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Promoters

Prof. Dr. Björn Heindryckx
G-FaST, Department for Reproductive Medicine, Department of Human Structure and Repair, Ghent University Hospital, Belgium

Dr. Frauke Vanden Meerschaut
Department for Reproductive Medicine, Ghent University Hospital, Belgium

Guidance committee

Prof. Dr. Dominic Stoop
Department for Reproductive Medicine, Ghent University Hospital, Belgium

Prof. Dr. Sofie Symoens
Center for Medical Genetics (CMGG), Department of Biomolecular Medicine, Ghent University Hospital, Belgium

Prof. Dr. Luc Leybaert
Physiology group, Department of Basic and Applied Medical Sciences, Ghent University, Belgium

Examination committee

Prof. Dr. Fransiska Malfait - Chair
Center for Medical Genetics (CMGG), Department of Biomolecular Medicine, Ghent University Hospital, Belgium

Dr. Ilse De Croo
Department for Reproductive Medicine, Ghent University Hospital, Belgium

Prof. Dr. Sandra Janssens
Center for Medical Genetics (CMGG), Department of Biomolecular Medicine, Ghent University Hospital, Belgium

Prof. Dr. Katrien Smits
Department of Internal Medicine, Reproduction and Population Medicine, Faculty of Veterinary Medicine, Ghent University, Belgium

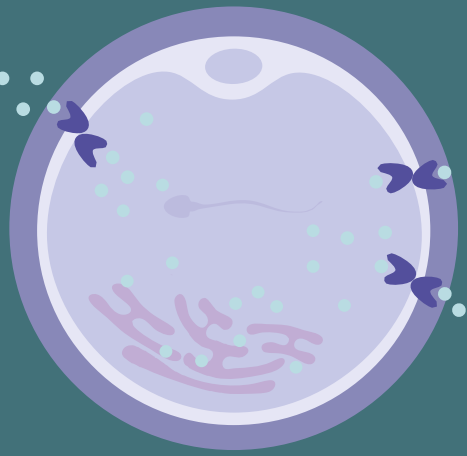
Dr. Özlem Okutman
Service de Gynécologie-Obstetrique, Clinique de Fertilité Hôpital Erasme, Université Libre de Bruxelles (ULB), Belgium

Prof. Dr. Willem Verpoest
Department of Reproductive Medicine, Utrecht University Medical Center (UCM), Utrecht University, The Netherlands

Dr. Minerva Ferrer-Buitrago
Crea Centro Médico de Fertilidad y Reproducción Asistida (CREA), Valencia, Spain

Overcoming Recurrent ICSI Failure: From Diagnosis to Treatment

Genetic Screening, Assisted Oocyte Activation Efficacy and Alternative Therapies



Arantxa Cardona Barberán

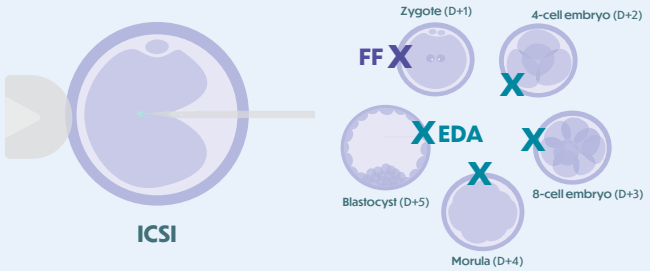
Ghent-Fertility And Stem cell Team (G-FaST)
Department for Reproductive Medicine, UZ Gent
Department of Human Structure and Repair,
Ghent University, Belgium

Public defense to obtain the degree of "Doctor in Health Sciences"
September 4, 2025

Summary

Infertility affects around 17.5% of couples worldwide.
While **intracytoplasmic sperm injection (ICSI)** has helped many couples conceive, some still face repeated failures.

ICSI failure can manifest as **low fertilization rates or complete fertilization failure (FF)**. Alternative, when normal fertilization occurs, some embryos stop developing, a condition known as **embryo developmental arrest (EDA)**. Without viable embryos, these couples have limited options to become genetic parents and depend on gamete donation or adoption.



At UZ Ghent, some **diagnostic tests** (MOAT, MOCA, HOCA) are available to identify whether the oocyte or sperm causes FF. However, these tests need special equipment, animal facilities and human oocytes, limiting their use in many IVF clinics. Recently, **genetic testing** has become a more practical and affordable strategy to diagnose the causes of ICSI failure, by identifying mutations linked to FF and EDA.

Normally, when sperm meets the oocyte, it releases a protein called PLCZ1 that triggers **calcium oscillations** that are crucial for the fertilization process. **Assisted oocyte activation (AOA)** is a treatment that uses **calcium ionophores** (calcimycin and ionomycin) to artificially raise calcium levels in the oocyte to facilitate fertilization. AOA is often used to treat FF after ICSI, but its benefit for EDA is still unclear. In addition, **clinical results after AOA vary widely** because of differences in treatment protocols and solutions used.

Another promising technique is **maternal spindle transfer (ST)**, an experimental treatment that involves transferring the genetic material from a poor-quality oocyte to a healthy donor oocyte. Recently, ST was shown to rescue recurrent IVF/ICSI failures caused by **defective oocyte cytoplasmic quality** and may help couples with female-related FF and EDA who do not benefit from AOA.

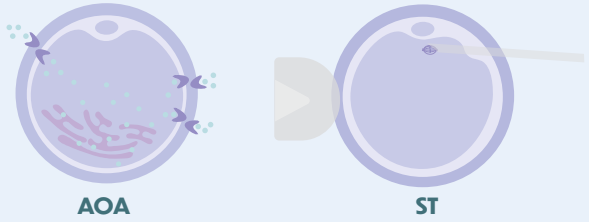
This thesis explored different aspects of the diagnosis and treatment of FF and EDA conditions. The main objectives and results obtained were the following:

1. **To perform targeted genetic screening of male and female genes linked to FF and EDA conditions.**
- In males, mutations in *PLCZ1* and *ACTL7A* were common in FF cases (29% and 15%, respectively) and were correlated with altered calcium signals during fertilization.
- In females, only a few variants of uncertain significance were found.

2. **To assess the effectiveness of AOA treatment in FF (fertilization rate <33%) and EDA (normal fertilization and blastocyst rate <15%) patients.**
- AOA helped all couples with FF, especially those with male genetic defects.
- AOA did not improve outcomes in EDA patients, as their sperm trigger normal calcium responses.

3. **To compare different calcium ionophore-based solutions in terms of activation and calcium-releasing potential.**
- A new ready-to-use ionomycin solution (RTU-I) performed similarly than our freshly prepared in-house ionomycin (IH-I), offering a standardized and quality-controlled product for AOA.
- Ready-to-use calcimycin (RTU-C) showed poor results, highlighting the need for regular batch quality testing.

4. **To investigate Patl2-related female infertility and potential treatments (AOA vs ST) in the *Patl2*$^{-/-}$ mouse model.**
- Palt2 protein is necessary for oocyte quality, as *Patl2*$^{-/-}$ oocytes showed poor embryo development after ICSI.
- Single-cell proteomics revealed abnormal cytoplasmic content. Consequently, ST, but not AOA, restored embryo development in these oocytes.



In conclusion, **AOA is recommended for FF after ICSI**, particularly in patients with calcium signaling defects (e.g., *PLCZ1* or *ACTL7A* mutations), **but not for EDA cases**. AOA treatment with ionomycin (RTU-I or IH-I) works better than with calcimycin (RTU-C).

While male-targeted genetic screening is a useful diagnostic tool, the implementation of **a whole exome sequencing gene panel is expected to enhance diagnostic yield** for both sexes.

In cases of female-related **FF or EDA where AOA fails, ST** shows promise. However, further safety validation is required, and oocyte donation remains the current recommendation.

PhD thesis

Do you want to know more?
You can request a copy of the complete thesis to:

arantxa.cardonabarberan@ugent.be

Short Curriculum Vitae

2019-2025 PhD in Health Sciences / Research Assistant
Ghent University, Belgium

2018-2019 Clinical embryologist
Clínica Eugin, Barcelona

2017-2018 MSc Cytogenetics and Reproductive Biology
Universitat Autònoma de Barcelona, Spain

2016-2017 Erasmus + mobility program/Research Internship
Wageningen University, The Netherlands

2013-2017 BSc Biotechnology
Universitat Politècnica de València, Spain

Arantxa Cardona Barberán

arantxacb10@gmail.com

GHENT UNIVERSITY · FACULTY OF MEDICINE AND HEALTH SCIENCES · G-FaST Ghent Fertility and Stem cell Team · UZ GENT