

Nutritional and Reproductive Strategies to cope with Heat Stress on Dairy Cows

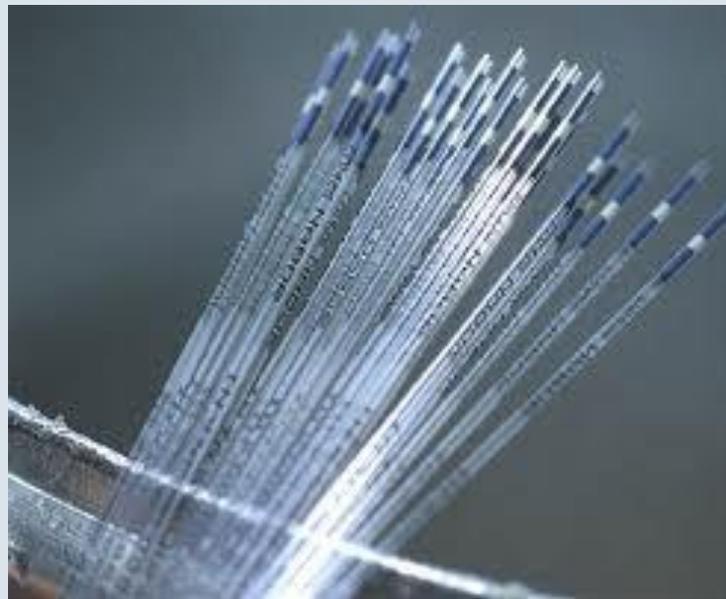
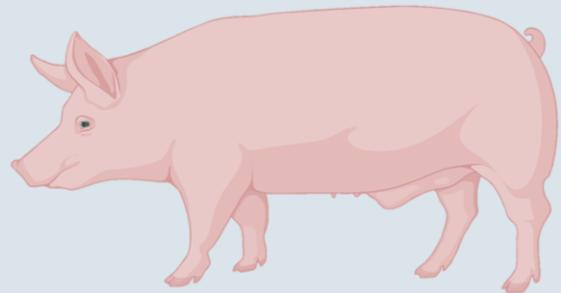
Laura Tovar Pascual
Supervisor: Diego Bucci
Co-Supervisor: Barbara Merlo, Ludovica M.E Mammi
39th cycle

Contents

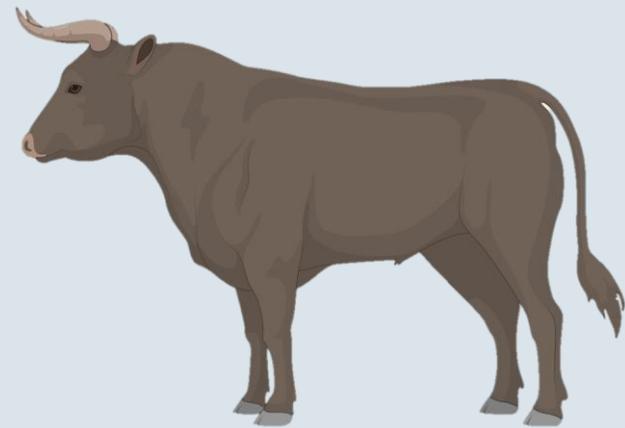
1. Laboratorio di Fisiologia dei Gameti e Biotecnologie Riproduttive (FGBR-ANFI)
Istituto Nazionale di Fecondazione Artificiale (INFA)
2. Laboratorio di Riproduzione e Biotecnologie Animali (LRBA-SERE)
3. Contribution to other activities
4. Period Abroad: University College Dublin (UCD)
5. Learned Techniques
6. Congresses
7. Future perspectives for 25-26



Boar semen exposed to infrared LED light (RLI)



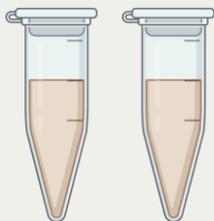
Heat-Stress and non Heat-Stress bull semen



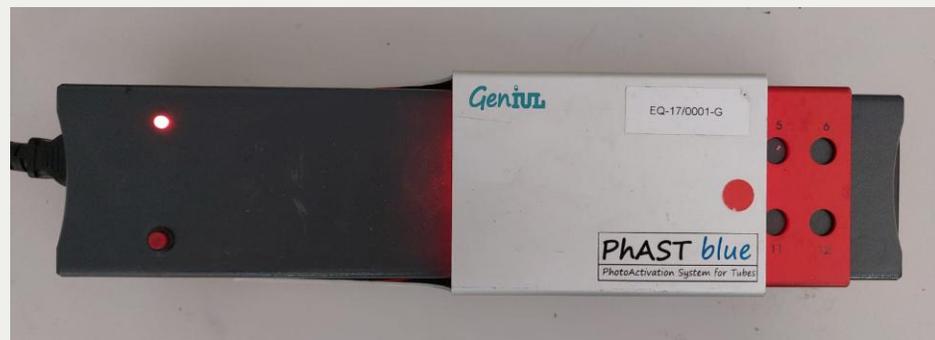
1. Fresh collected semen



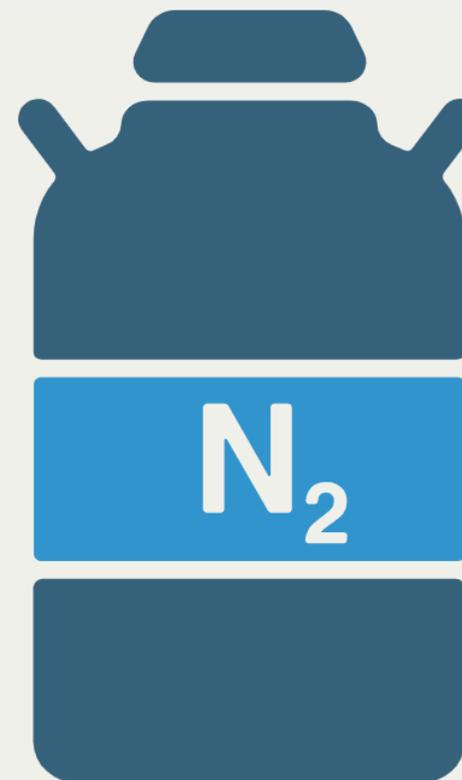
2. Distributed in different groups

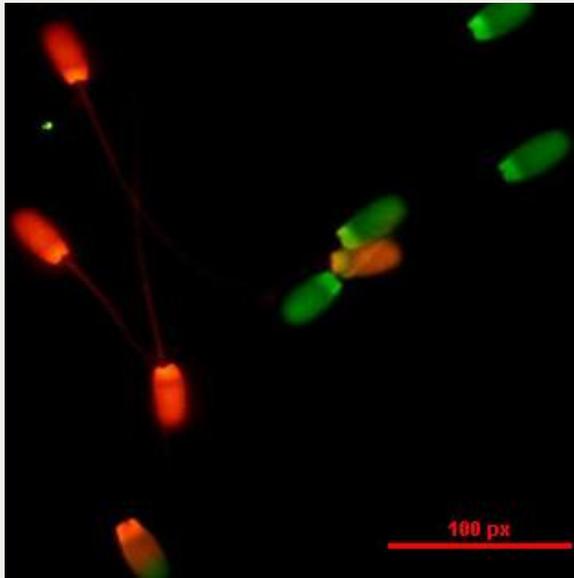
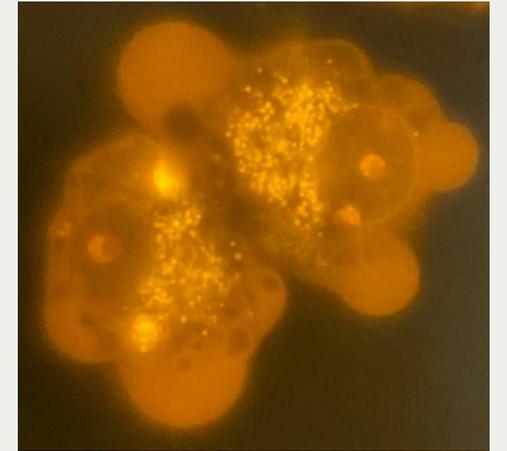


3. InfraRed Led Light exposure

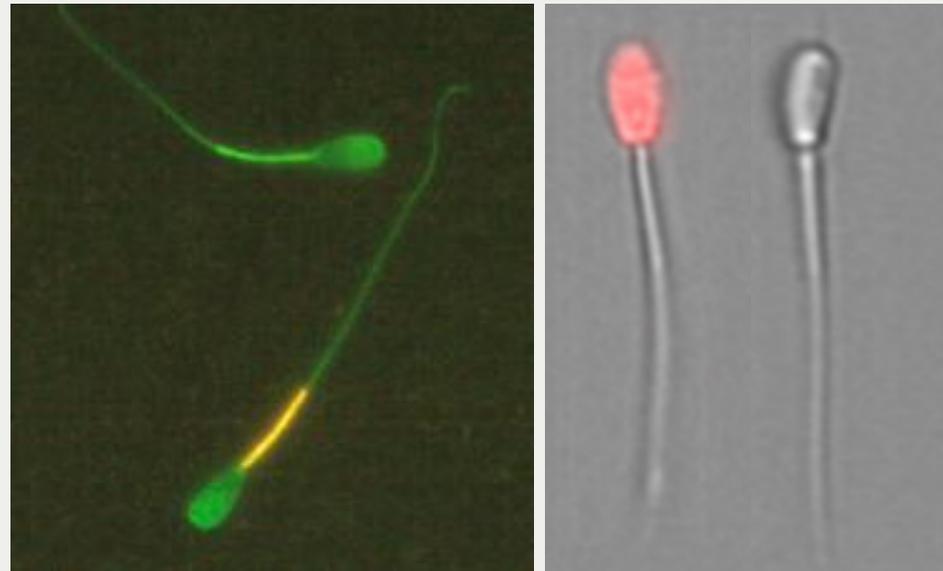


4. Cryopreserved until further analysis

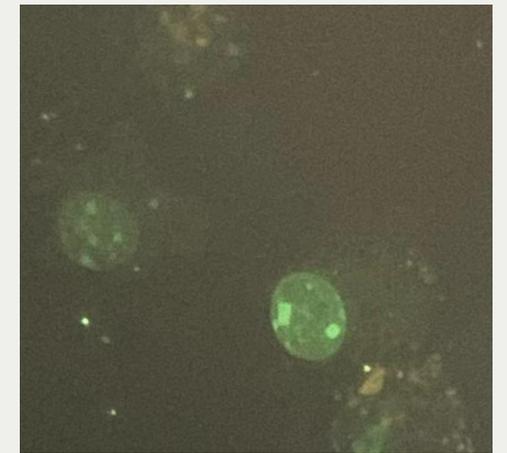




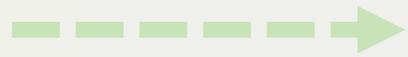
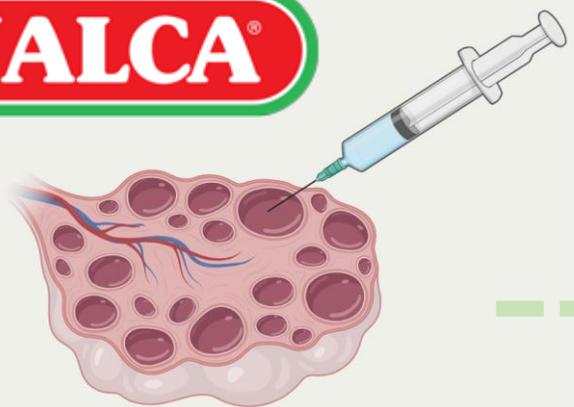
Viability (SybrGreen + PI)



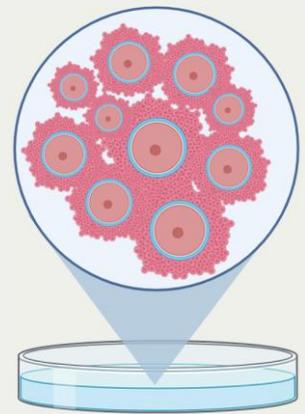
Mitochondrial activity (JC1 + DRAQ7)



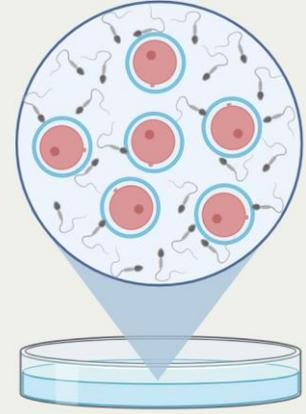
Granulosa cells



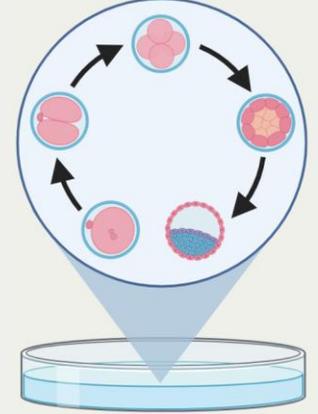
IVM



IVF

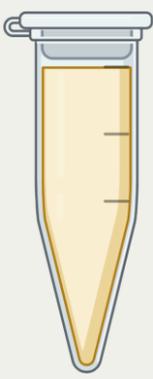
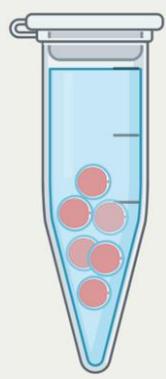


IVC

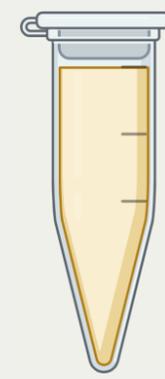
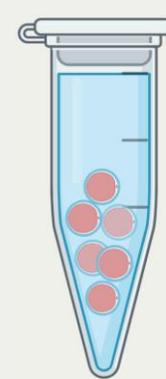


Stock -80°C

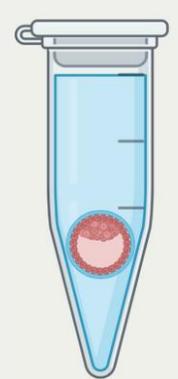
In vivo
Matured oocytes Follicular Fluid

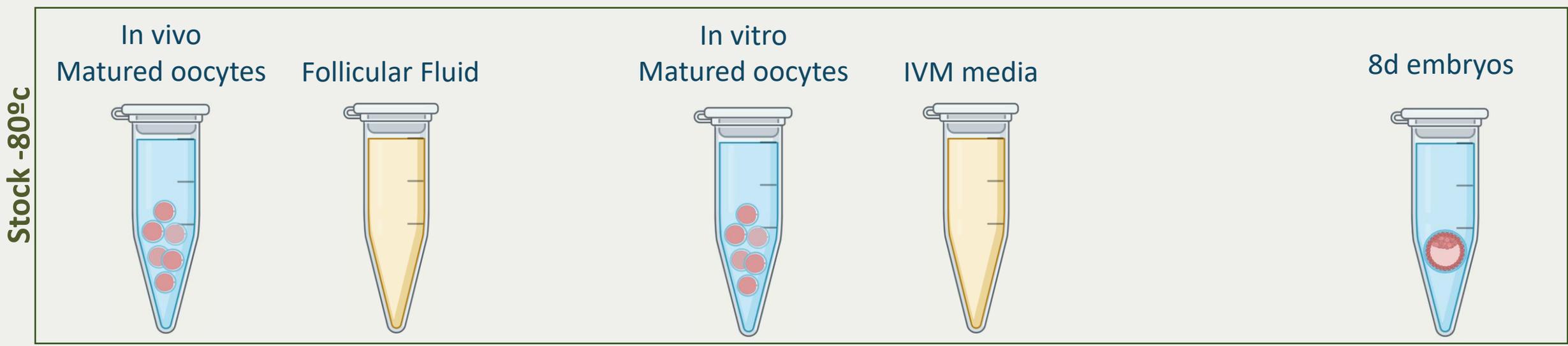


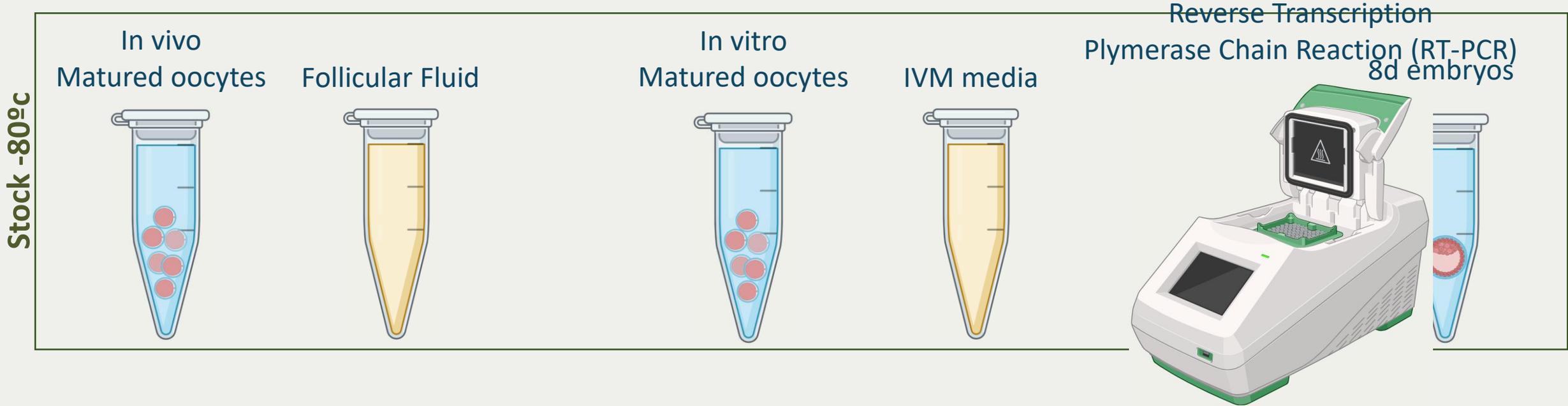
In vitro
Matured oocytes IVM media



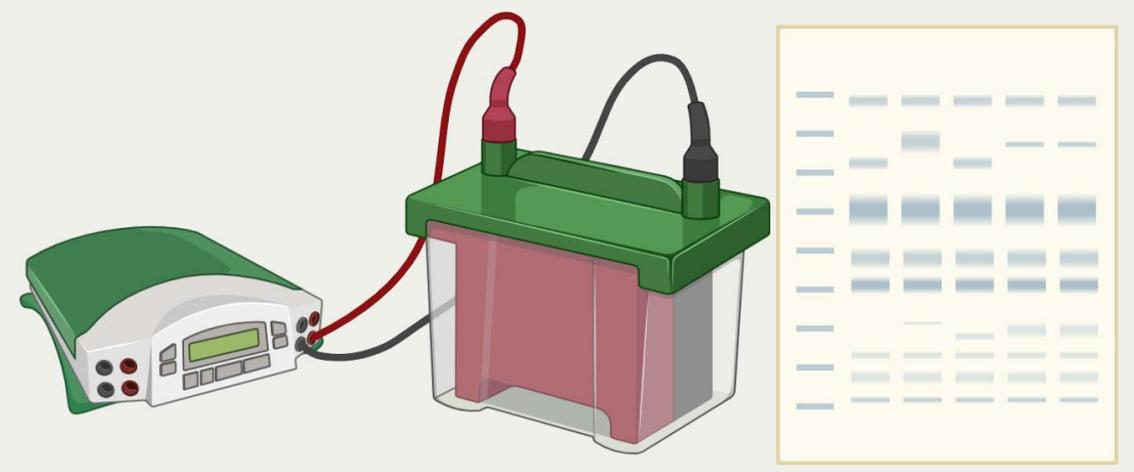
8d embryos







Methodology refinement
Heat Shock Proteins (HSP) 60,70,90



Theriogenology

Nanoplastics internalization impairs mitochondrial activity in equine sperm
--Manuscript Draft--

Manuscript Number:	THERIO-D-25-00677R1
Article Type:	Original Research Article
Keywords:	spermatozoa, nanoplastics, plastic pollution, mitochondria, oxidative stress, fertility, equine
Corresponding Author:	Jose Manuel Ortiz-Rodriguez, DMV, PhD University of Bologna Department of Veterinary Medical Sciences Ozzano dell'Emilia, Bologna ITALY
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Keywords:
Nanoplastic
Polystyrene
Bovine
Oocyte
Maturation
Fertilization

Research in Veterinary Science

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

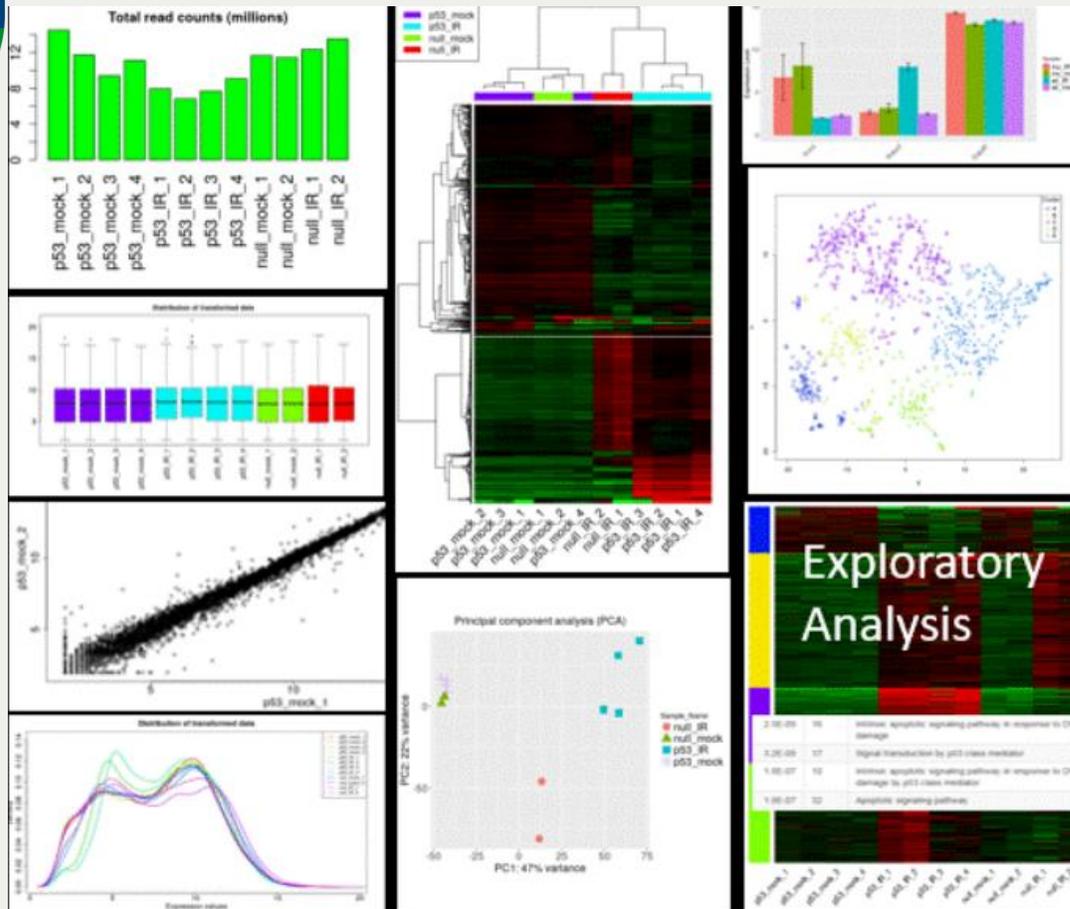
Manuscript Number:	
Article Type:	Research Paper
Section/Category:	Physiology
Keywords:	oocyte; nuclear maturation; cytoplasmic maturation; oocyte developmental competence; ROS; cumulus cells steroidogenesis
Corresponding Author:	Marcella Spinaci University of Bologna Department of Veterinary Medical Sciences Ozzano Emilia (Bologna), Bologna ITALY
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Polystyrene nanoparticles (PS-NPs) have emerged as a significant environmental concern due to their widespread presence and potential toxicity. This study investigates the effects of PS-NPs on bovine oocyte in vitro maturation (IVM) to assess their impact on reproductive health. During IVM, oocytes were exposed to varying concentrations of 100 nm PS-NPs (0, 5, 50, 100, and 200 µg/mL) or of fluorescent 30 nm PS-NPs (0, 5, 35, and 70 µg/mL). Higher PS-NPs concentrations (100 and 200 µg/mL for 100 nm PS-NPs and 70 µg/mL for 30 nm PS-NPs) significantly reduced nuclear maturation rates and increased degeneration. Similarly, cytoplasmic maturation was negatively affected at higher concentrations (P < 0.05). PS-NPs exposure also elevated reactive oxygen species (ROS) levels and reduced glutathione (GSH) content within the oocyte. Notably, PS-NPs were internalized by both oocytes and cumulus cells in a concentration-dependent manner. These findings confirm the reproductive toxicity of PS-NPs, emphasizing their potential to compromise mammalian fertility and raising concerns regarding their environmental and health implications.

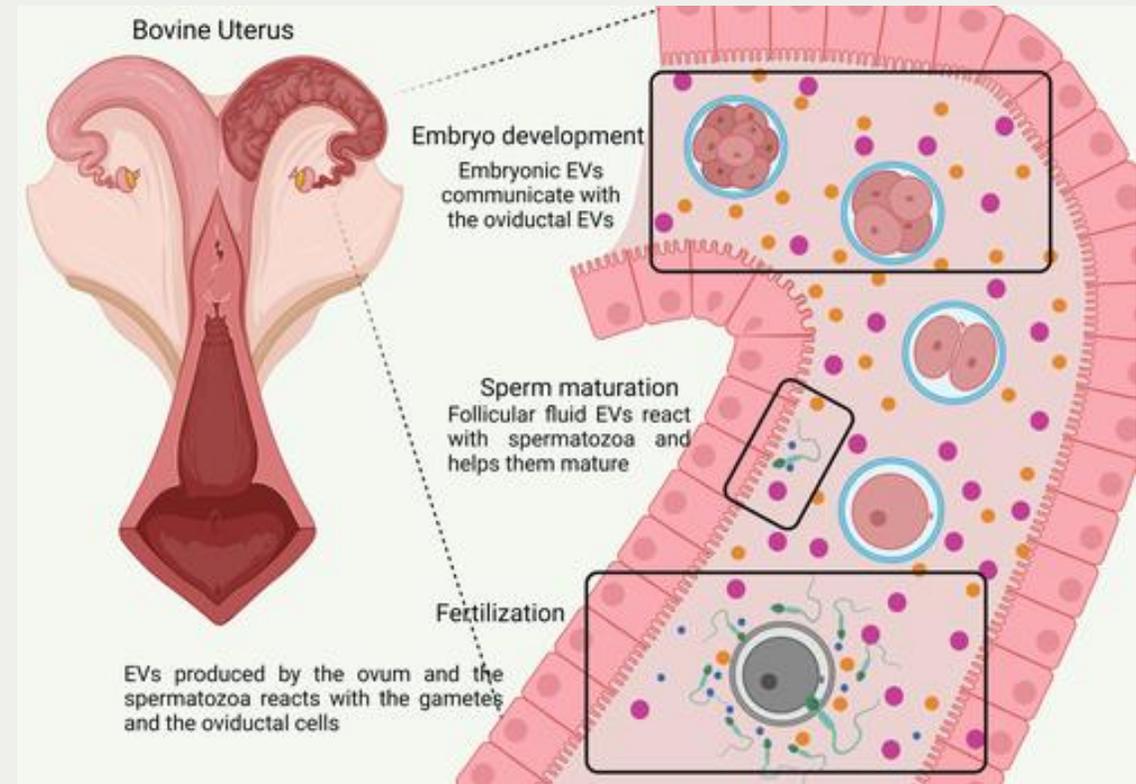
Under Review



1. mRNA Seq Exploratory analysis



2. miRNA content of endometrial EVs produced under heat stress conditions

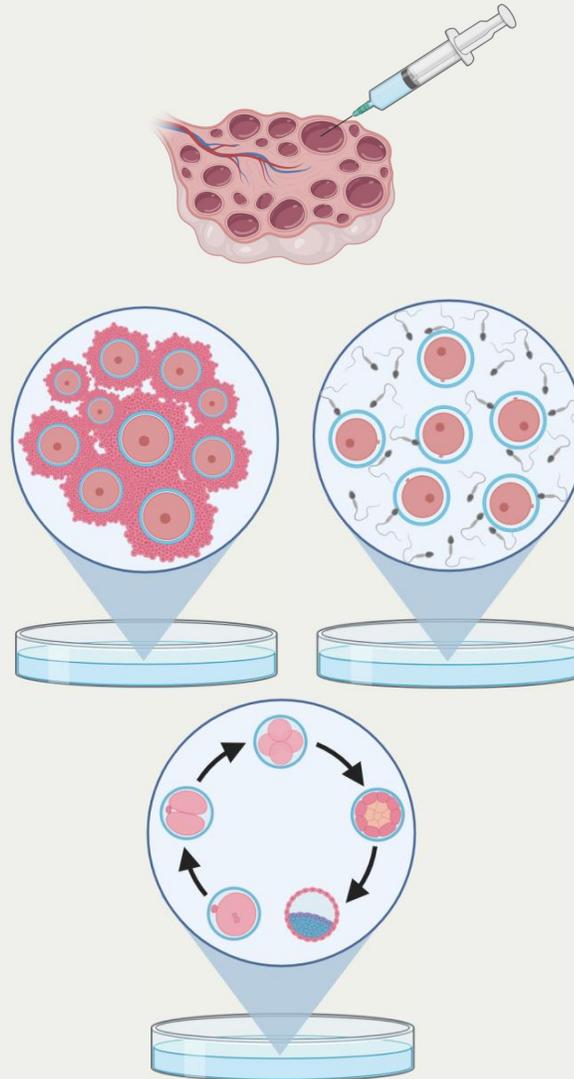


Godakumara, K., et al, 2022

C.A.S.A system and flow cytometry

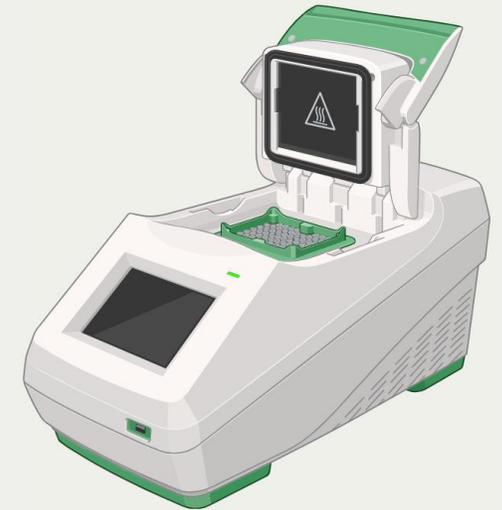


Reproductive Biotechnologies



Molecular analysis

RT-PCR





Seasonal effects on *in vitro* oocyte maturation and embryo development in Holstein dairy cattle in northern Italy

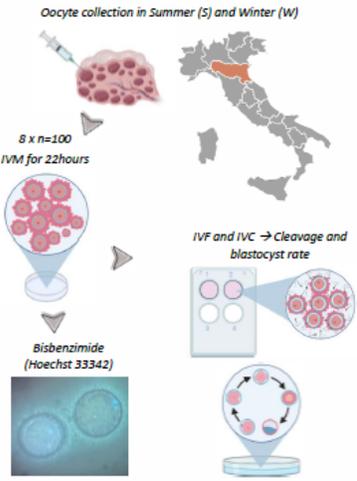
Laura Tovar-Pascual, Eleonora Iacono, Diego Bucci, Ludovica Mammi, Barbara Merlo
Department of Veterinary Medical Sciences, Alma Mater Studiorum-University of Bologna, Bologna, Italy



Background
Dairy cattle are particularly vulnerable to heat stress, which negatively affects their reproductive performance, resulting in substantial economic losses.

Objective
To study the influence of natural seasonal thermal variation, regardless of any potential mitigation strategies, on *in vitro* nuclear maturation and oocyte developmental competence in culled Holstein dairy cows in the Emilia-Romagna region (northern Italy, latitude 44.5°N).

Materials and Methods



Results

Group	Total Hoechst Oocytes	MII (%)	Inmature (%)	Degenerate (%)
Summer	70	40 (57.1±22.9) ^a	17 (22.8±26.0) ^a	13 (20.1±12.7) ^a
Winter	103	86 (79.5±14.1) ^b	13 (15.6±10.8) ^b	4 (4.8±7.0) ^b

Table 1. Data are presented as total number and % are described as Mean±SD. Significant seasonal differences were observed across all evaluated parameters (p<0.05). Different letters indicate significant differences between groups.

Group	Total IVC Oocytes	Blastocyst d7/Tot (%)	Blastocyst d8/Tot (%)
Summer	491	10 (2.3±2.3) ^a	24 (5.5±4.2) ^a
Winter	587	55 (8.9±6.7) ^b	65 (11.3±6.4) ^b

Table 2. Data are presented as total number and % are described as Mean ± SD. Different letters indicate significant differences between groups.

Contributing Factors

- Holstein cows have elevated metabolic requirements, which may increase their vulnerability to heat-induced reproductive challenges.
- Many of the animals used for oocyte retrieval may have already experienced reproductive impairments, as they were selected for culling based on compromised fertility or health status.

Key Findings

- Summer-associated heat stress impairs reproductive potential at the oocyte level in Holstein dairy cattle in northern Italy.
- Delayed nuclear maturation during the early stages of IVF may reflect the reduced developmental competence, particularly in summer.

Conclusion

- Compared to similar studies in beef cattle, the impact of even moderate heat stress appears more pronounced on Holstein dairy cows.
- A more detailed morphological and molecular evaluation of embryos could clarify how heat stress alters early developmental competence.
- Monitoring oocyte quality and embryo cleavage patterns seasonally may help optimize embryo transfer success rates.

Data were analysed using a binomial Generalized Linear Model (GLM) with a logit link function; statistical significance was set at p<0.05.

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Winter	587	55 (8.9±6.7) ^b	65 (11.3±6.4) ^b

Group	Cleavage Rate (%)	Blastocyst d7/CL (%)	Blastocyst d8/CL (%)
Summer	290 (68.6±14.9) ^a	10 (3.6±3.8) ^a	24 (8.1±7.2) ^a
Winter	442 (83.3±6.9) ^b	55 (10.7±8.2) ^b	65 (13.5±8.0) ^b

Table 2. Data are presented as total number and % are described as Mean ± SD. Different letters indicate significant differences between groups.

Reproduction in Domestic Animals

OC 5.4 | Impact of egg yolk- and commercial liposome-based extenders on fresh and frozen-thawed ram sperm

L. Rombaut¹; F. Ferreira²; J. P. Barbas³; N. Carolino³; L. Gavin-Plagne⁴; L. Lopes-da-Costa⁵; R. M.L.N. Pereira³
¹Biotechnology and Genetic Resources Unit, INIAV-National Institute of Agrarian and Veterinarian Research; CIISA-Centre for Interdisciplinary Research in Animal Health, University of Lisbon; ²Biotechnology and Genetic Resources Unit, INIAV-National Institute of Agrarian and Veterinarian

Artificial Insemination (AUB-INFA), University of Bologna, Italy; ³Faculty of Veterinary Medicine – Reproductive Biology Unit, Ghent University, Belgium

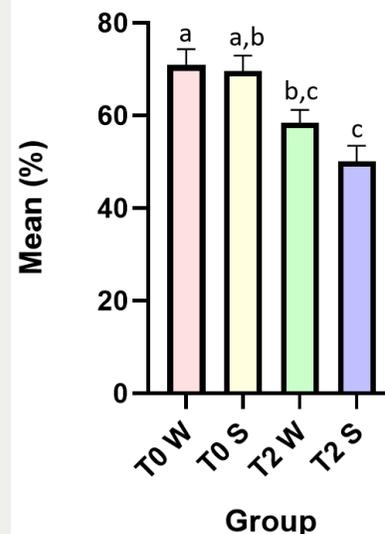
Heat stress in cattle has an increasing influence on fertility in both males and females with climate change contributing. Altered testicular thermoregulation may affect spermatogenesis, with a consequent decrease in sperm quality potential. This study examines the seasonal impact on sperm cryopreservation of Holstein bulls in the Netherlands. A total of 24 ejaculates



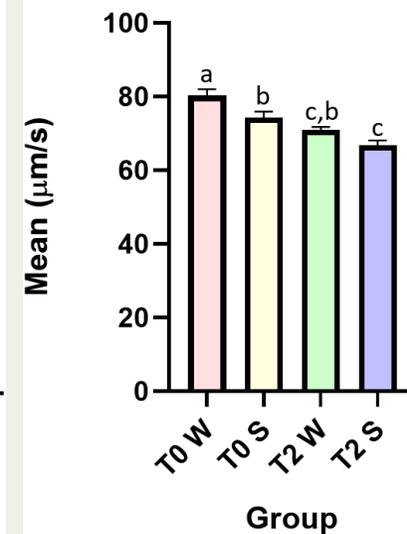
L. Tovar Pascual¹; J. Ortiz-Rodriguez²; S. Dindo³; D. Buccì³; B. Mislei²; G. Mari²; G. Rizzoto³
¹Department of Veterinary Medical Sciences, Alma Mater Studiorum-University of Bologna, Italy; ²National Institute of

after combination with different stations were also assessed. To control for repeated observations of the recipient mare, five random datasets were created and a random effects binary logistic regression model was applied to model the pregnancy outcome.

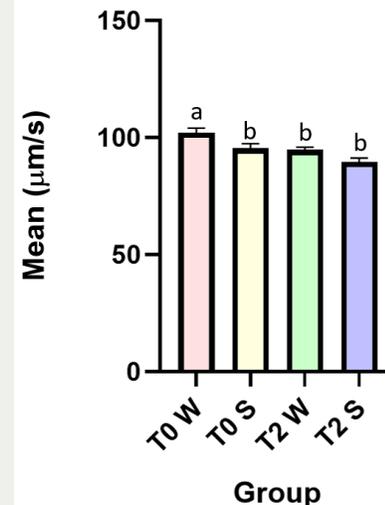
Total Motility



Straight-Line Velocity (VSL)



Average Path Velocity (VAP)



Different letters indicate significant differences between groups p-value < 0.05

OC 5.3 | Polystyrene nanoplastics impair stallion sperm function

S. Dindo¹; L. Tovar-Pascual¹; G. Mari²; M. Spinaci¹;
J. Ortiz-Rodriguez¹

¹Department of Veterinary Medical Sciences, Alma Mater Studiorum-University of Bologna; ²National Institute of Artificial Insemination (AUB-INFA), University of Bologna, Italy

The worldwide diffusion of nanoplastics (NP) has become a major threat to environmental health. The accumulation of these particles in mammalian tissues potentially triggers a range of biological effects, including oxidative stress and inflammation. Given that NP have been isolated in reproductive organs such as testicles, and research focusing on the consequences of NP on animal reproductive performance is limited, this study explores the impact of 30 nm polystyrene NP on stallion mature spermatozoa. A total of 15 frozen-thawed ejaculates from five stallions were divided into three samples either without (CTR) or with different concentrations of NP (50 µg/mL and 200 µg/mL) and incubated at 38°C. Sperm viability, mitochondrial activity and reactive oxygen species (ROS) production were evaluated by flow cytometry before treatment (T0) and after 3 h of incubation (T3). Statistical analysis was performed using Kruskal-Wallis one-way analysis on ranks. Results are presented as median and interquartile range. A significant decrease in sperm viability was observed in the 200 µg/mL NP sample compared with CTR at T3 (25.6% (22.1–30.8) vs. 45.8% (41.4–49.5), respectively, $p < 0.05$). Moreover, the 200 µg/mL NP induced a reduction in the percentage of sperm with high mitochondrial membrane potential after 3 h compared with CTR at T0 (24.0% (12.5–31) vs. 48.7% (31.6–61.5), respectively, $p < 0.05$) in association with a decrease in hydrogen peroxide (H₂O₂) concentration (median fluorescence intensity (MFI): $12.3 (6.4–15.2) \times 10^3$ vs. $13.9 (8.2–21.8) \times 10^3$, $p < 0.05$). Our results suggest that NP negatively affect stallion sperm mitochondrial activity, potentially impairing sperm survival. These findings confirm the emerging concerns in animal reproduction linked to plastic pollution.

Internalization of Nanoplastics in equine spermatozoa

Jose Manuel Ortiz-Rodriguez¹, Sofia Dindo¹, Vito Antonio Baldassarro¹, Laura Tovar-Pascual¹, Beatrice Mislis^{1,2}, Diego Bucci¹, Marcella Spinaci¹

¹Department of Veterinary Medical Sciences, Alma Mater Studiorum-University of Bologna, Bologna, Italy
²National Institute of Artificial Insemination (AUB-INFA), University of Bologna, Bologna, Italy

Introduction

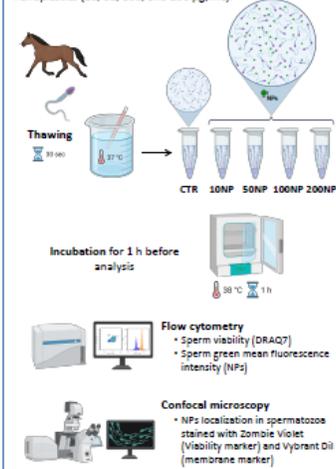
Plastic pollution is a growing global concern due to its potential effects on both human and animal health. The disaggregation of large plastic fragments into microplastics (MPs) and nanoplastics (NPs) facilitates their widespread dissemination in the environment. These particles can be internalized by organisms and subsequently accumulate in biological tissues, where they may induce toxicological effects such as inflammation and oxidative stress. While the mechanisms underlying the interaction between plastic particles and cells remain uncertain, recent human studies point to a potential association between increased subfertility and the exposure of gametes to NPs.

OBJECTIVE

The aim of this study was to evaluate the *in vitro* internalization of nanoplastics (NPs) in mature equine spermatozoa

Methodology

Five frozen-thawed ejaculates from different stallions were divided into five aliquots and incubated either without (CTR) or with different concentrations of 30 nm green-fluorescent nanoplastics (10, 50, 100, and 200 µg/mL).

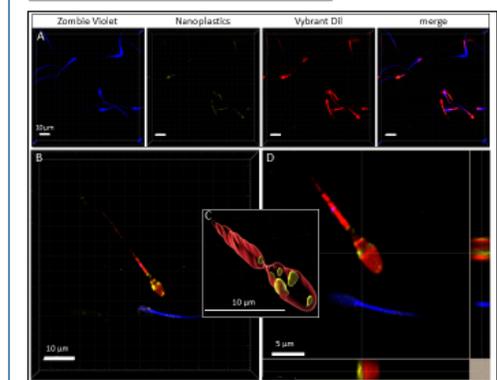
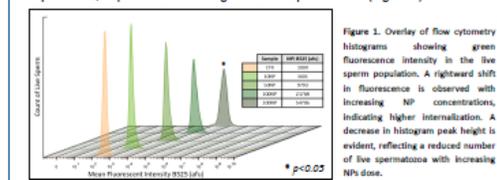


CONCLUSION

Sperm internalize NPs in a dose-dependent manner, with preferential accumulation in the post-acrosomal and midpiece regions. This distribution could suggest potential interference with mitochondrial function and fertility. Further studies, including ongoing work by our group, aim to clarify these effects.

Results

A significant, dose-dependent increase in green mean fluorescence intensity (MFI) was detected in live sperm by flow cytometry (Figure 1), indicating greater NP internalization. Sperm viability showed a decreasing trend with higher NP uptake. Confocal microscopy further revealed a preferential accumulation of NPs in the midpiece and/or post-acrosomal regions of the spermatozoa (Figure 2).



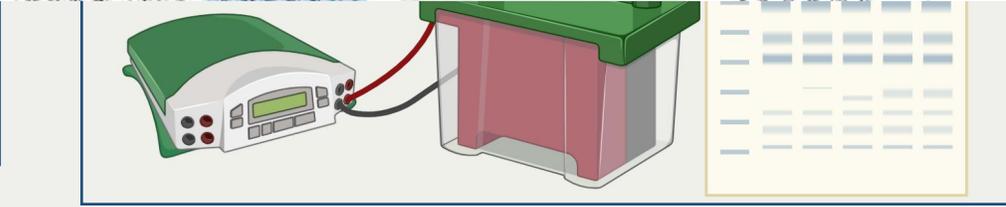
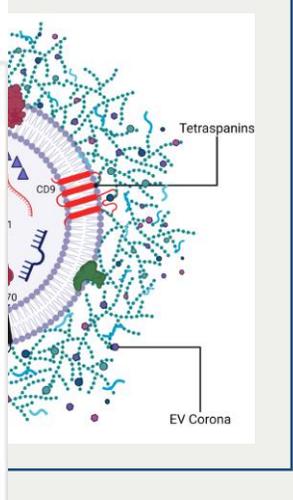
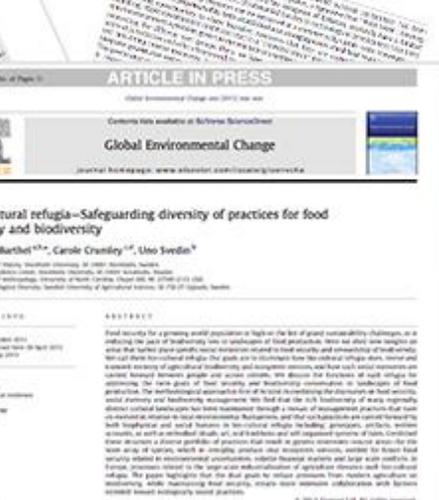
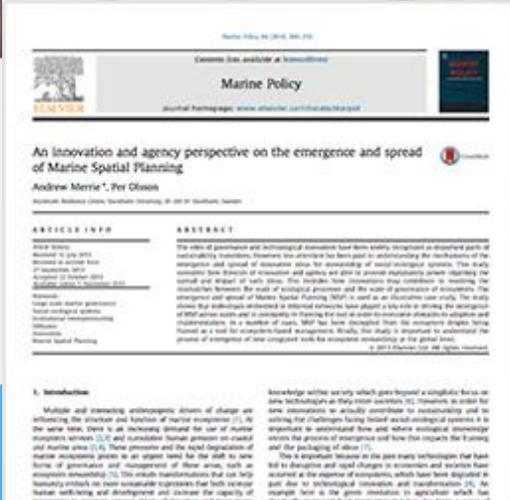
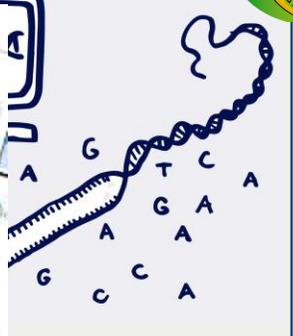


Characterisation of the biological materials



Data processing

Period abroad



Nutritional and Reproductive Strategies to cope with Heat Stress on Dairy Cows

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