



ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

DIPARTIMENTO
DI SCIENZE MEDICHE
VETERINARIE

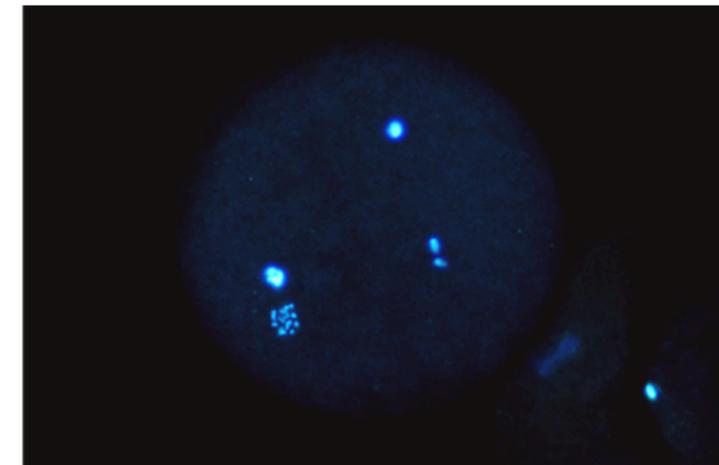
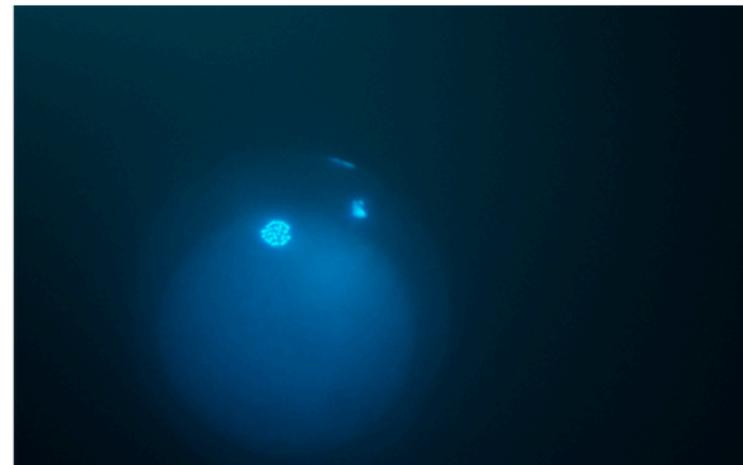
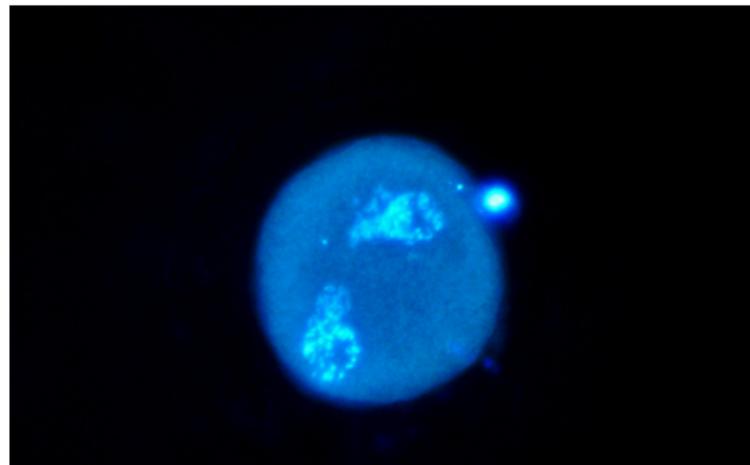
Assisted reproduction and regenerative medicine: a possible partnership for improving fertility

Emilia Attolini

40° Cycle (A.A. 24-25)

Supervisor: Prof.ssa Merlo

Co-Supervisors: Prof.ssa Giardino, Prof. Bucci, Prof. Nesci



Mesenchymal stromal/stem cells conditioned medium (MSC-CM)

Conditioned medium is the medium where the stem cells are cultured

It contains:

- Growth factors: VEGF, HGF, IGF, FGF
- Cytokines: IL-6, IL-10, TGF- β
- Extracellular vesicles / exosomes
- Antioxidants and metabolites

Beneficial factors:

- Stimulates cell proliferation and differentiation
- Promotes tissue regeneration
- Reduces oxidative stress and inflammation
- Enhances cell survival and development

IVEP:

- Mimics natural maternal microenvironment
- Improves blastocyst quality and development
- Increases viability and implantation potential

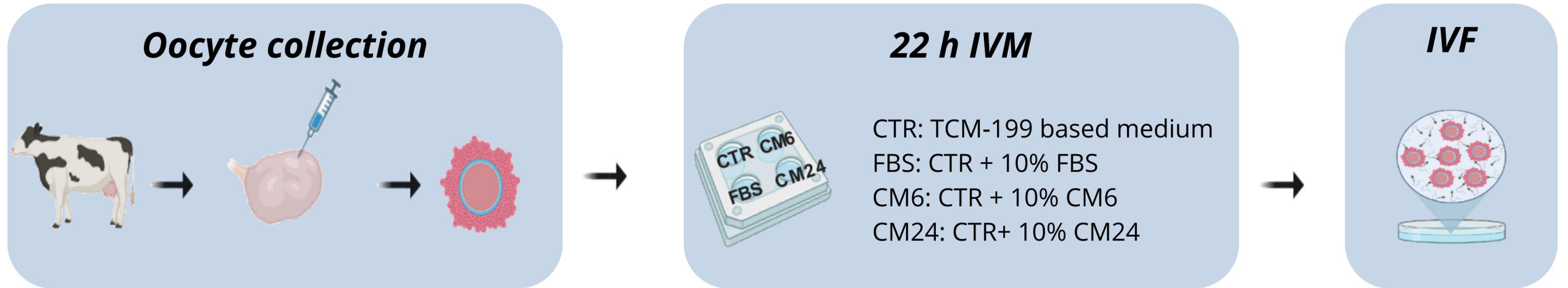
1) Effects of WJ-MSC-CM on bovine IVM

AIM:

To assess the effect of conditioned medium derived from equine Wharton's jelly mesenchymal stromal/ stem cells (WJ-MSC-CM) on

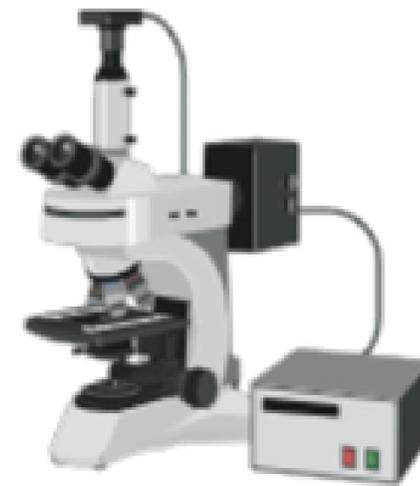
- bovine oocyte nuclear and cytoplasmic maturation
 - embryo production
- compare it with that of FBS

Assessment of nuclear and cytoplasmic maturation of bovine oocytes



Nuclear maturation:

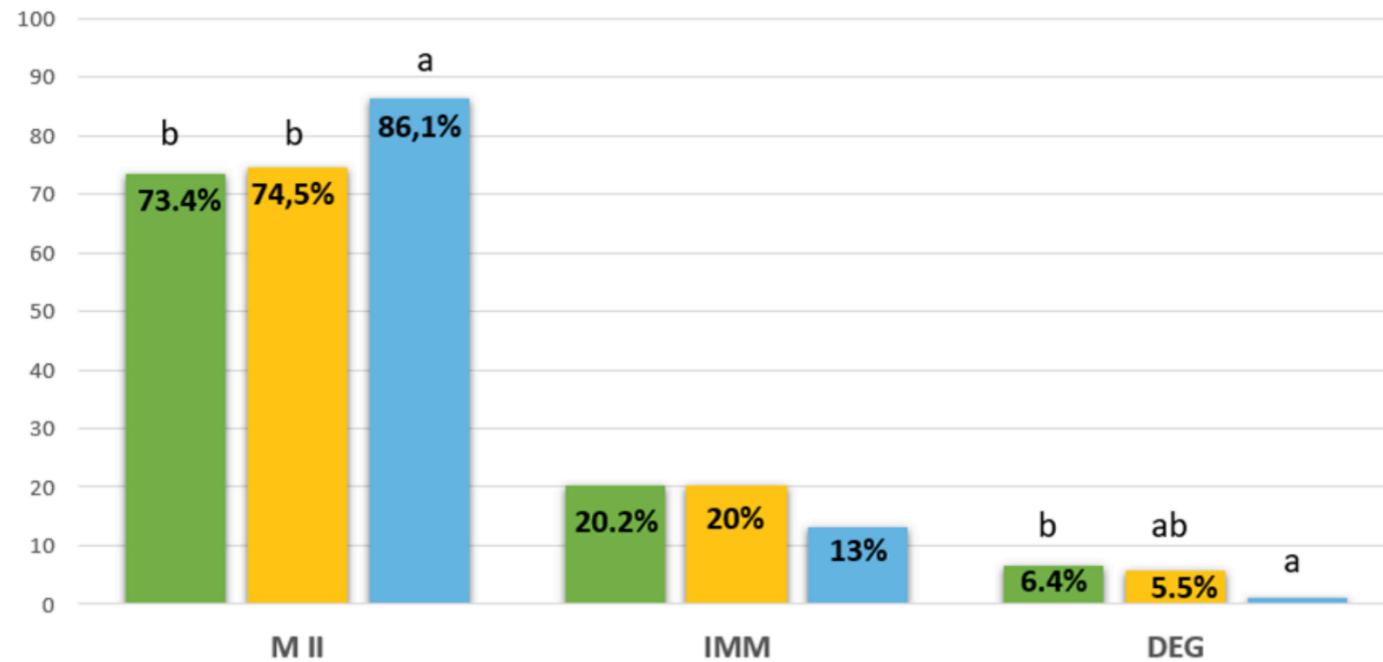
- Mature
- Immature
- Degenerate



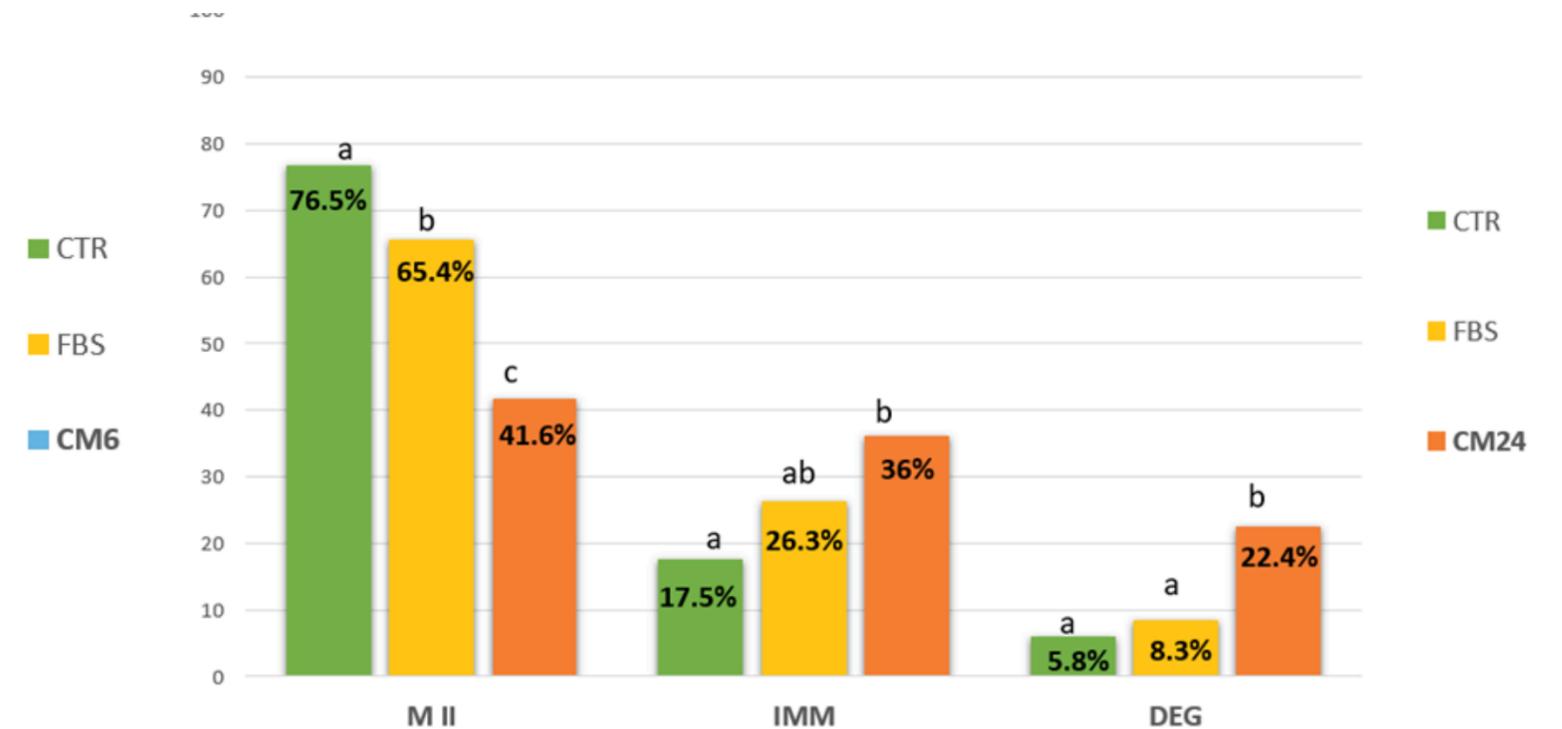
Cytoplasmic maturation:

- Penetration rate
- Monospermy rate
- Fertilization efficiency
- Pronuclei (PN) formation

RESULTS: nuclear maturation

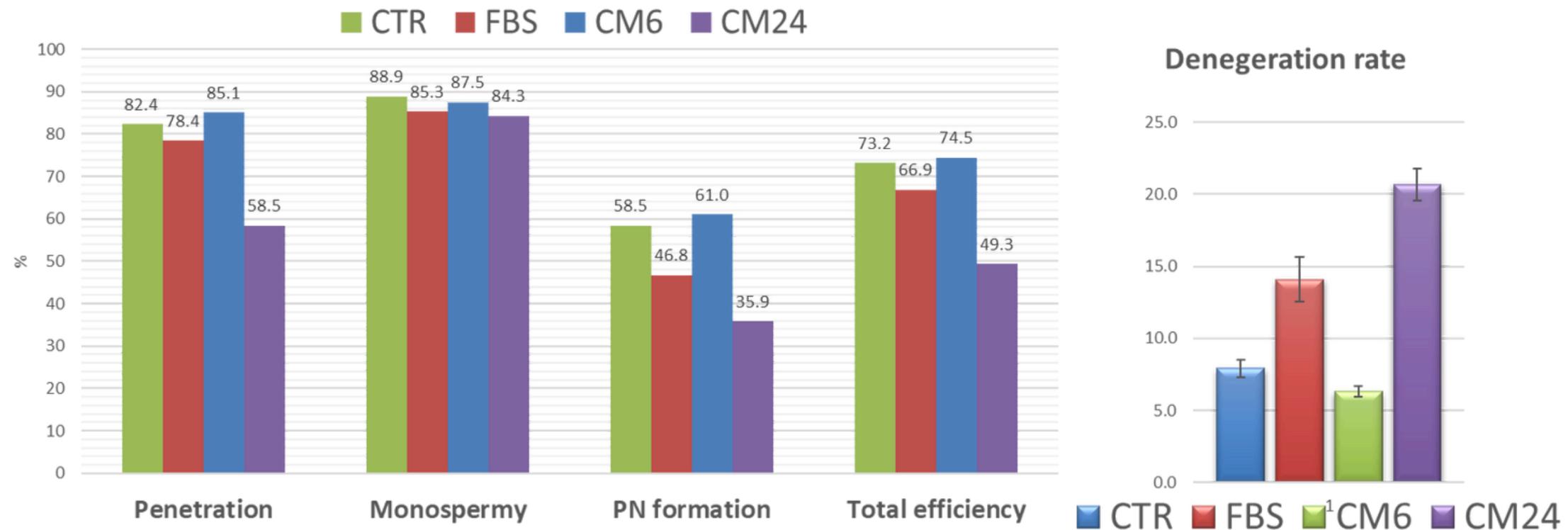


- The CM6 group exhibited the highest maturation rate
- The degeneration rate was significantly lower in CM6 compared to CTR and FBS



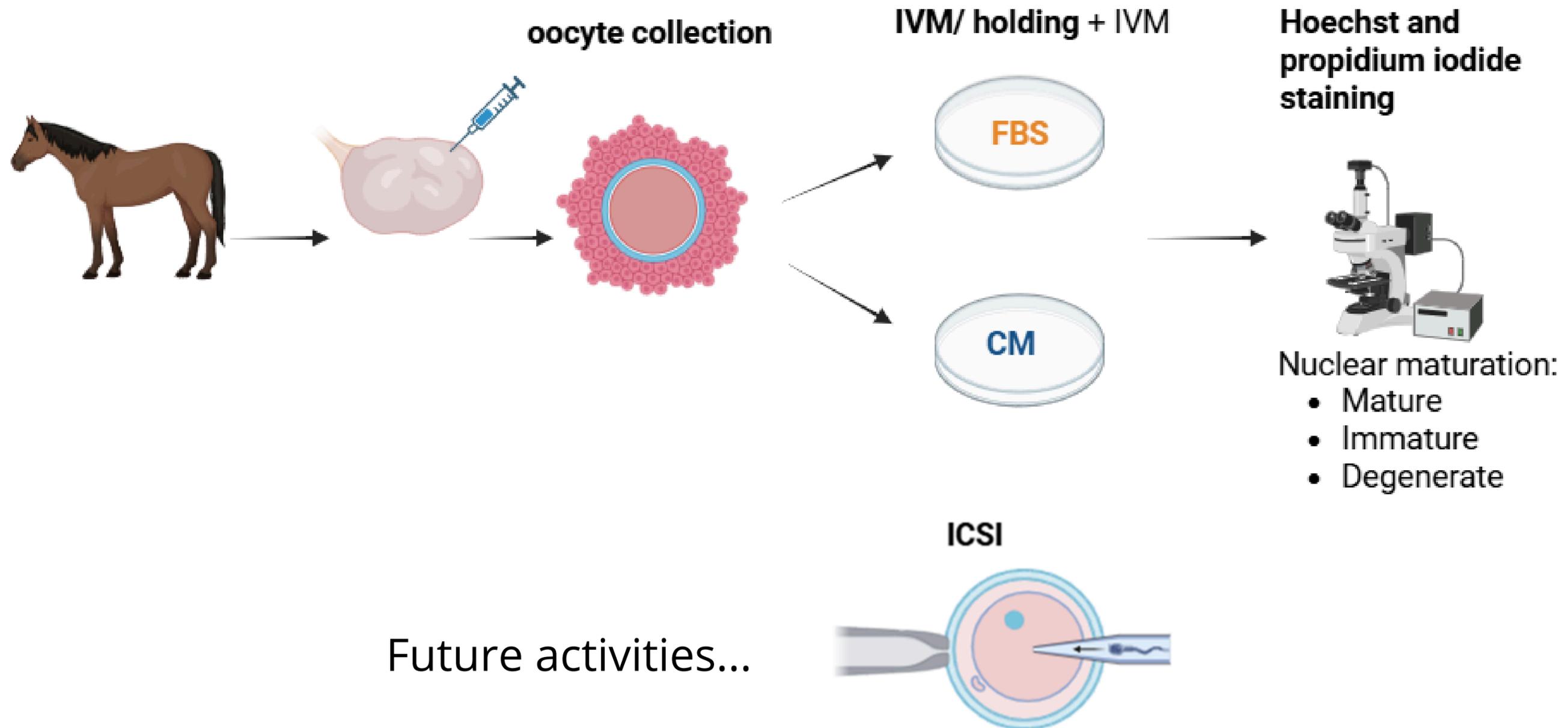
- The CTR had the highest maturation rate
- The proportion of immature oocytes was lower in CTR than in CM24
- Degeneration was significantly higher in CM24

RESULTS: cytoplasmic maturation

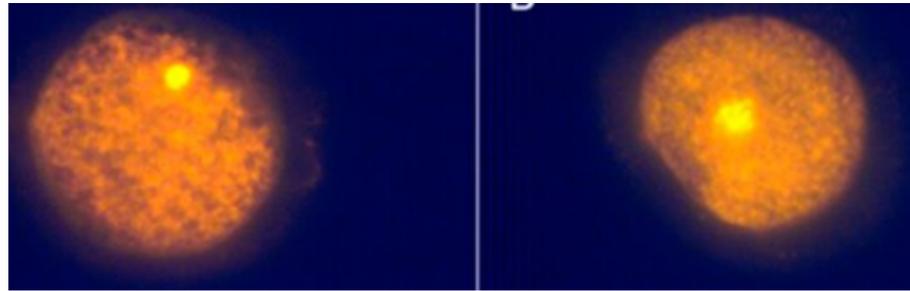


- CM6 showed a lower degeneration rate compared to FBS and CM24
- Penetration rate and fertilization efficiency were lower in CM24 compared to CTR, FBS and CM6
- PN formation was significantly higher in CTR and CM6

2) Effects of WJ-MSC-CM on equine IVM



Preliminary results



	20h IVM	26h IVM	holding + 20h IVM	holding + 26h IVM
FBS	15,1% MII 50,1% IMM 34% DEG	32,7% MII 36,5% IMM 30,8% DEG	21% MII 30,6% IMM 48,4% DEG	18,5% MII 46,3% IMM 35,2% DEG
CM	25% MII 51,8% IMM 23,1% DEG	45,7% MII 26% IMM 28,3% DEG	20,3% MII 31,9% IMM 47,8% DEG	22,2% MII 41,3% IMM 36,5% DEG

AIM:

Standardize a protocol for analysing bovine oocytes metabolism before and after maturation using the Seahorse Analyzer to better characterize oocyte bioenergetics.

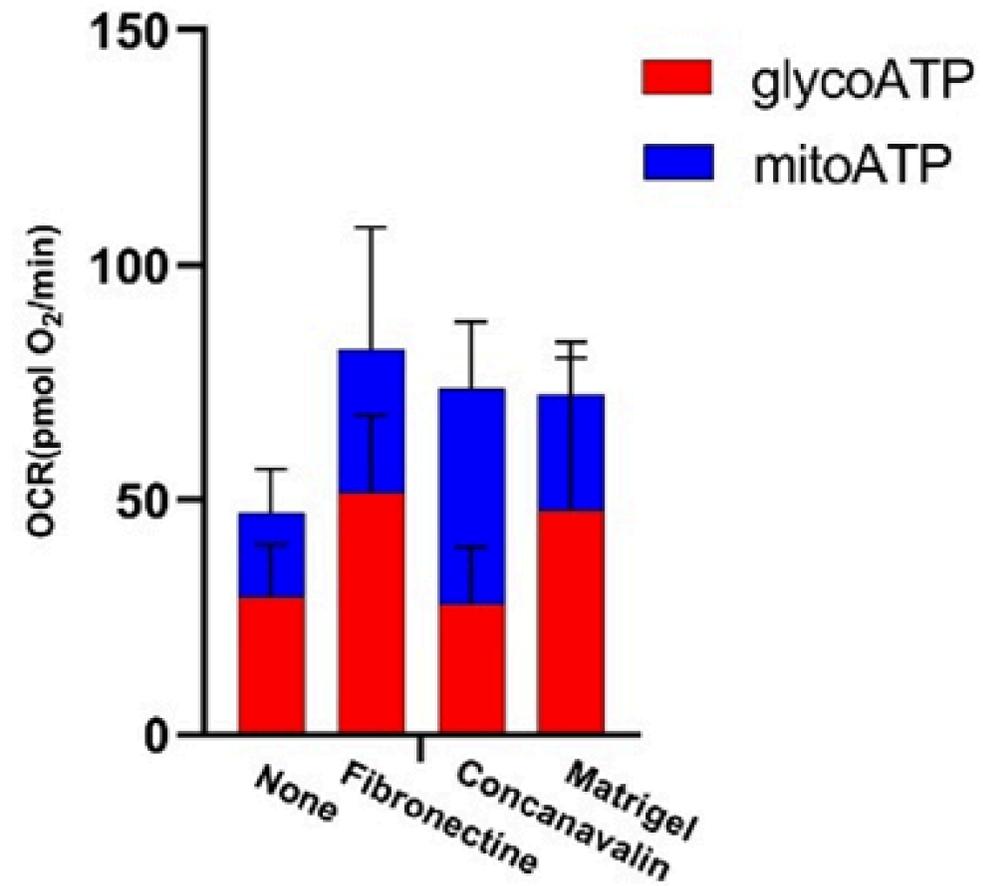
Experimental design

- Groups of five COCs before maturation and after 22 hours of IVM
 - Three different media: TCM 199, DMEM/F12 and HSOF
- Uncoated wells or wells coated with one of three different materials: fibronectin, concanavalin A or Matrigel®

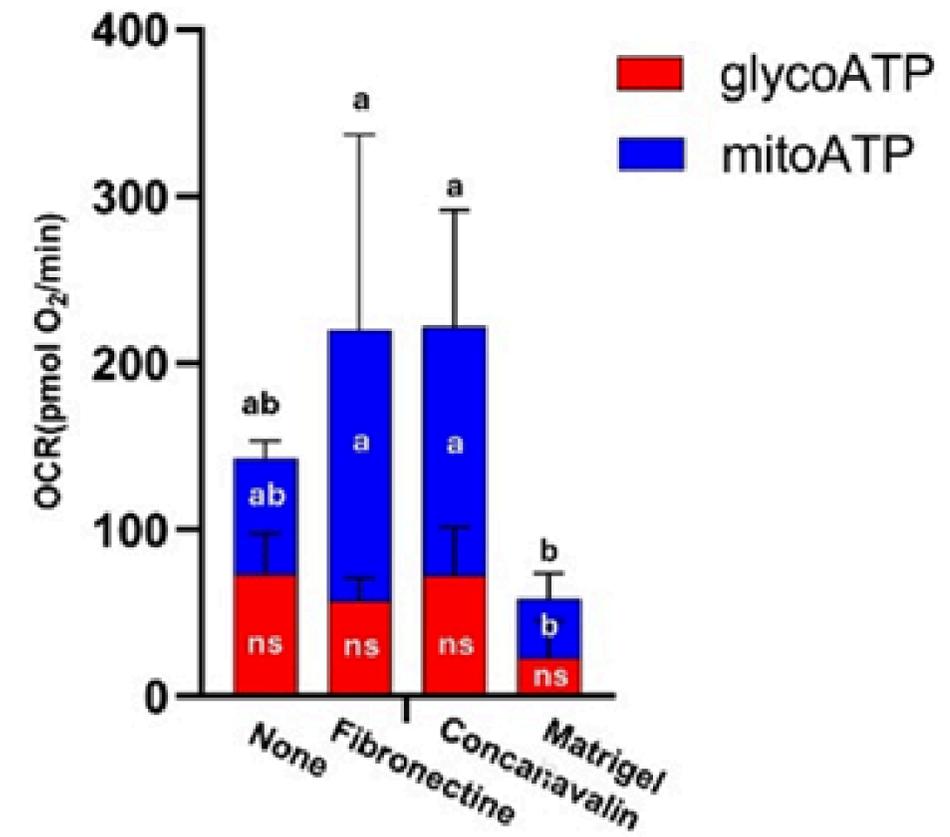


RESULTS

Immature COCs rely on glycolysis



Mature COCs: A shift from glycolytic to oxidative metabolism occurs during bovine COC IVM



Other activities

1. Effects of polystyrene nanoparticles on bovine oocyte in vitro maturation

2. Effect of heat stress on bovine holstein oocytes

3. Effect of heat stress on ovarian dynamics in Holstein lactating cows



4. Clinical activity at INFA and experimental dairy farm

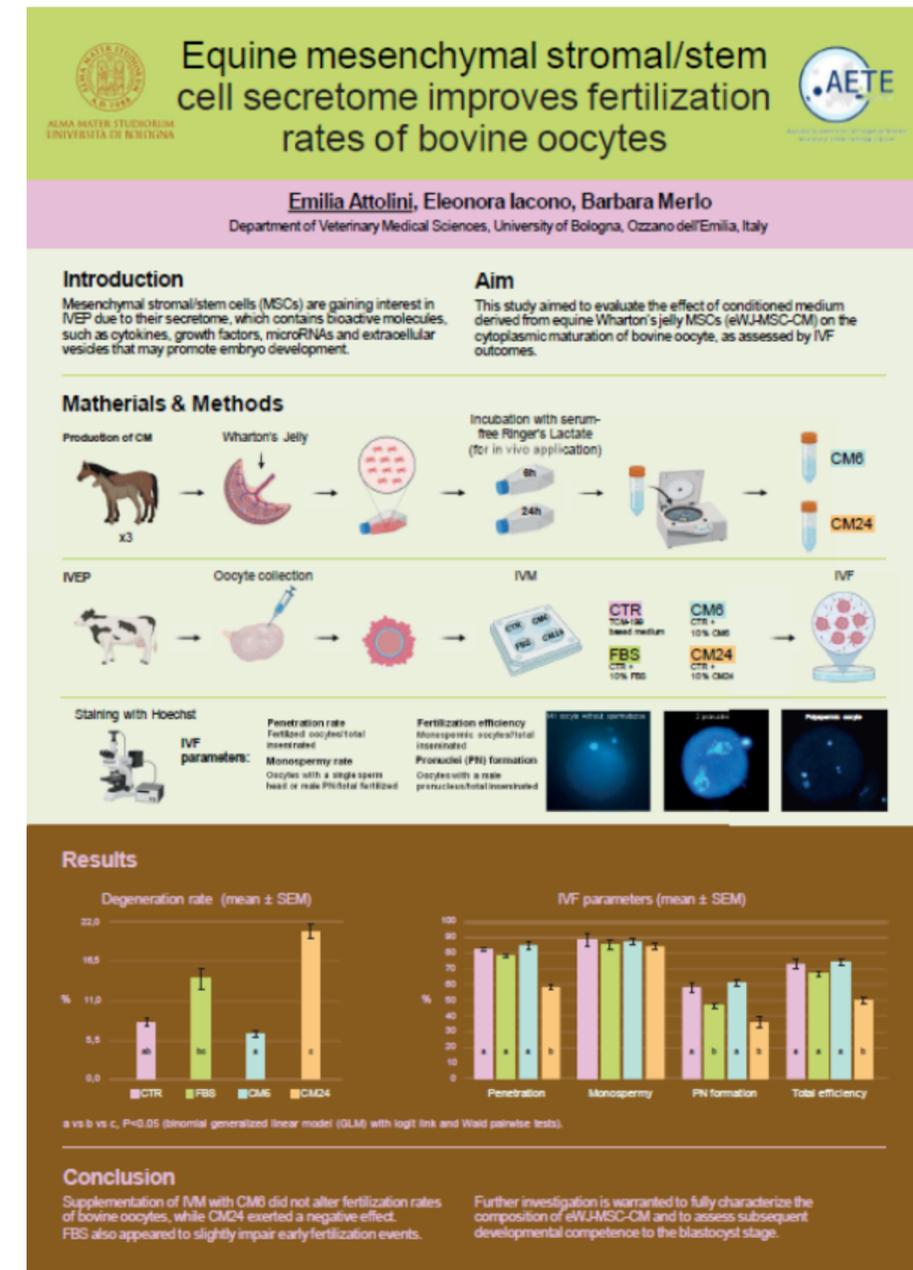
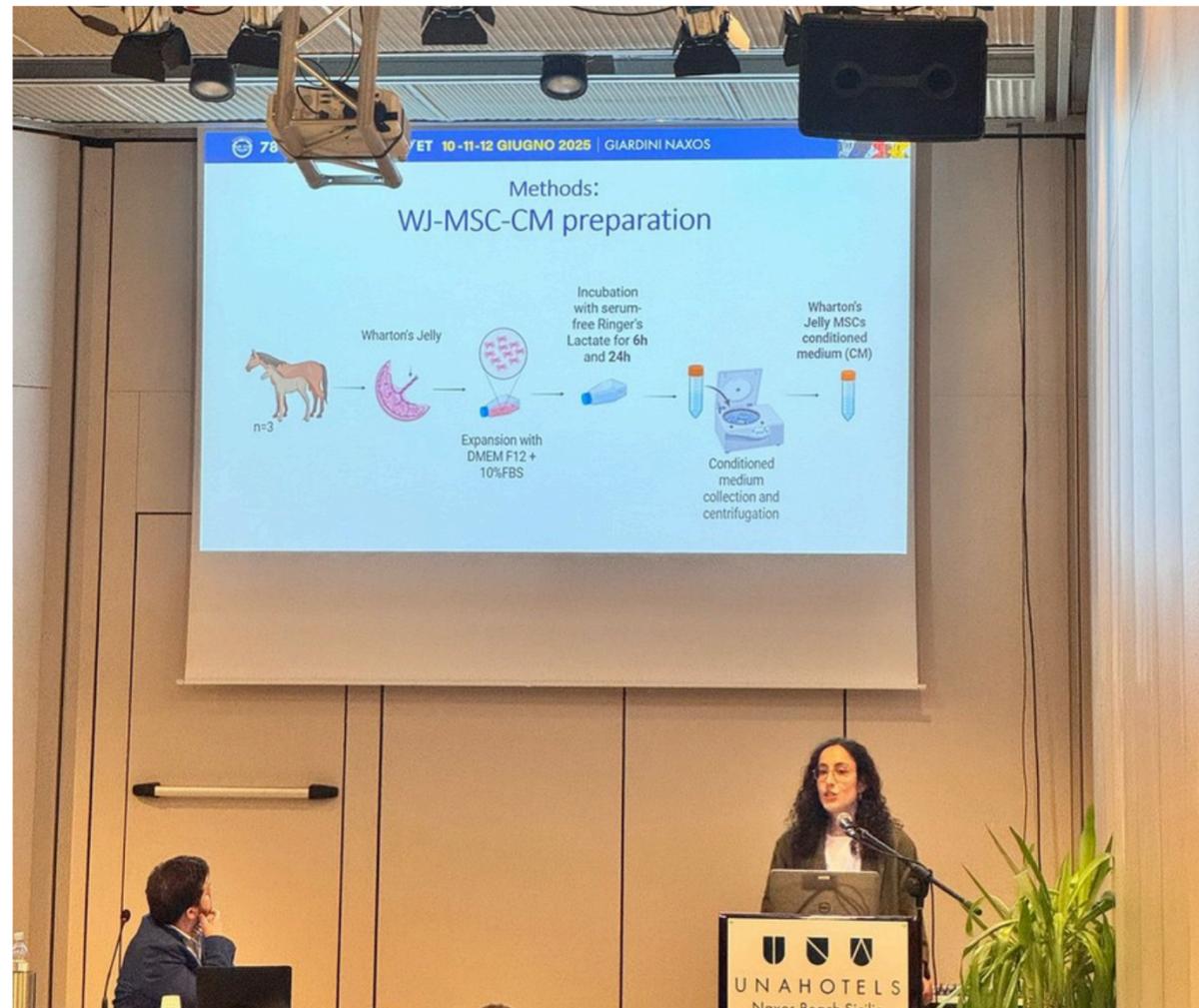


5. Non-surgical embryo recovery in Lacaune ewes



Congresses

- 1.30° Congress SIVE, Bologna, February 2025
- 2.78° Congress SISVET, Giardini Naxos, June 2025
- 3.41st AETE Annual Meeting in Cork, Ireland



Publications



Article

Post-Insemination Infusion of Wharton's Jelly Mesenchymal Stromal/Stem Cells-Derived Conditioned Medium: A Novel Approach for Improving Pregnancy Outcomes in Problem Mares

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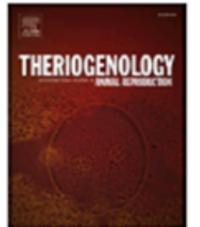
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Original Research Article

Effects of polystyrene nanoparticles on bovine oocyte in vitro maturation

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In progress:

1. "Comparative analysis of metabolism in mature and immature bovine cumulus-oocyte complexes and standardization of bioenergetic analysis." Cristina Algieri, Emilia Attolini, Eleonora Iacono, Salvatore Nesci, Barbara Merlo.
2. "Assessment of a cervical relaxation protocol in Lacaune ewes and cervical passage efficiency by first-time trained veterinarians in non-surgical embryo recovery." Beatrice Mislei, Giulia Ballotta, Emilia Attolini, Barbara Merlo, Eleonora Iacono, Gaetano Mari, Jeferson Ferreira da Fonseca

Future activities

- Further testing of the CM in bovine and equine IVM, up to the blastocysts stage
- Testing the CM from adult MSCs
- Use of the secretome in the cryopreservation of oocytes and embryos
- Analysis of the conditioned medium in order to characterize it
- Use of the Seahorse analyzer on oocytes matured under different conditions, including CM, and subsequently on embryos
- Analysis of the data collected and drafting of related scientific publications



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THANKS FOR YOUR ATTENTION

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