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INVITATION PUBLIC DEFENSE

Interplay between subclinical mare health conditions and oocyte competence

Mohamed Hedia

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PROMOTERS

Prof. dr. Katrien Smits
Faculty of Veterinary Medicine, UGent

Prof. dr. Ann Van Soom
Faculty of Veterinary Medicine, UGent

Prof. dr. Jo Leroy
Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, UAntwerpen

Curriculum Vitae

Mohamed Hedia, born on August 22nd, 1992, in Cairo, Egypt, is a veterinarian and researcher specializing in reproductive biology and embryology. He earned his Bachelor of Veterinary Sciences from Cairo University in July 2014. Recognizing his academic excellence, he was appointed as a teaching assistant in the Department of Theriogenology. Over five years in this role, he gained teaching and mentoring experience while developing his research interests in reproductive science.

In December 2018, he obtained his Master's degree in Animal Reproduction from Cairo University, focusing his research on the effects of heat stress on male reproduction in rams. Driven by his passion for advanced research, Mohamed applied in December 2019 for a highly competitive Egyptian governmental scholarship to pursue his doctoral studies abroad. In September 2021 he joined the Reproductive Biology Unit, Ghent University and the Gamete Research Centre, University of Antwerp as a joint PhD candidate. His research investigated why some healthy mares fail to consistently produce embryos through OPU-ICSI. He studied if hidden health issues in mares, like oxidative stress, inflammation, and endotoxemia, affect oocyte quality and reduce success rates.

Alongside his PhD research, Mohamed has been actively engaged in academic supervision at Ghent University. He has supervised two master's students, guiding them in project design and thesis preparation. He has also contributed for over three years to the laboratory work of the clinical OPU-ICSI program at the Faculty of Veterinary Medicine, Ghent University. Additionally, Mohamed serves as a reviewer for peer-reviewed scientific journals in his field.

Mohamed has authored 24 peer-reviewed publications, contributed to eight conference proceedings, and delivered four talks at international conferences. He also participates in international training activities, including serving as a teaching staff member for the annual "Equine OPU-ICSI" and "Sex in a Dish" workshops at Ghent University.

Where?

The defense will take place on **August 25, 2025 at 17.00h**

Auditorium A

Faculty of Veterinary Medicine
Ghent University, Campus Merelbeke
Salisburylaan 133, Merelbeke

How to attend?

The public defense is open to everyone and does not require registration.

If you would like to attend the **reception**, please register by **August 22nd** via email: Mohamed.Hedia@UGent.be

The defense can also be attended via live stream. To receive a personal invitation., please send an email to Mohamed.Hedia@UGent.be

Members of the Jury

Prof. dr. Myriam Hesta
Chairman of the Jury
Faculty of Veterinary Medicine, UGent

Dr. Pascale Chavatte-Palmer
Research director at the University of Paris-Saclay, UVSQ, INRAE, and the National Veterinary School of Alfort, BREED Research Unit, France

Dr. Ilse Goovaerts
Senior clinical embryologist and quality manager at the Center for Reproductive Medicine, University Hospital of Antwerp, Belgium

Prof. dr. Geert Opsomer
Faculty of Veterinary Medicine, UGent

Dr. Jan Govaere
Faculty of Veterinary Medicine, UGent

Summary

Although ovum pick-up (OPU) followed by intracytoplasmic sperm injection (ICSI) is a well-established technique for the laboratory production of equine embryos nowadays, fluctuations in its outcomes remain a challenge, particularly in clinically healthy mares. Lipid metabolism, inflammation, oxidative stress, and endotoxemia are key factors influencing fertility in mares. Research in other species has shown that disruptions in lipid metabolism, particularly elevated free fatty acids in follicular fluid, negatively impact oocyte quality and embryo development. Chronic inflammation, often linked to health conditions, impairs ovarian function and oocyte competence. Oxidative stress, caused by an imbalance of reactive oxygen species and antioxidants, disrupts reproductive processes such as folliculogenesis and oocyte maturation. Endotoxemia, resulting from infections or leaky gut, alters the follicular microenvironment, further compromising oocyte quality. These factors are closely linked to maternal health and influence the follicular environment and oocyte competence. While these factors have been well-documented in both animals and humans, their specific impact on OPU-ICSI results in mares remains underexplored.

Here, we hypothesized that oxidative stress, inflammation, and lipid metabolism disturbances affect the follicular environment and oocyte quality in donor mares. Specifically, we propose that lipid metabolites, inflammatory markers, and oxidative stress indicators in follicular fluid correlate with their serum levels and influence oocyte developmental competence in otherwise healthy mares. Additionally, lipopolysaccharides in the follicular fluid may impair oocyte development during in vitro maturation. The aim is to investigate these factors step by step in the following chapters.

A first study (Chapter 3) investigates how systemic lipid metabolism, inflammation, and oxidative stress are reflected in the follicular environment of mares. Serum and follicular fluid samples were collected from slaughterhouse mares, measuring concentrations of total cholesterol, triglycerides, non-esterified fatty acids, interleukin-6, and oxidative stress indicators in follicles of different sizes. The results showed strong correlations between serum and follicular fluid levels of non-esterified fatty acids and interleukin-6. Moderate correlations were also observed for cholesterol and oxidative stress index, particularly in medium-sized follicles. These findings suggest that systemic inflammatory and oxidative stress conditions in donor mares are reflected in the follicular environment and may affect oocyte quality and developmental competence.

In a second study (Chapter 4), we collected blood samples during OPU in mares to measure serum concentrations of interleukin-6, reactive oxygen metabolites, biological antioxidant potential, and the oxidative stress index. These systemic markers were then correlated with oocyte and embryo development outcomes following intracytoplasmic sperm injection. The results showed that none of these systemic markers were associated with embryo formation. However, higher levels of reactive oxygen metabolites were linked to delayed embryo development. These findings suggest that while systemic oxidative and inflammatory markers do not directly affect embryo formation, higher oxidative stress may still influence the timing of embryonic development, emphasizing the need to monitor donor mare health during assisted reproduction.

Next, the presence of lipopolysaccharide (LPS) in the follicular fluid of clinically healthy slaughterhouse mares was investigated (Chapter 5), highlighting endotoxemia as an emerging factor in maternal health. The potential impact of lipopolysaccharide on oocyte developmental competence was explored in two experiments. In the first, follicular fluid was collected from large follicles to measure lipopolysaccharide, estradiol, progesterone, tumor necrosis factor-α, and interleukin-6 levels. Lipopolysaccharide concentrations were negatively correlated with progesterone, tumor necrosis factor-α, and interleukin-6, suggesting a potential immunosuppressive effect within the follicular environment. In the second experiment, cumulus-oocyte complexes were exposed to lipopolysaccharide during in vitro maturation before fertilization via intracytoplasmic sperm injection. While no significant differences were observed in maturation, cleavage, or blastocyst rates between the lipopolysaccharide-treated and control groups, the findings raise concerns about potential subclinical effects. These results highlight the presence of detectable lipopolysaccharide in equine follicular fluid and its association with altered cytokine and steroid profiles, adding endotoxemia to the list of maternal health factors investigated in this thesis, and warranting further research to explore its implications for fertility outcomes.

Finally, we explored the effects of lipopolysaccharide exposure during in vitro maturation on the proteomic profile of single equine oocytes (Chapter 6). Despite the lack of significant disruptions in global protein expression, the study revealed subtle changes in pathways related to cytosolic components, carbon metabolism, and protein folding in equine oocytes. Besides, this first report on the single-oocyte level, with over 4488 protein identifications, sheds light on the novel proteomic features of mature equine oocytes that can potentially highlight the molecular mechanisms underlying oocyte competence and embryo development in horses.

In Chapter 7, the key findings and their implications for equine reproductive health are discussed. The research presented highlights important aspects of follicular environment regulation, particularly in relation to lipid metabolism, inflammation, and endotoxemia, and how these factors influence oocyte quality and developmental competence. The implications for OPU-ICSI programs should be a double-faced intervention plan, either in the form of a peri-OPU supportive diet for donor mares or in the form of targeted supplements that ensures optimal in vitro conditions for oocytes during maturation and early embryos during culture. Besides, study limitations and future research directions are mentioned.