PhD PROJECT PROPOSAL

CENTURI
TURING CENTRE
FOR LIVING SYSTEMS

The mechanics of ovulation

Supervisor 1

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Supervisor 2

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Abstract (10 lines)

The origin of new life is a fundamental question in biology. During ovulation, the maternal contribution to this new life – the egg – is released from an ovarian follicle. Prior to egg release, the follicle undergoes dramatic morphological changes, culminating in the physical rupture of its wall. However, the role of mechanical forces in ovulation is unknown, representing a major gap in understanding the process and its related causes of infertility.

Ovulation is highly dynamic and occurs within the body, making it challenging to study directly. Recently, we developed a novel model system using live microscopy in isolated mouse follicles, offering a systematic and quantitative approach to study ovulation. In this project, we will extend the system for use with atomic force microscopy (AFM) and micropipette aspiration to map the mechanics of ovulation. This interdisciplinary project, combining complementary expertise in reproductive cell biology and biophysics, will provide key insights into the mechanical control of ovulation and could lead to the development of new fertility therapies.

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Keywords

Ovulation, ovary, follicle, oocyte, egg, reproduction, fertility, mechanics, force, stiffness, surface tension, atomic force microscopy (AFM), micropipette aspiration, live microscopy.

Scientific question and Objectives (10 lines)

For efficient and robust ovulation to occur, the follicle must undergo phases of expansion and contraction prior to rupture. With such large-scale morphological changes in a short period of time, it seems clear that this would reflect changes in the mechanical properties of the follicle. However, the role of mechanical forces in ovulation is unknown, representing a significant gap in our understanding of the process and its related causes of infertility. We hypothesise that the expansion and contraction phases of ovulation each correspond to modulations of the mechanical properties of the follicle which, when combined, ensure robust ovulation. Specifically, we predict that both follicle stiffness and surface tension increase during follicle expansion and that these mechanical changes are sensed by follicle cells, causing them to initiate contraction and rupture. By tracking detailed cellular movements and integrating this with direct mechanical measurements made using AFM and micropipette aspiration, we will uncover how follicle mechanics drives the process of ovulation.

Proposed approach and research plan (20 lines)

We will use AFM to measure follicle stiffness and micropipette aspiration to probe follicle surface tension during ovulation. Follicles will be cultured using the ex vivo ovulation protocol and collected at 0, 2, 4, 6, 8, 10, 12, and 14 hours after the addition of hCG. At the desired time point, follicles will be transferred either to an AFM located in the DyNaMo lab or to a confocal microscope equipped with a micropipette aspiration setup. The AFM experiments will be conducted under the supervision of Felix Rico (DyNaMo), an expert in AFM technologies, while the micropipette aspiration and confocal microscopy will be done under the supervision of Christopher Thomas (IBDM), an expert in reproductive cell biology and advanced microscopy. By taking local measurements across different follicular compartments, we will investigate whether follicle stiffness and surface tension increase during ovulation and assess their mechanistic role in linking expansion with contraction. We will combine these measurements with treatments that block follicle expansion and contraction (4-MU; hyaluronic acid synthase inhibitor and JKC-301; endothelin receptor A antagonist) as well as treatments that induce increased expansion and contraction (injection of high molecular weight dextran into the antrum of the follicle and addition of endothelin 2). Additionally, we will investigate how the cells of the follicle dynamically organise during ovulation and how this relates to follicle mechanics. We will perform an immunofluorescent screen in ovulating follicles for key adhesion and contractility proteins, and use live confocal microscopy to track the movement of individual follicular cells and quantify transitions in cell density and shape during ovulation. Together, these experiments will provide a detailed characterisation of how the dynamic organisation of individual follicular cells leads to tissue-scale mechanical changes that drive ovulation.

Interdisciplinarity and Implication of the two labs (15 lines)

This interdisciplinary project combines reproductive cell biology and advanced quantitative live microscopy with direct force measurements to investigate the mechanical control of ovulation. Christopher Thomas, a group leader at the IBDM and reproductive cell biologist, specialises in developing ex vivo strategies to reconstitute ovarian processes for advanced quantitative live imaging.

Felix Rico, an Associate Professor in the Physics Department at Aix-Marseille University, has extensive expertise in developing and applying AFM for direct force measurements on cells and tissues. His work focuses on the mechanics and adhesion properties of biological systems, including the development of AFM-based strategies to study the mechanics of living systems. This project leverages the complementary expertise in reproductive cell biology and biophysics of the two supervisors, enabling it to achieve its full interdisciplinary potential.

Specify with whom the person recruited will collaborate and on what aspects

The core experiments for the project will be conducted jointly between the supervisory labs of Christopher Thomas and Felix Rico. Key support will also be provided within the IBDM by Thomas Lecuit, Pierre-François Lenne, Delphine Delacour, Elsa Bazellières, Robert Kelly, and Pascale Durbec, who will contribute critical expertise in biophysical characterisations, and within the DyNaMo lab by Claire Valotteau and Michael Sebbagh.

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PhD student's expected profile

We are seeking a motivated PhD student with a background in cell biology or biophysics. The ideal candidate should have experience or a strong interest in advanced live imaging techniques and biophysical assays. A collaborative mindset and enthusiasm for interdisciplinary research are essential.

Is this project the continuation of an existing project or an entirely new one?

This is a new project.

Two to five references related to the project

* denotes equal first author. † denotes corresponding author

1. **Thomas C***, Marx T*, Penir S, Schuh M†. *Ex vivo imaging reveals the spatiotemporal control of ovulation.* Nature Cell Biology. 26, 1997–2008 (2024). doi.org/10.1038/s41556-024-01524-6.

2. **Thomas C***, Marx T*, Penir S, Schuh M†. *Ex vivo imaging of ovulation in mouse ovarian follicles.* Protocols.io. (2024). doi.org/10.17504/protocols.io.14egn6nxql5d/v1.

3. Guevorkian K, Maître JL†. *Micropipette aspiration: A unique tool for exploring cell and tissue mechanics in vivo.* Methods Cell Biol. 2017;139:187-201. doi: 10.1016/bs.mcb.2016.11.012.

Two main publications from each PI over the last 5 years

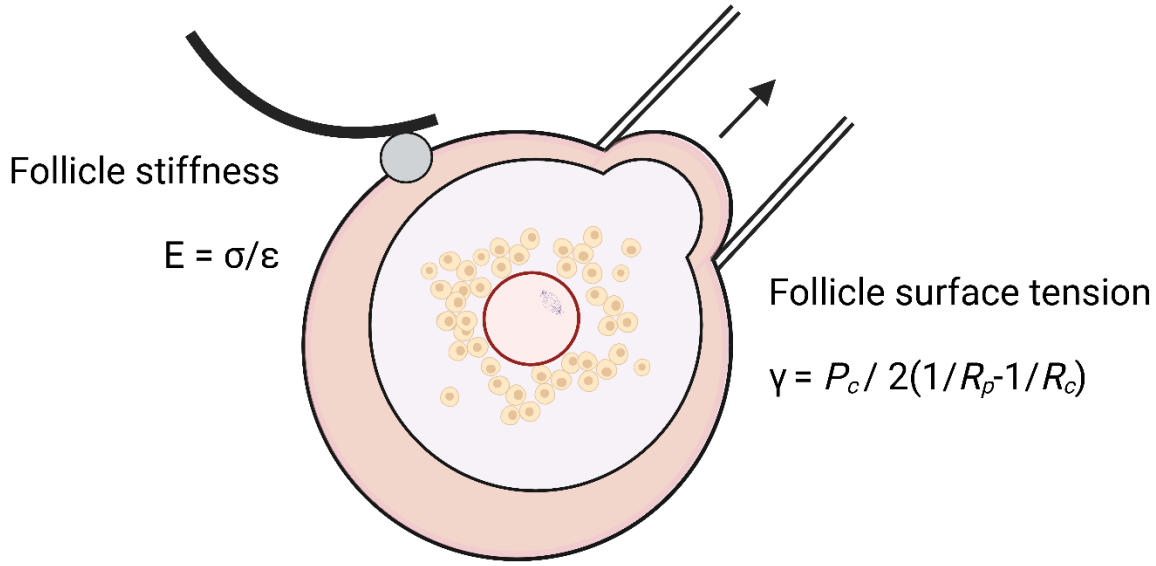
1. **Thomas C***, Marx T*, Penir S, Schuh M†. *Ex vivo imaging reveals the spatiotemporal control of ovulation.* Nature Cell Biology. 26, 1997–2008 (2024). doi.org/10.1038/s41556-024-01524-6.

2. **Thomas C†**, Wetherall B, Levasseur MD, Harris RJ, Kerridge ST, Higgins JMG, Davies OR, Madgwick S†. *A prometaphase mechanism of securin destruction is essential for meiotic progression in mouse oocytes.* Nat Commun. 2021 Jul 14;12(1):4322. doi: 10.1038/s41467-021-24554-2.

3. Eroles M, Lopez-Alonso J, Ortega A, Boudier T, Gharzeddine K, Lafont F, Franz CM, Millet A, Valotteau C, **Rico F†**. *Coupled mechanical mapping and interference contrast microscopy reveal viscoelastic and adhesion hallmarks of monocyte differentiation into macrophages.* Nanoscale. 2023 Jul 27;15(29):12255-12269. doi: 10.1039/d3nr00757j.

4. López-Alonso J, Eroles M, Janel S, Berardi M, Pellequer JL, Dupres V, Lafont F, **Rico F†**. *PyFMLab: Open-source software for atomic force microscopy microrheology data analysis.* Open Res Eur. 2024 Jul 24;3:187. doi: 10.12688/openreseurope.16550.1.

Project's illustrating image



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Internal use only – *the following information will be used by the CENTURI administration team only and will not be published*

Supervisor HDR 1:

☐ Obtained HDR

☐ Will be obtained by the beginning of the contract

☐ Not obtained

Supervisor 2 HDR*

☐ X Obtained HDR

☐ Will be obtained by the beginning of the contract

☐ Not obtained

If appropriate: Supervisor 3

Please know that a third supervisor cannot be mentioned on a doctoral contract and is only indicated in the project

Envisaged Ecole Doctorale (doctoral school): Ecole Doctorale Sciences de la vie et de la santé - ED 62

Time allocated to each institute*:

It is reminded that in the spirit of Centuri PhD call, the work should be done and should benefit the closest to equality between the two labs of supervisors 1 and 2. We highly recommend a 60/40% repartition at least

Name of institute 1: Institut de Biologie du Développement de Marseille Time allocated: 60%

Name of institute 2: Aix-Marseille Université, Inserm, DyNaMo Time allocated: 40%

It is the "project owner's" responsibility to check with his/her doctoral school that he/she is able to supervise a new student.

Reminder: Rules for supervisors

Groups which already have a PhD or postdoc funded by CENTURI can submit projects, but the number of simultaneous supervisions (of CENTURI PhD students / postdoctoral fellows) by a researcher is limited to 3. Lead supervision counts as 1; co-supervision counts as 0.5. Amongst these 3 supervisions, researchers are allowed to be the lead supervisor for up to 2 PhD students / postdoctoral fellows. As such, a researcher could be:

- the lead supervisor for 2 PhD students / postdoctoral fellows and the co-supervisor for 2 PhD students / postdoctoral fellows.
- the lead supervisor for 1 PhD student / postdoctoral fellow and the co-supervisor for 4 PhD students / postdoctoral fellows.
- the co-supervisor for 6 PhD students / postdoctoral fellows.

Additional rule applies for supervisors:

- a supervisor cannot submit more than 1 project / PhD Call (lead or co-supervisor)