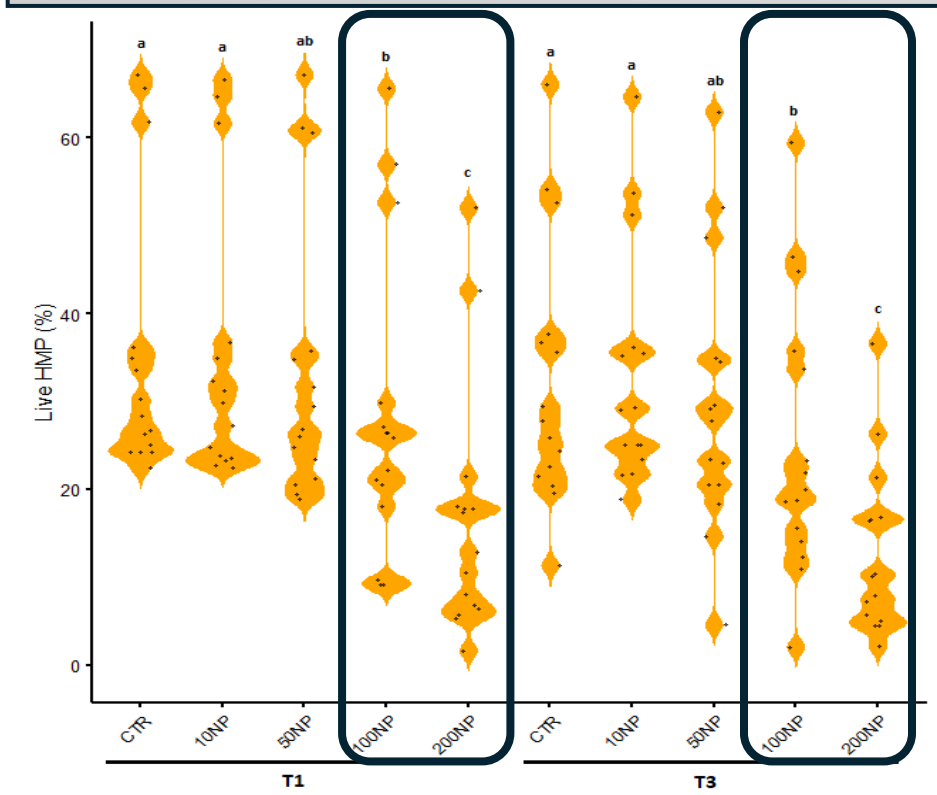
✓ Marked reduction in sperm survival

✓ Synergistic effect over time

↓ Total motility
Progressive motility

p < 0.001
between treatment-time combinations

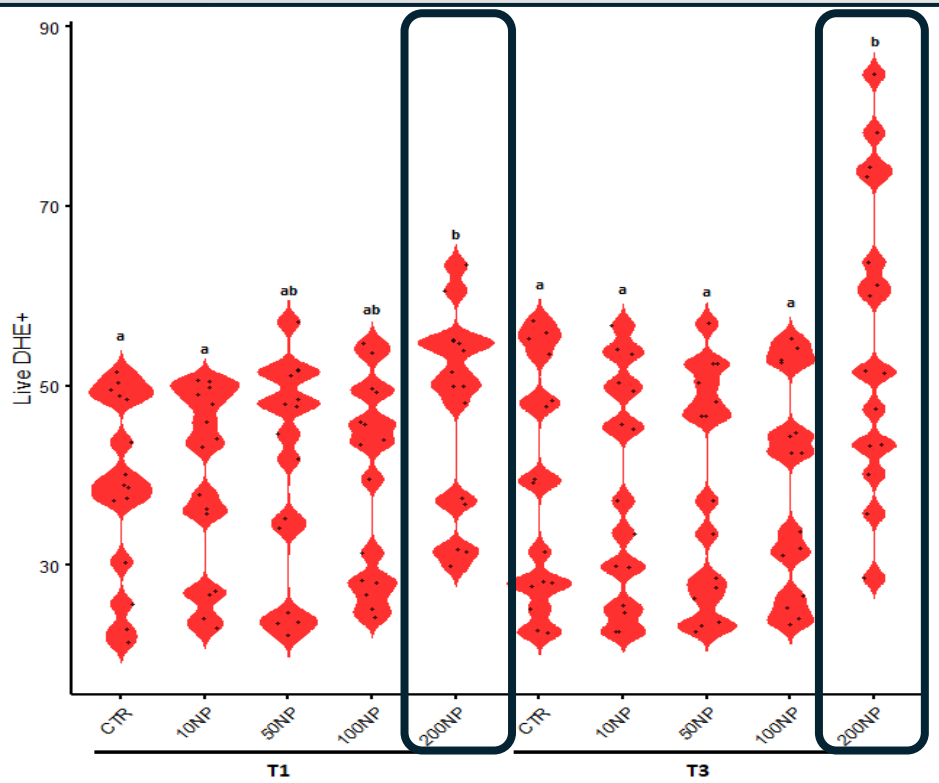
MITOCHONDRIAL ACTIVITY



p < 0.05
p < 0.001 for 200NP within the same time point

Significant reduction in % of **live sperm** exhibiting **high mitochondrial membrane potential**

ROS PRODUCTION



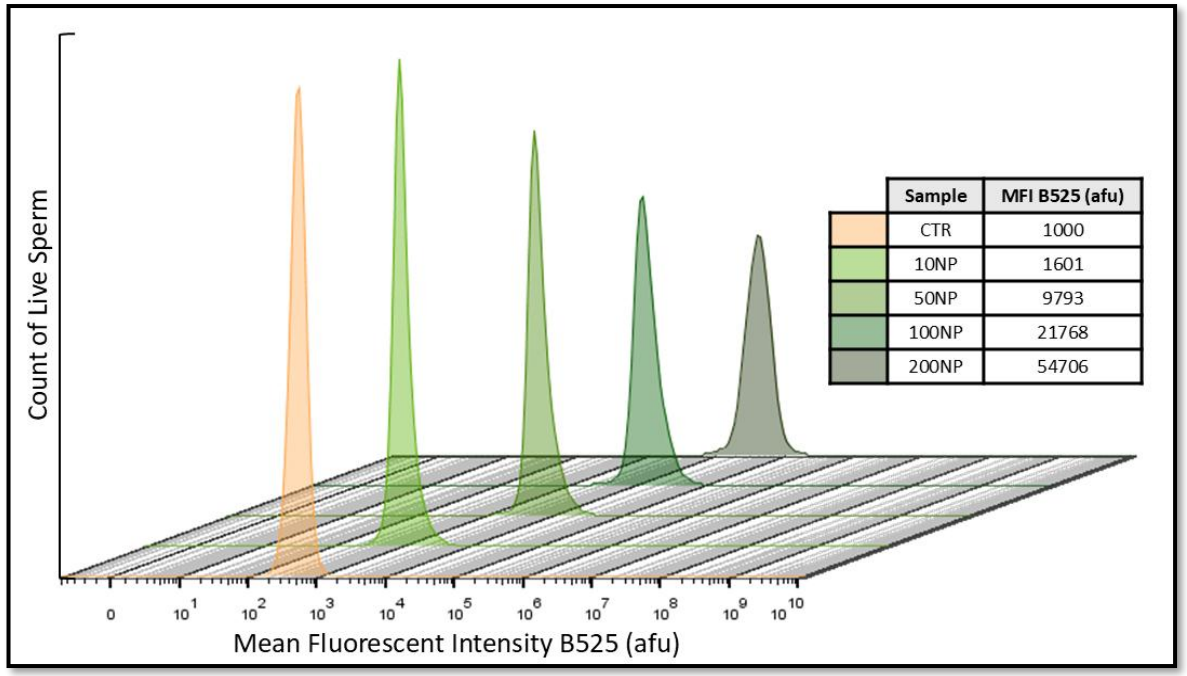
p < 0.05 for T1
p < 0.001 for T3 within the same time point

Marked increase in Superoxide ion concentration

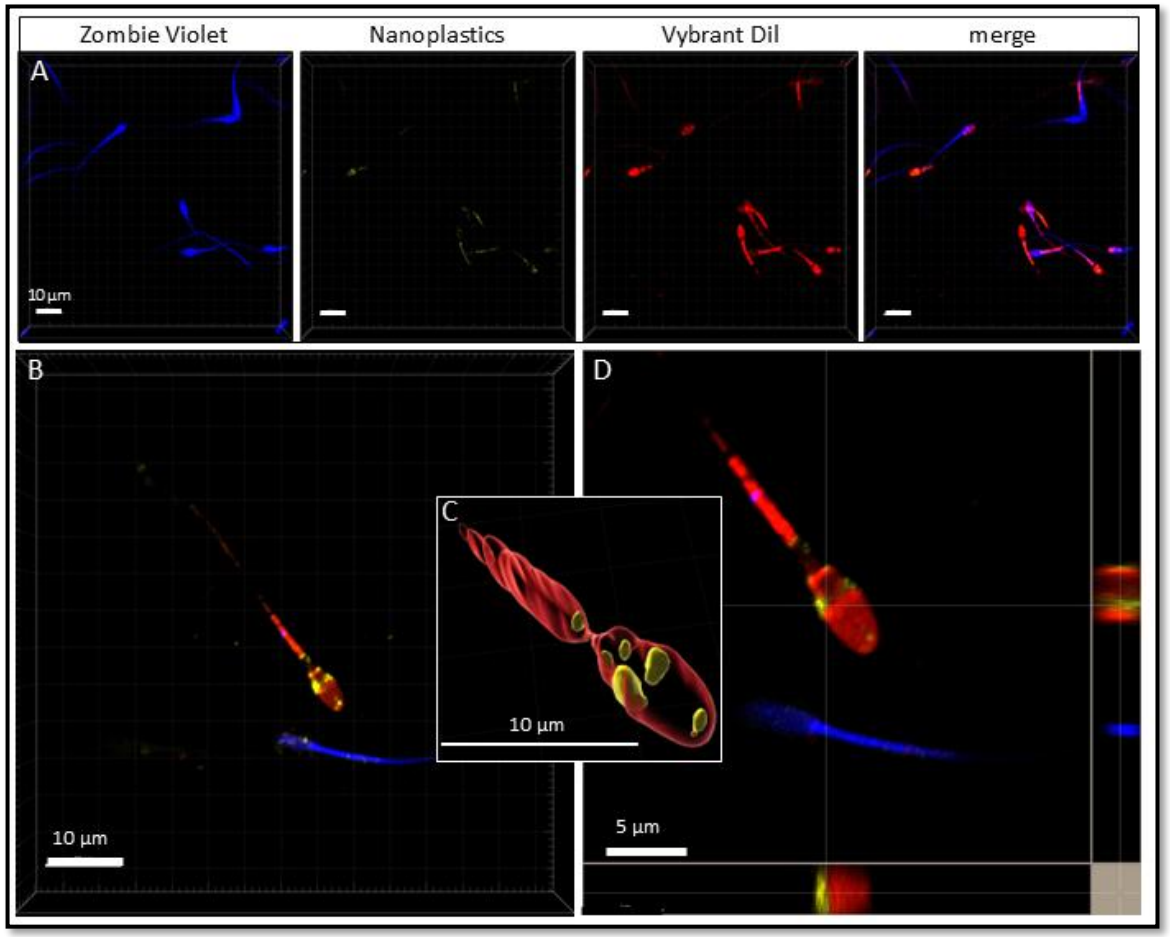
*no significant differences in Hydrogen Peroxide concentration

RESULTS

NP INTERNALIZATION



Sample	MFI B525 (afu)
CTR	1000
10NP	1601
50NP	9793
100NP	21768
200NP	54706



Sperm internalize NP in a dose-dependent manner, with a higher concentration observed in the **post-acrosomal** and **midpiece** regions

PROJECT 2

Research in Veterinary Science

EFFECT OF POLYSTYRENE NANOPLASTICS ON IN VITRO MATURATION OF PIG CUMULUS-ENCOSED OOCYTES

--Manuscript Draft--

Manuscript Number:	
Article Type:	Research Paper
Section/Category:	Physiology
Keywords:	oocyte; nuclear maturation; cytoplasmic maturation; oocyte developmental competence; ROS; cumulus cells steroidogenesis
Corresponding Author:	Marcella Spinaci University of Bologna Department of Veterinary Medical Sciences Ozzano Emilia (Bologna), Bologna ITALY
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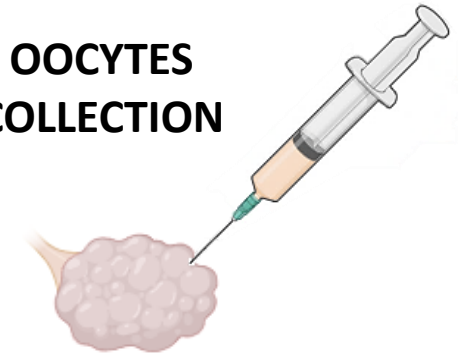
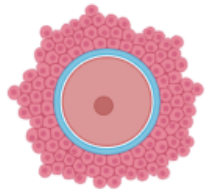
	Alessandro Marino Volsa
	Cinzia Cappannari
	Diego Bucci
	Jose Manuel Ortiz-Rodriguez

UNDER REVIEW

OOCYTES COLLECTION



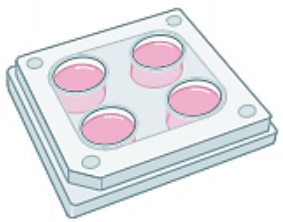


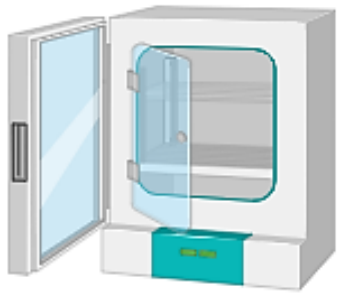
IVM

+

100nm PS-NP

CTR
10, 50, 100, 200 µg/mL

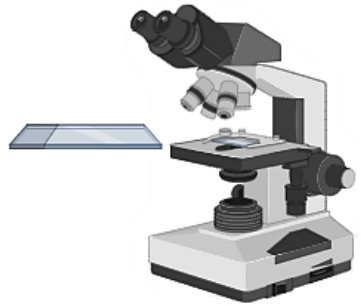




39 °C

44 h

NUCLEAR MATURATION



CYTOPLASMIC MATURATION

IVF
with frozen-thawed semen

Parthenogenetic activation

GSH and ROS levels

CellTracker Blue
H2DCFDA

CCs STEROIDOGENESIS

Progesterone (P4)
estradiol-17β (E2)

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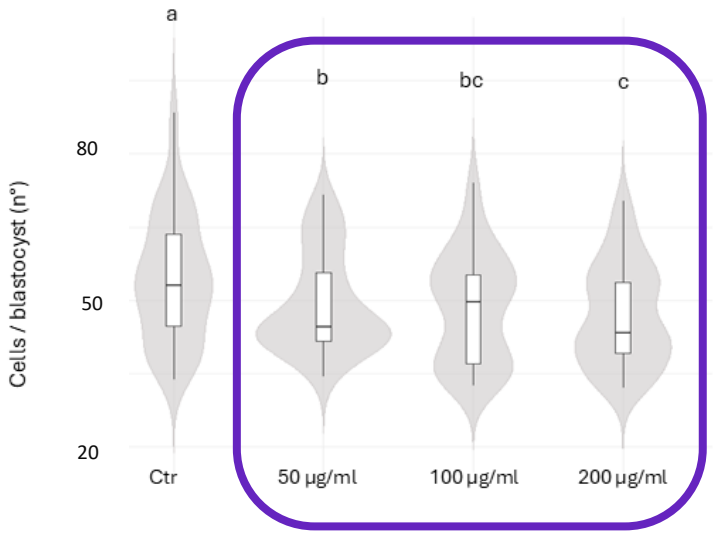
MATERIALS & METHODS

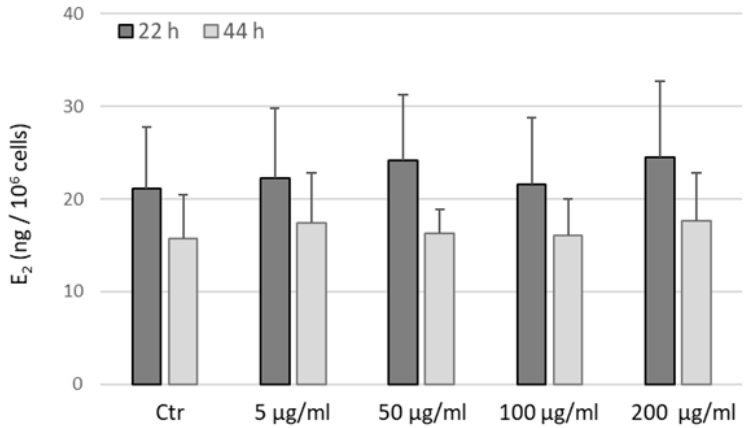
NUCLEAR and CYTOPLASMIC MATURATION, E2 & P4 levels

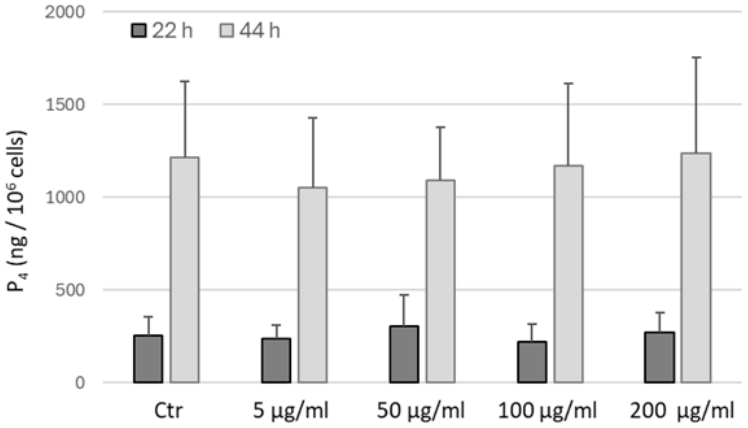
No influence:

- ✓ % MII
- ✓ % fertilized oocytes
- ✓ Cleavage rate, Blastocyst rate

Dose dependent significant **decrease** in the mean **number of blastomere per blastocyst**

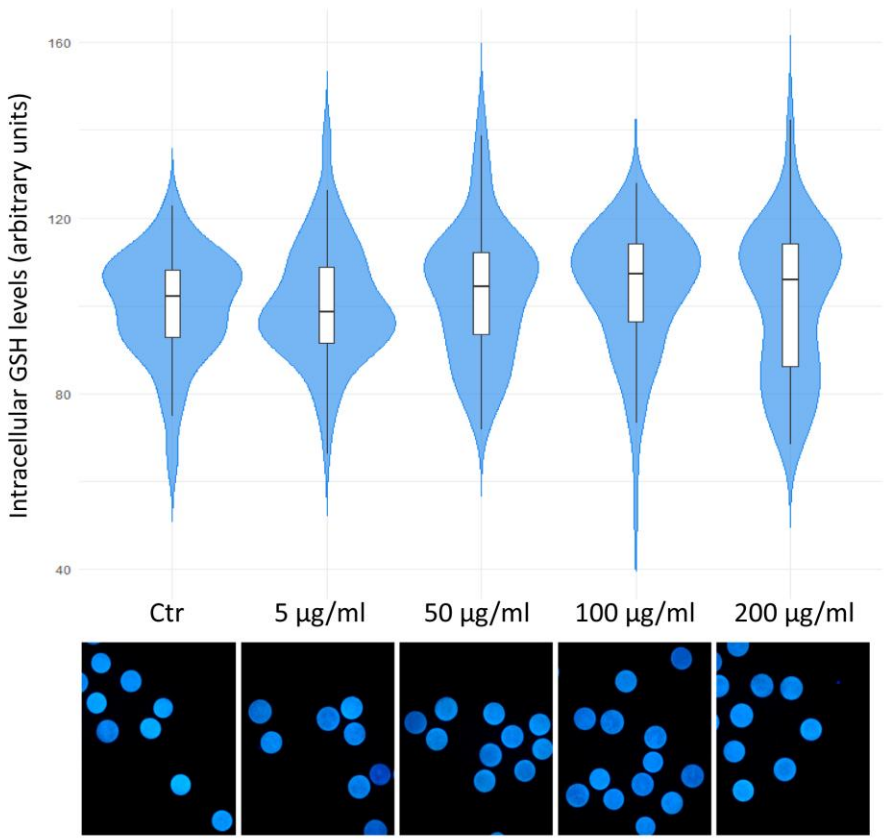




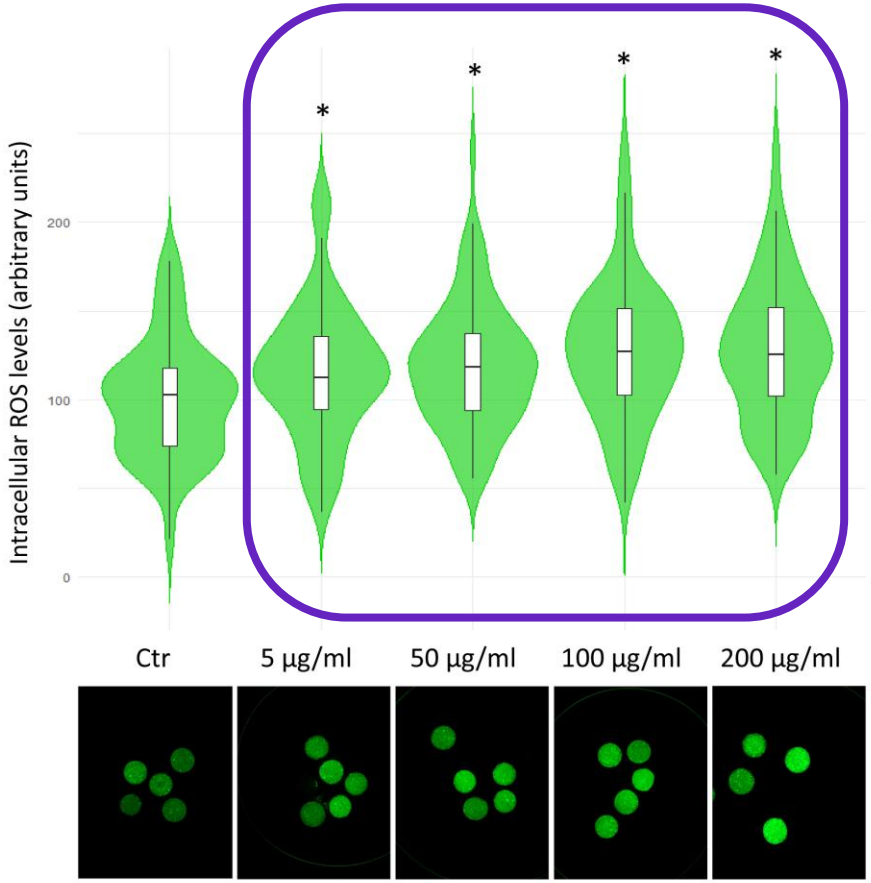


No influence on E2 and P4 levels

GSH and ROS levels



CellTracker Blue



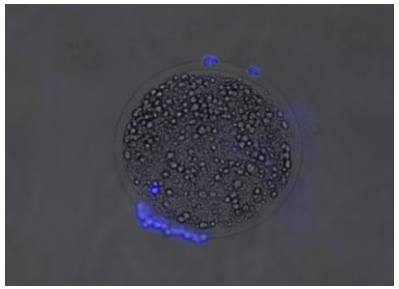
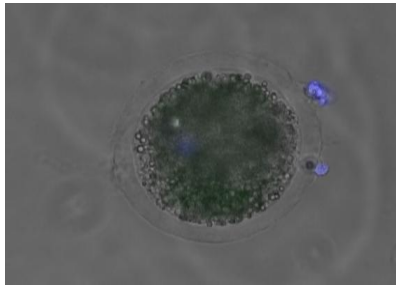
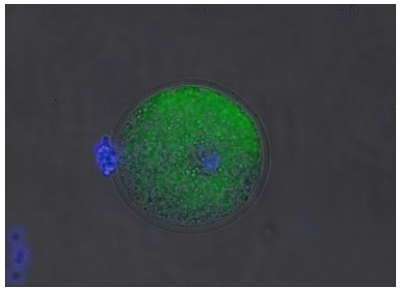
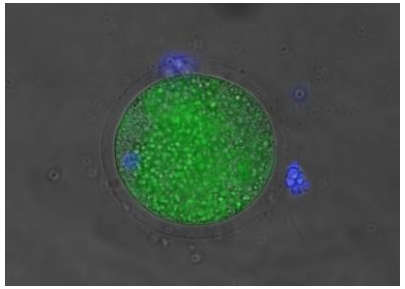
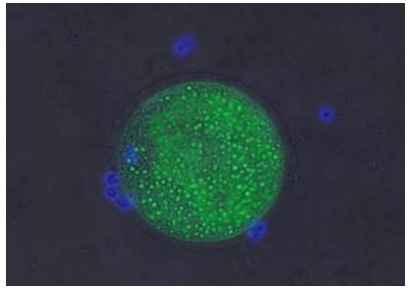
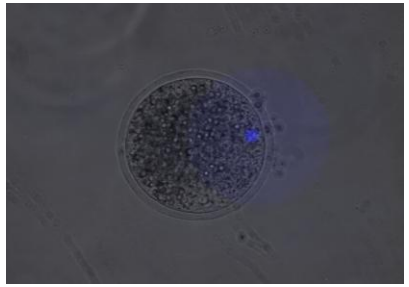
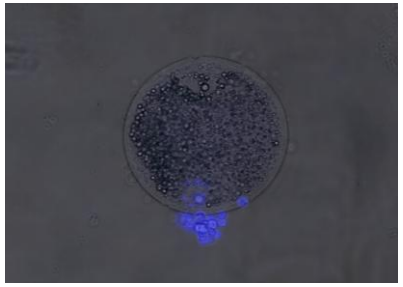
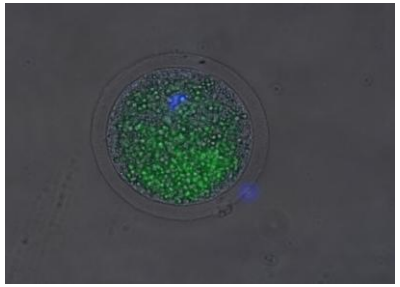
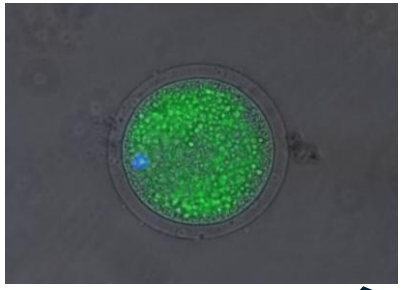
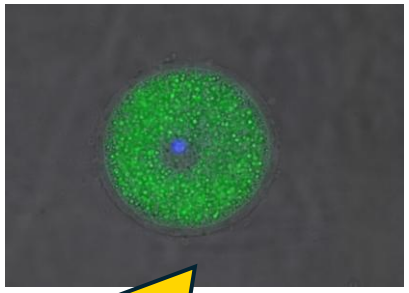
H2DCFDA

Increased intracellular ROS levels in exposed oocytes ($p<0.01$)

No influence on GSH

What is the role of cumulus cells in the uptake of NP?

WORK IN PROGRESS

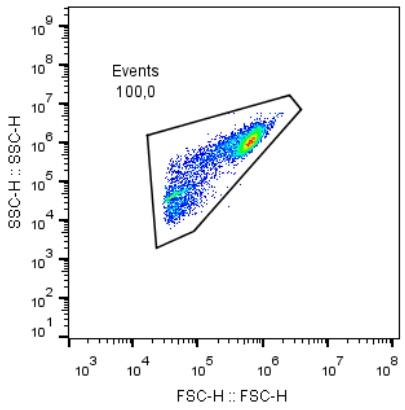
	Ctr	5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml
DENUDED OOCYTES					
4 h IVM					
CUMULUS ENCLOSED OOCYTES					

30 nm Fluorescent yellow-green PS-NP

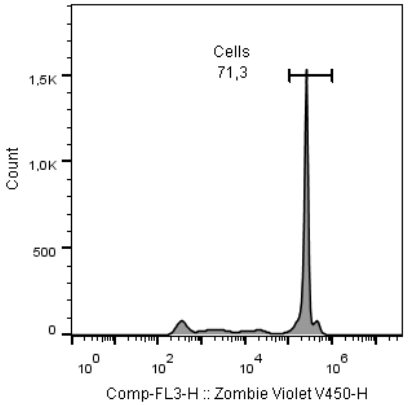
RESULTS

CUMULUS CELLS – IVM 44h

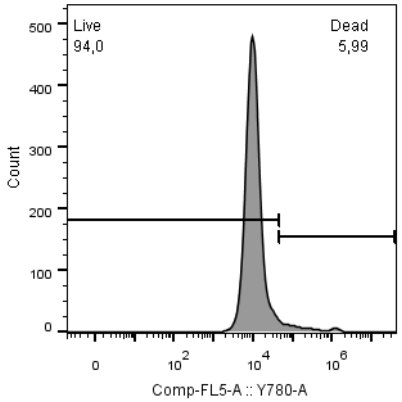
WORK IN PROGRESS



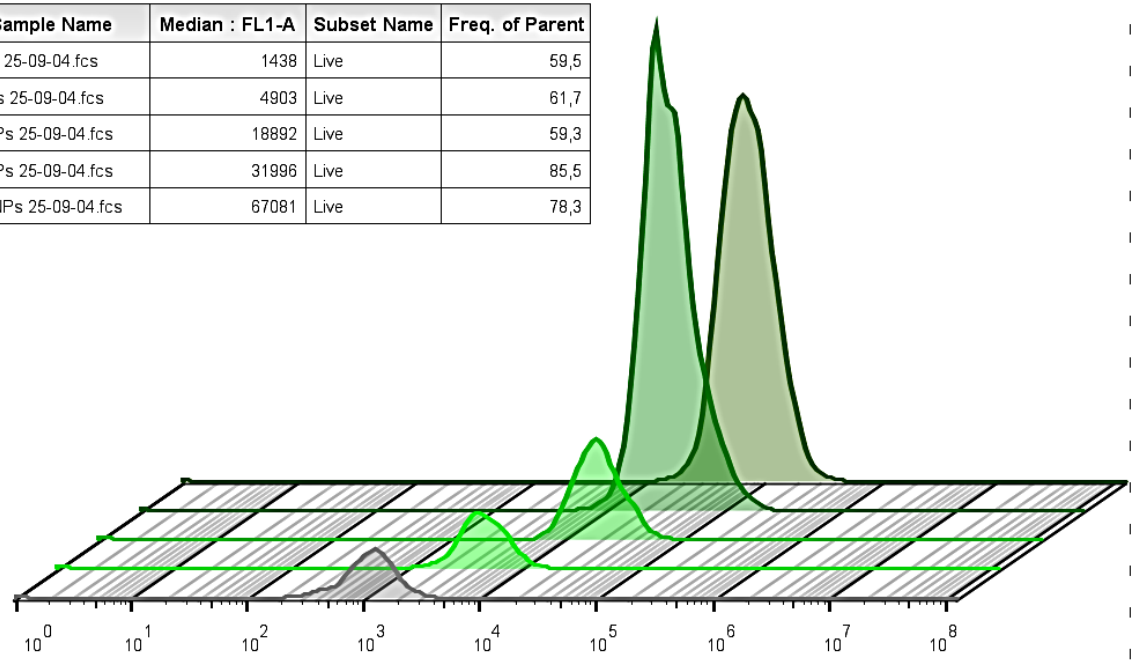
Cells marker (Hoechst 33342)



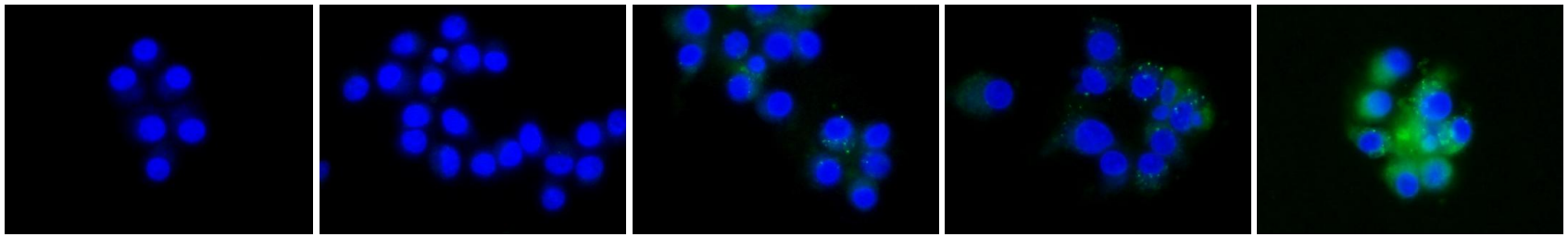
Cells viability (Draq7)



	Sample Name	Median : FL1-A	Subset Name	Freq. of Parent
	CTR 25-09-04.fcs	1438	Live	59,5
	5NPs 25-09-04.fcs	4903	Live	61,7
	25NPs 25-09-04.fcs	18892	Live	59,3
	50NPs 25-09-04.fcs	31996	Live	85,5
	100NPs 25-09-04.fcs	67081	Live	78,3



Ctr | 5 µg/ml | 25 µg/ml | 50 µg/ml | 100 µg/ml



30 nm Fluorescent yellow-green PS-NP

RESULTS

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Conclusions

- ✓ Both **stallion sperm** and **pig oocytes** can **internalize PS-NP**
- ✓ PS-NP **negatively** affect stallion **sperm mitochondrial activity**, potentially impairing sperm survival
- ✓ PS-NP cause **oxidative stress** in sperm and oocytes, by **increasing ROS** production and can possibly **interfere with embryonic cell proliferation**
- ✓ Further studies are necessary to explore the consequences of the global spread of NP on reproductive physiology

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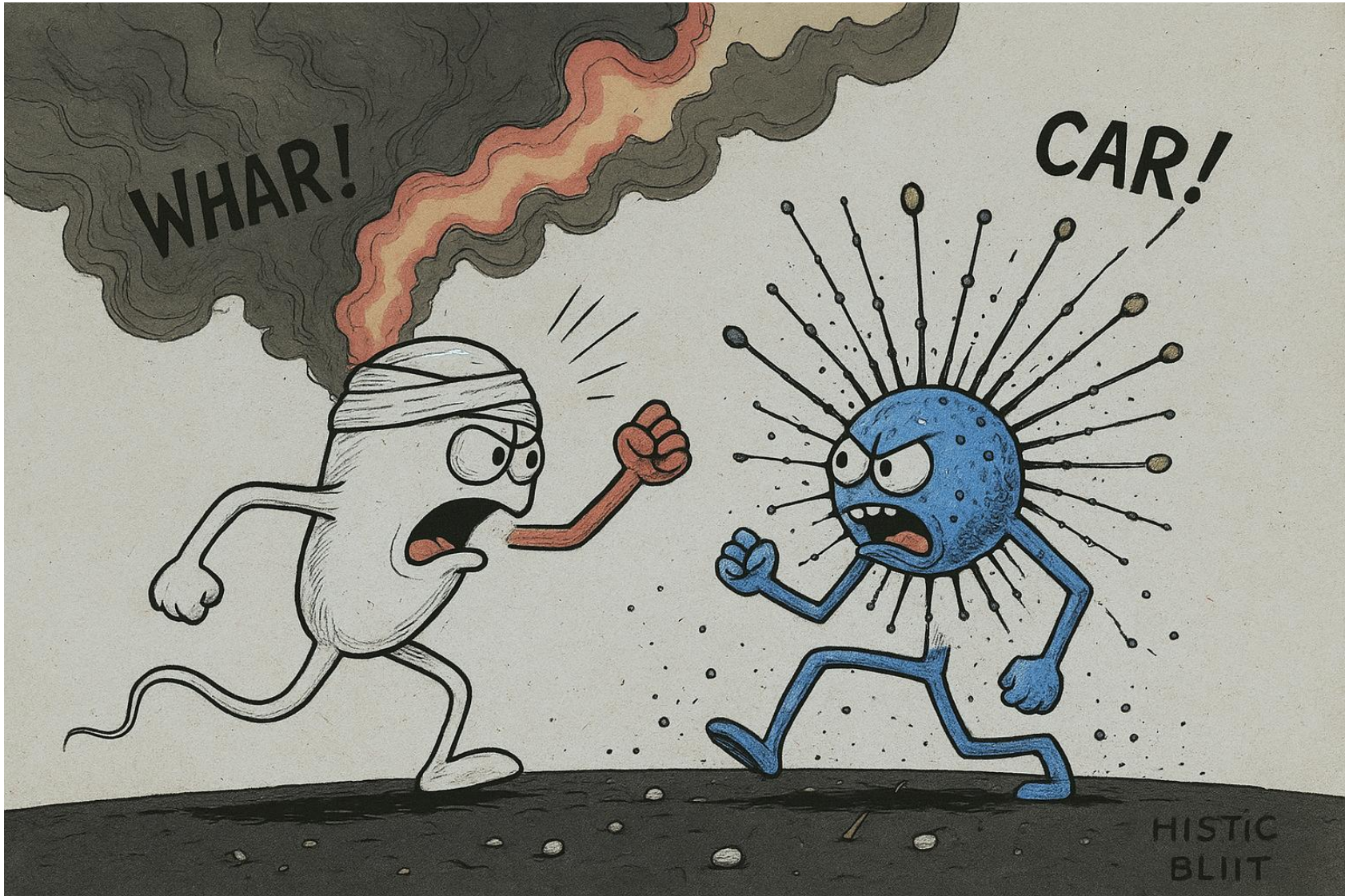
Project Collaborations

Assessing the impact of seasonal heat stress on sperm cryopreservation in Holstein bulls	Ghent University, Belgium
Seasonal influence on the oxidative status of seminal plasma proteins and semen parameters of Belgian Blue bulls	University of Padova, Italy
The effects of co-incubating spermatozoa with seminal extracellular vesicles (sEVs) from different ejaculate fractions on fertilization, early embryo quality, and development	University of Murcia, Spain
Use of natural extracts as a substitute for antibiotics in preserving bull semen	University of León, Spain

Thank you for your attention

sofia.dindo2@unibo.it



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