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Transforming EU food systems with innovative
strategies for sustainable packaging

Assessment of the impact of exposing *Mytilus galloprovincialis* gametes to environmental microplastics on fertility and larval quality: Initial evidence of detecting Prothymosine- α in invertebrates

Ilef Romdhani, Alessandra Gallo, Massimo Venditti, Mohamed Rida Abelouah, Rita Varchetta, Hana Najahi, Khouloud Boukadida, Raffaele Boni, Aicha Aitallah, Sergio Minucci, and Mohamed Banni



¹ Laboratory of Agrobiodiversity and Ecotoxicology, Higher Institute of Agronomy, University of Sousse, Tunisia

² Higher Institute of Biotechnology, University of Monastir, Tunisia

³ Department of Experimental Medicine, University Degli Studi Della Campania Luigi Vanvitelli, Via Santa Maria di Costantinopoli, 16, 80138 Napoli, Italy

⁴ Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy

⁵ Laboratory of Aquatic Systems: Marine and Continental Environments, Faculty of Sciences, Ibn Zohr University, Agadir, Morocco

⁶ Department of Sciences, University of Basilicata, Viale dell'Ateneo Lucano, 10, 85100 Potenza, PZ, Italy



An underwater scene with sunlight filtering through the water surface, creating a bright, shimmering effect. The water is a deep blue color, and the sunlight creates a lens flare effect. The word "Problematic" is overlaid on the image, with "Proble" in white and "matic" in dark blue.

Proble**matic**

10,000,000

tons of plastic enter the oceans annually

(Jambeck et al. 2015)

+ 80%

marine litter is plastics

(European Parliament 2019)



PLASTIC



Macro > 5 mm

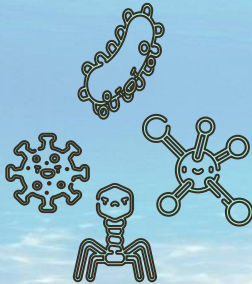
Meso [1 cm- 5 mm]

Micro < 5 mm

Nano < 1 μ m



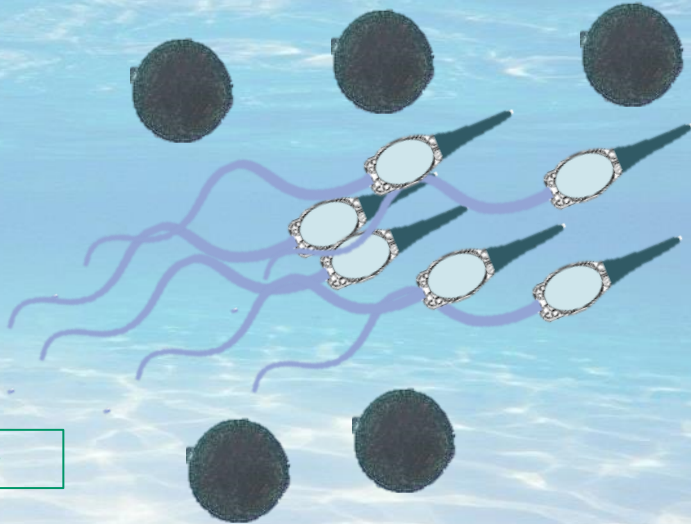
Environmental/
physical
stressors



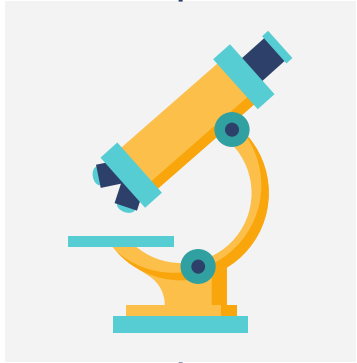
Broadcast-spawning



Mytilus galloprovincialis



Study objectives



MP characterization and quantification

Chemistry



Oxidative stress status and motility

Physiology



DNA integrity and Bax/Bcl-2

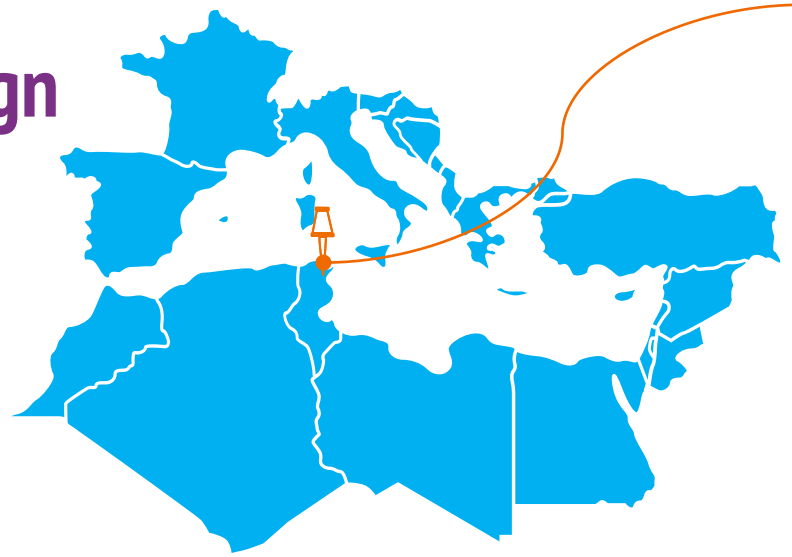
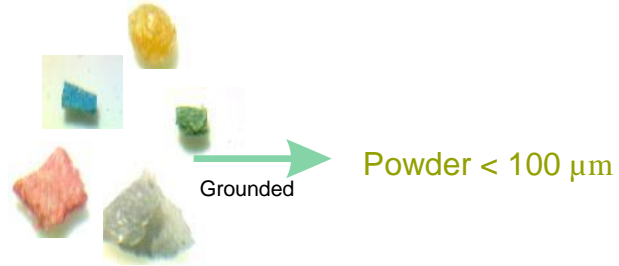
Apoptosis



Detection and expression variation

Prothymosin-a

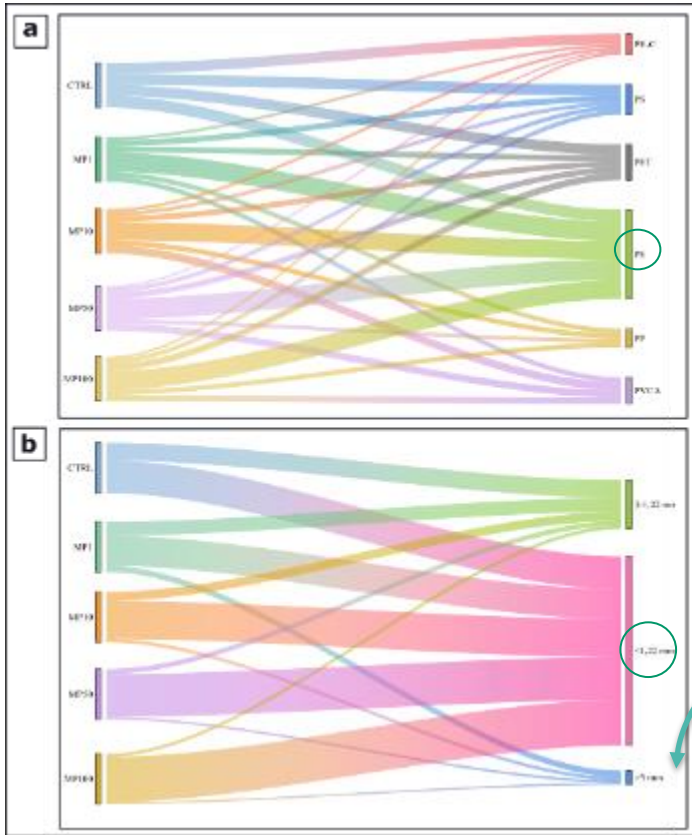
Experimental design



Cap-Serrat Bizerte ($37^{\circ}13'28.5''\text{N}$ $9^{\circ}13'23.4''\text{E}$)



Plastic characterization



Microscopic visualization

Shapes

Colors

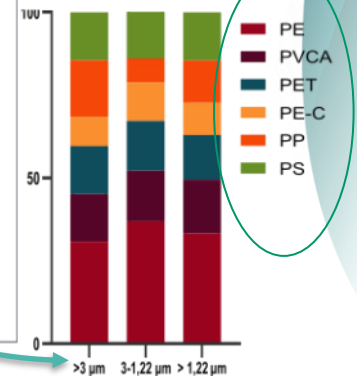
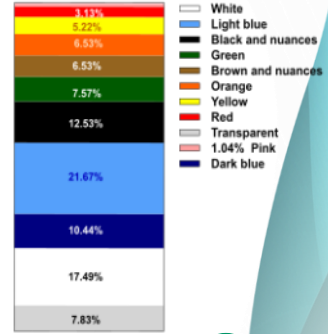
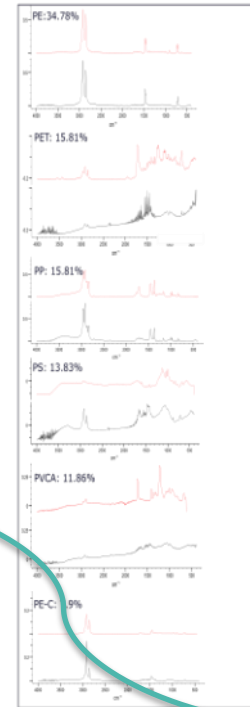
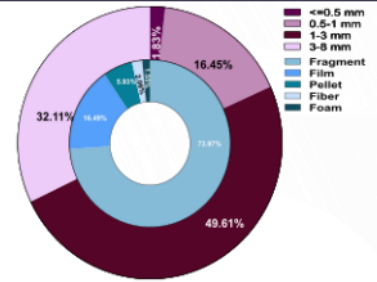
FTIR

Types

μ RAMAN

In SPZ

In stock solution



Gamete physiology

Spectrofluometric analysis

Viability

Decreased

Mitochondrial Membran Potential

Increased,
hyp: Oxidative stress
compensation

ROS

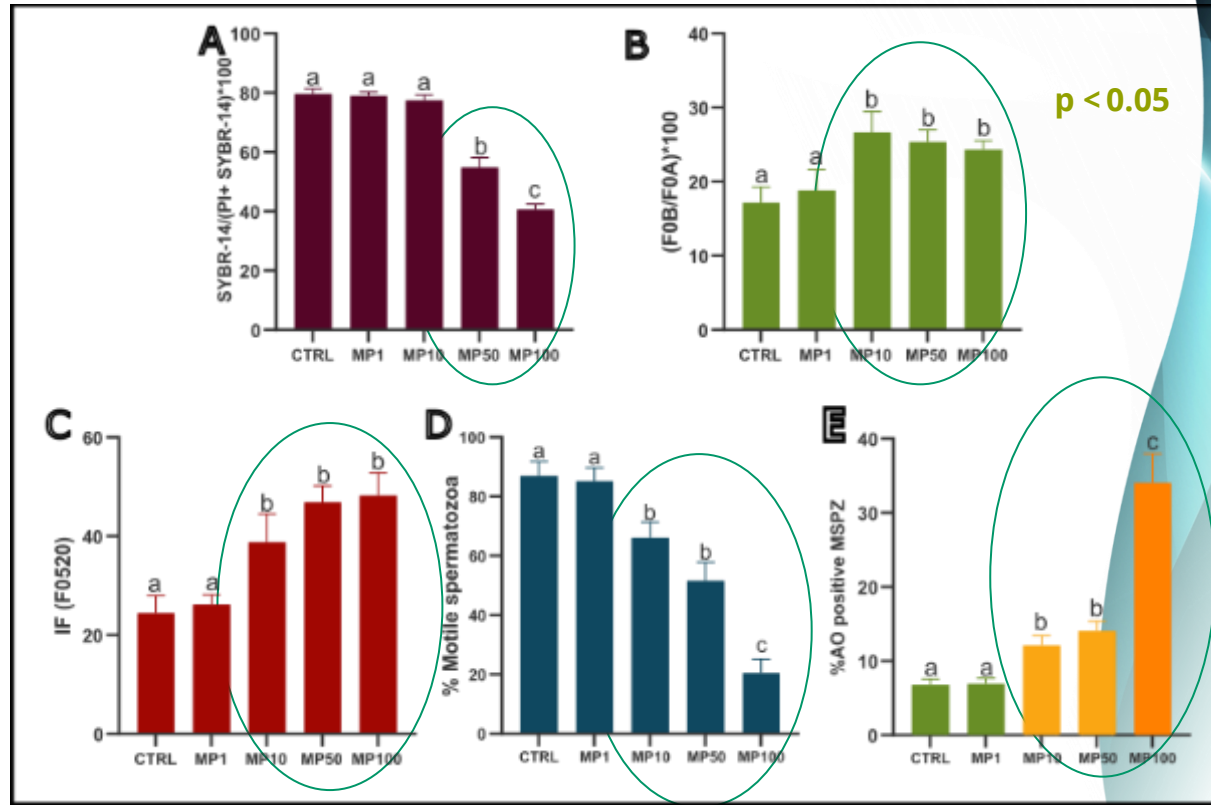
Increased

% motile SPZ

Decrease

% + AO cells

Increased
: DNA
damage



- **SPZ are particularly vulnerable to oxidative stress: limited antioxidative potential** (Aitken et al., 2020)
- **Association of ROS with DNA damage in male gametes** (Aitken et al., 1998, Kardivel et al., 2009)
- **Reduced motility**

EMP induces (MP10+, MP50 and MP100++)
gamete quality: ↓ Fertilization rate

Intrinsic apoptosis pathway

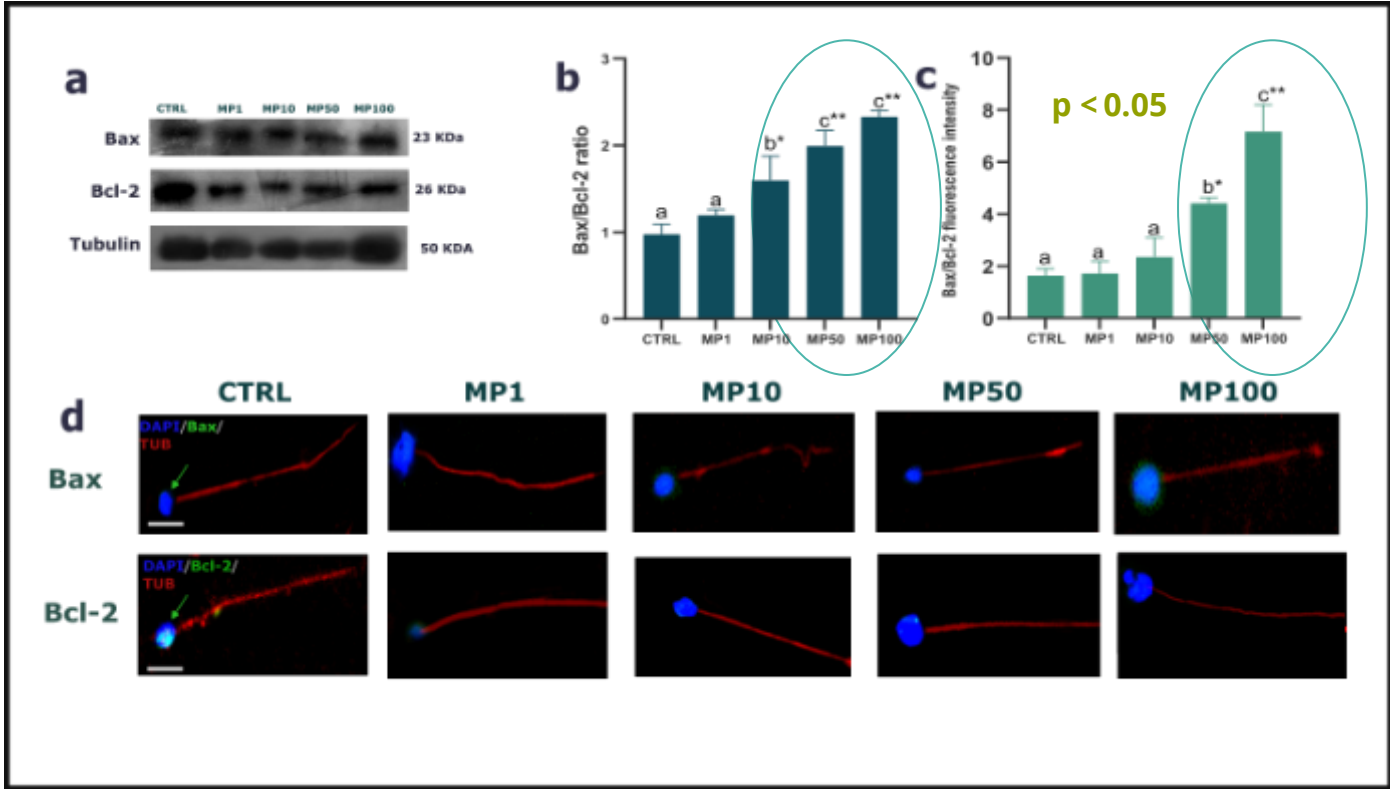
Apoptosis: Bax/Bcl-2

Pro-apoptotic/ Anti-apoptotic

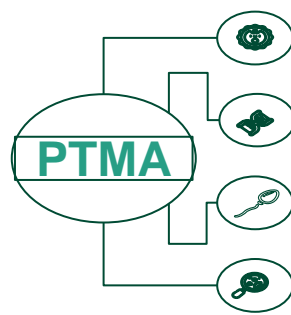
Pro-apoptotic effect of EMP (MP50, MP100)

WB
Quantification

IF
Localisation
Quantification



Prothymosine- α



Cell proliferation, immune response, and chromatin remodelling (Venditti et al., 2022)

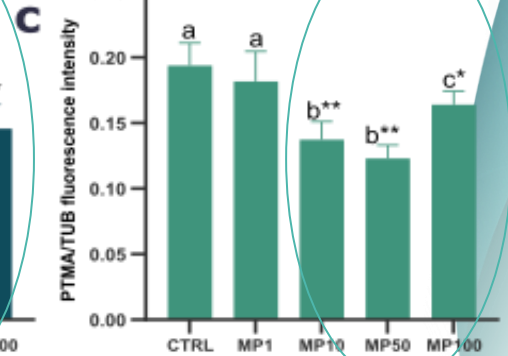
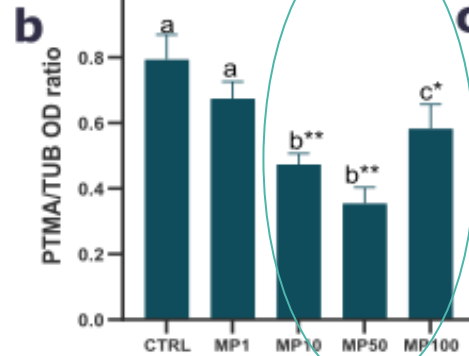
Safeguarding against apoptosis during cell cycle regulation in mammalian testis differentiation

Potential role in fertilization: Acrosomal localization in mammals SPZ

Dearth of documented evidence regarding the presence, localization, and role of PTMA in molluscs

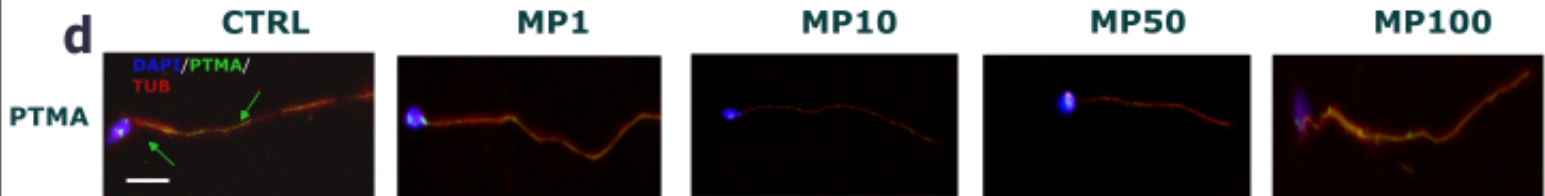
WB

Quantification



IF

Localisation
Quantification



Conclusion



1 Significant threat to broadcast spawning species by impairing gamete quality

2 Elucidates the mechanism of EMP spermotoxicity involving the increase of mitochondrial activity necessary to cope with the energy demand changes

3 The ROS increase affects sperm motility, probably altering membrane fluidity, and also induces DNA damage leading ultimately to sperm death through apoptosis activation

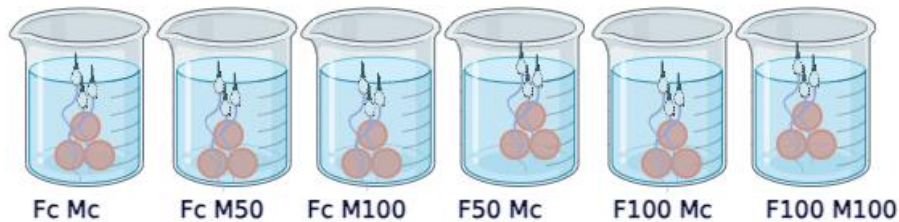
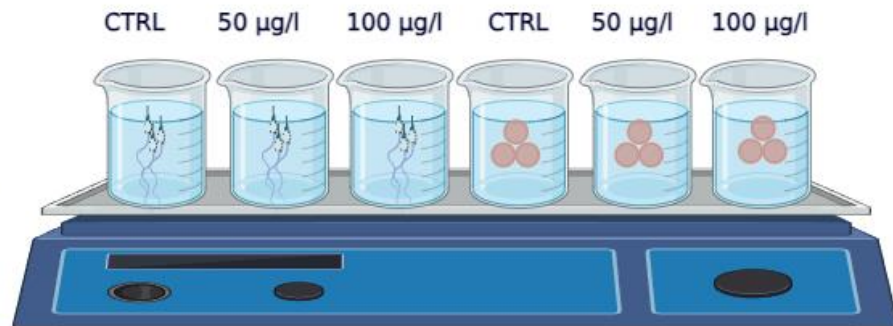
4 Our study represents the first evidence of the detection of PTMA in mollusc spermatozoa. PTMA content showed significant variations concerning EMP exposure



Spawning thermal stimulation
15-20C (30 min)

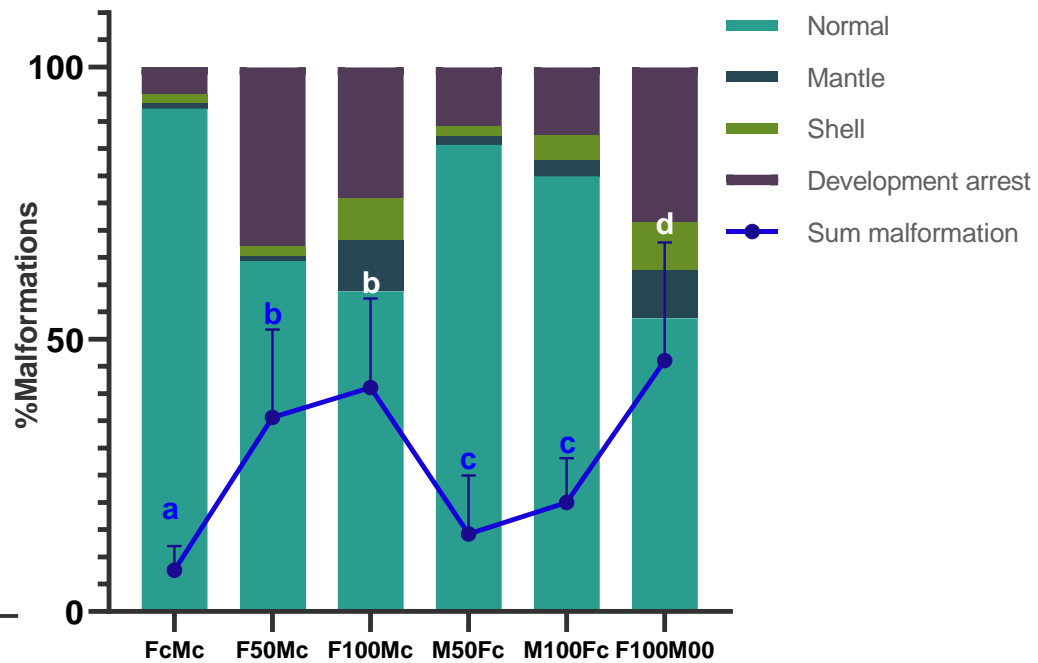
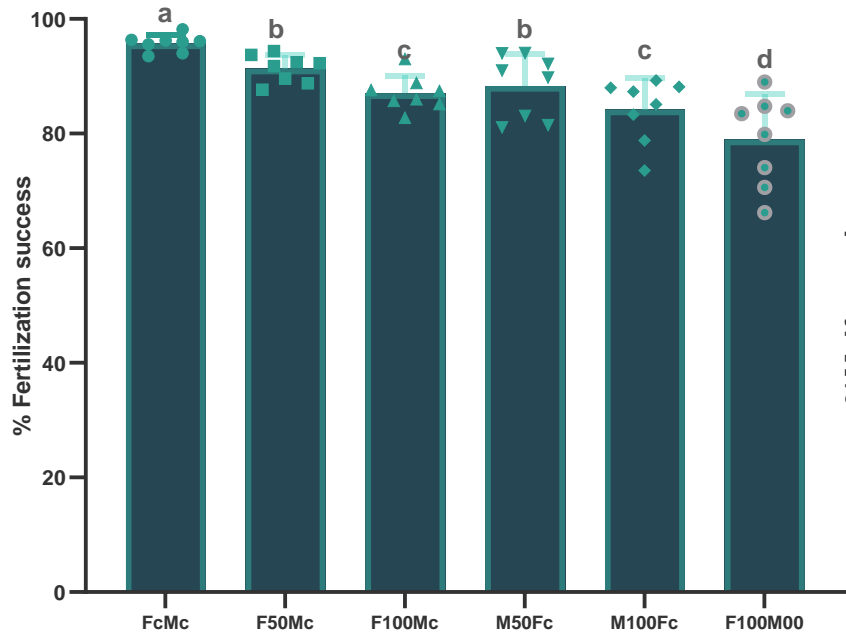


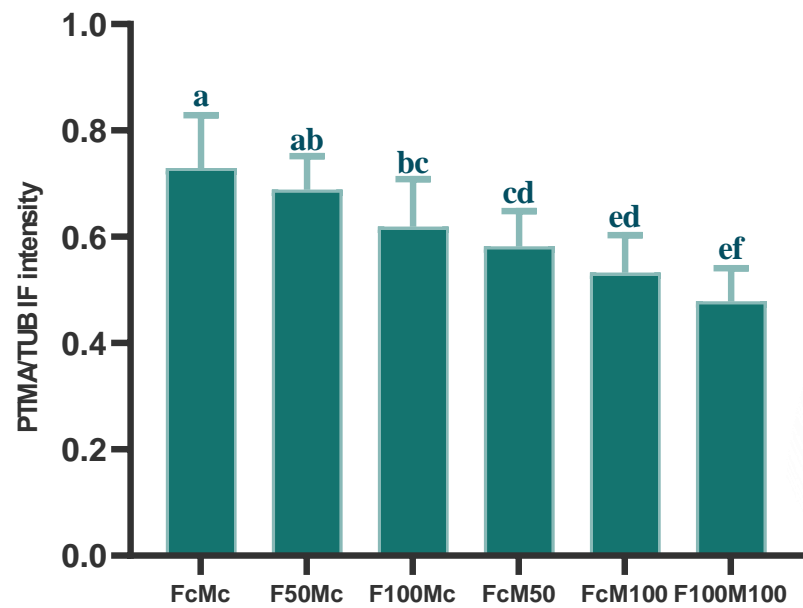
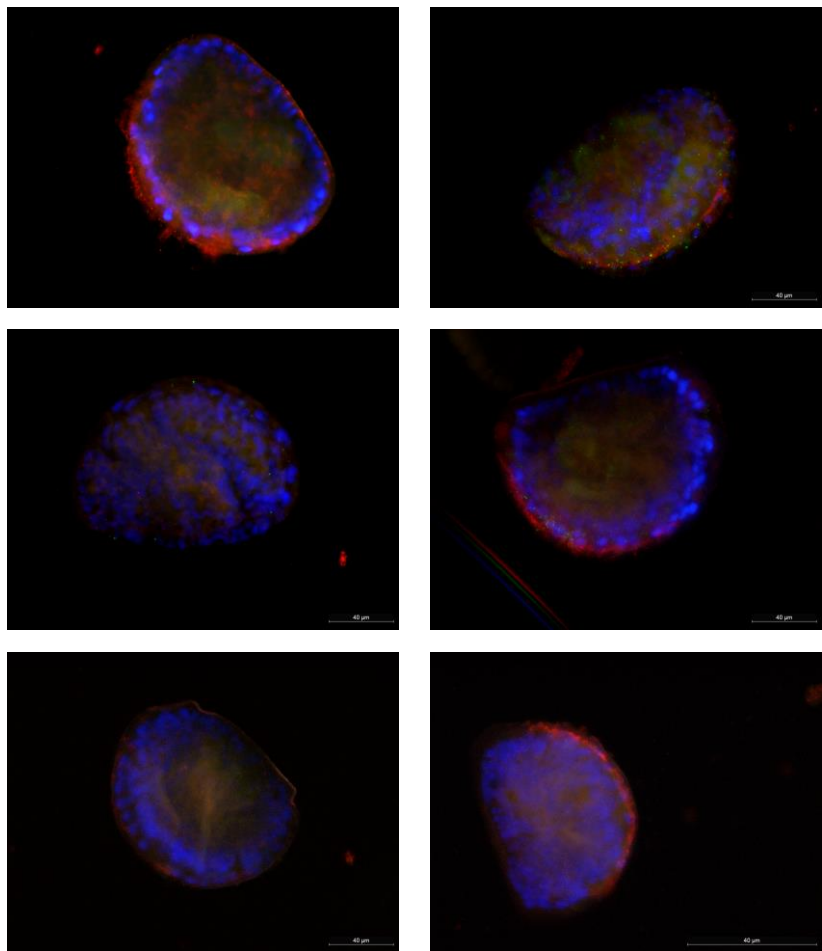
1h

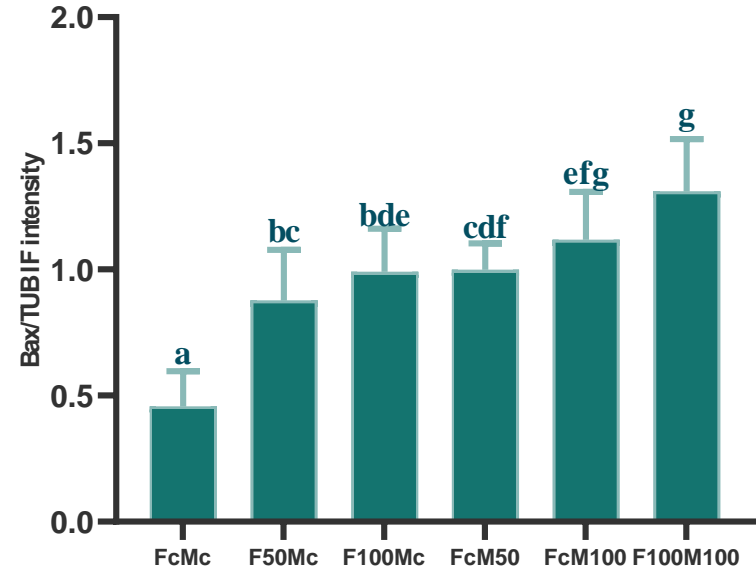
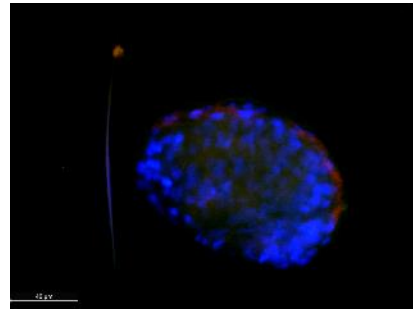
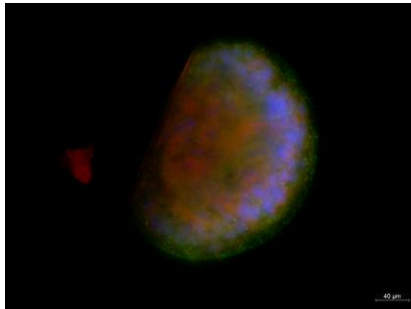
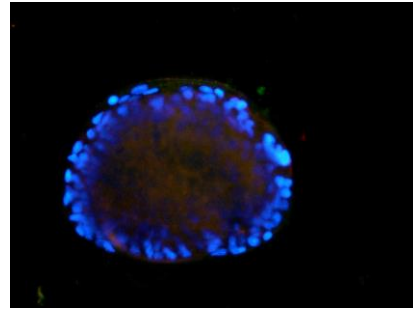
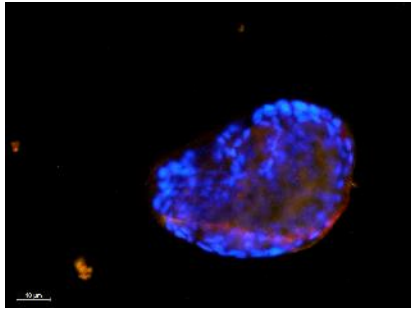
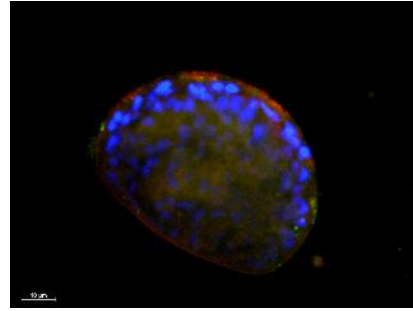
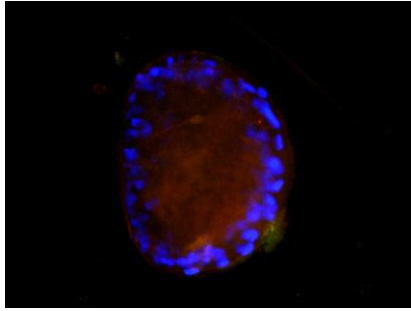


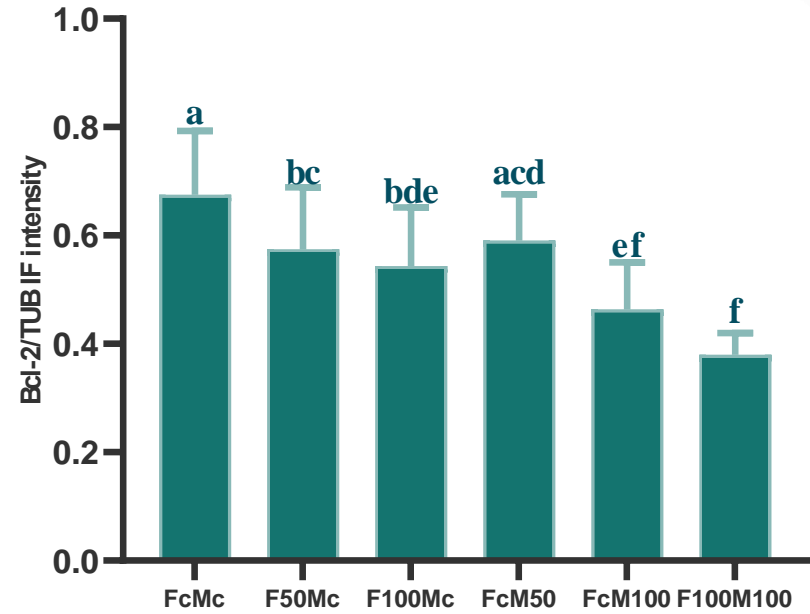
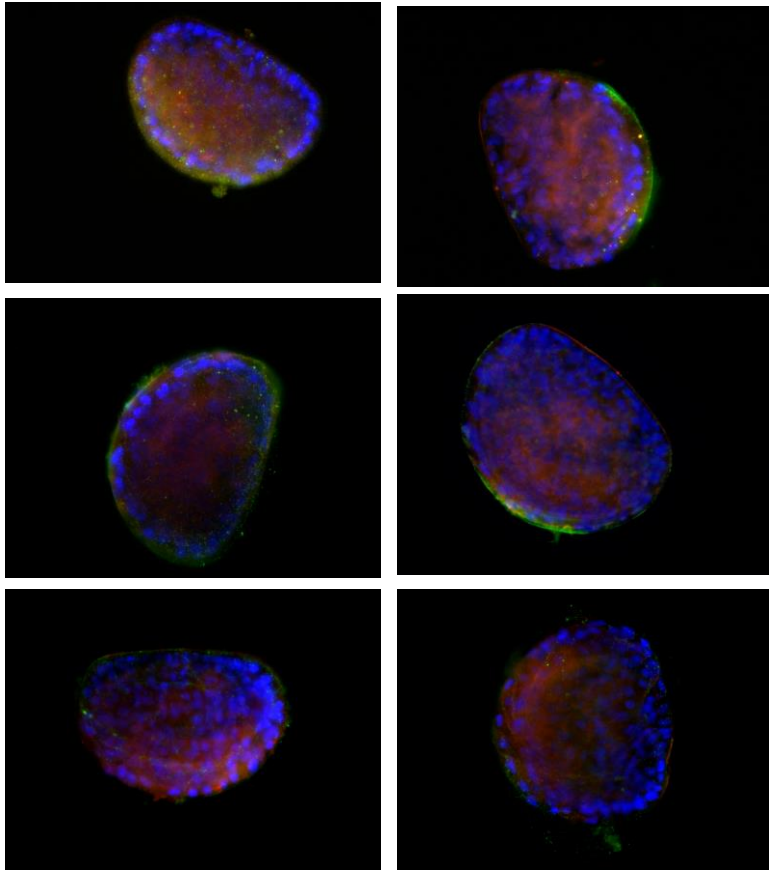
48h













Thank you
For your attention

Contact: m_banni@yahoo.fr