0000000234 - OvulAtlas

1. EXCELLENCE (4 pages max)

1.1. Pre-proposal's context, positioning and objective(s)

The origin of new life is one of the most fundamental questions in biology. In mammals, the maternal contribution to this new life, **the egg, is released from an ovarian follicle during ovulation.** Despite its critical role in reproduction, our mechanistic understanding of the steps that drive follicle maturation and the expulsion of the egg remains surprisingly limited. A **key challenge** lies in the **limited methodological toolkit** and the **lack of established model systems**, which restricts quantitative, functional and reproducible studies. Moreover, **ovulation is a highly dynamic process** that occurs within the body, making it challenging to directly study both the event and its underlying mechanisms(1). Most of our knowledge comes from fixed samples, endpoint assays, and low spatial/temporal resolution readouts. **Such approaches cannot resolve the dynamics and causal relationships that couple follicle maturation and egg release.** To uncover the fundamental principles of ovulation, we need methods that directly measure and manipulate these processes as they unfold.

We recently developed a **model system that allows for direct visualisation and manipulation of the entire process of ovulation** in a precisely controlled setup(2). By combining long-term follicle culture with quantitative live imaging, it allows us to image the full ovulatory programme in single isolated mouse antral follicles (Figure 1a). In brief, follicles are mechanically isolated from whole mouse ovaries and cultured in medium containing FSH and the LH analogue human chorionic gonadotrophin (hCG) to mimic hormonal changes during the ovulatory cycle. To follow the behaviour of individual cells, the system takes advantage of CAG-TAG transgenic mice expressing a membrane (Myr-TdTomato) and a histone (H2B-GFP) marker(3). A combined approach of confocal and two-photon microscopy allows for the study of ovarian processes on both the cellular and whole follicle scale. Using this unique model system, we identified important structural and dynamic changes during ovulation and uncovered key molecular pathways that drive the ovulation process(2).

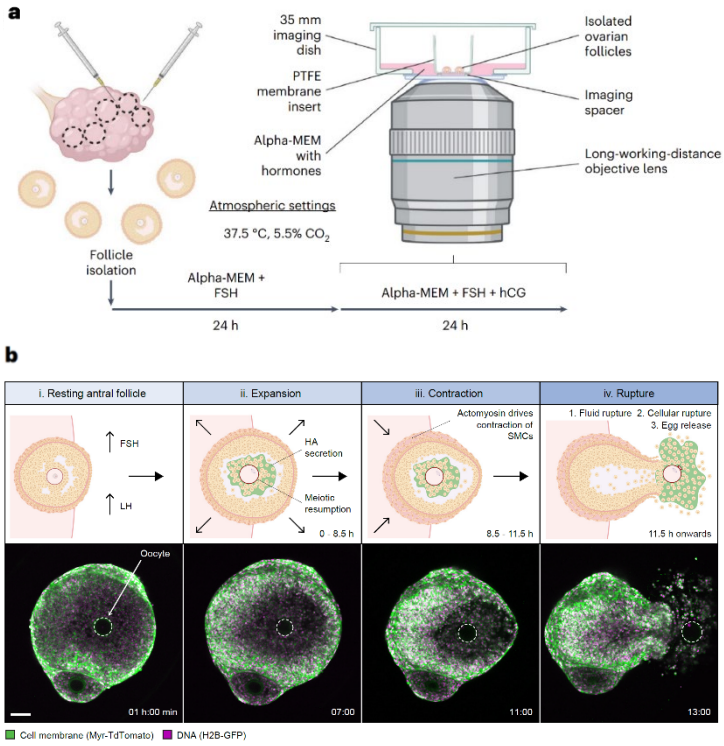

Figure 1. A model system for visualisation and manipulation of ovulation. (a) Follicles are isolated from mouse ovaries and cultured on the microscope with hormones that mimic the ovarian cycle. **(b)** Representative time-lapse confocal images of ovulation in isolated follicles. Cell membranes (Myr-TdTomato) in green; DNA (H2B-GFP) in magenta. Scale bar, 100 µm. Adapted from Thomas and Marx et al., 2024 (2).

We found that **ovulation, which takes 12 hours in mice, consists of three distinct phases** that occur synchronously in all follicles (Figure 1b). **Phase 1 – Follicle expansion**, which involves the secretion of hyaluronic acid into the space surrounding the oocyte, resulting in an increase in follicle volume. **Phase 2 – Follicle contraction**, which begins 8 hours post-stimulation and is triggered by progesterone and endothelin signalling. Importantly, this contraction, combined with the internal hydrostatic pressure generated by hyaluronic acid secretion, leads to increased mechanical stress on the oocyte-containing the cumulus mass. When this stress reaches a critical threshold, the follicle ruptures, and the oocyte is released during **phase 3 – Follicle rupture(2)**.

Despite uncovering dramatic tissue-scale changes in the ovulating follicle, **we still lack a cell-resolved understanding of how the follicle is organised**, how its compartments are defined, and how specific cell populations remodel, move, and interact across the 12-hour ovulatory programme. This gap is fundamental because, without a cellular map, we cannot resolve the dynamic cell behaviours that drive follicle rupture and oocyte release. **OvulAtlas will deliver the first dynamic cellular atlas of mouse**

ovulation, revealing how distinct cell populations reorganise in real time to drive follicle rupture and egg release.

OvulAtlas combines the expertise of **Christopher THOMAS** in ovarian cell biology and advanced quantitative microscopy with **Leo GUIGNARD**'s computational approaches for analysing morphogenesis at single-cell resolution from fluorescence microscopy(4,5). In **Objective 1**, we will leverage our existing scRNA-seq dataset generated from cultured follicles collected at 0, 3, 6, 9 and 12 h during ovulation. We will use key cell-type markers from the 15 annotated follicle cell types together with whole-mount follicle immunostaining and tissue clearing to map cell types to follicular compartments and determine how these populations change through ovulation. In **Objective 2**, we will establish live imaging and image-analysis pipelines to track the trajectories of individual cells within these mapped populations throughout ovulation, quantifying changes in position, shape, density, and collective behaviours. In **Objective 3**, we will deploy this quantitative framework under endocrine perturbation by targeting progesterone receptor (PGR) signalling to test causality and link molecular control to quantified single-cell behaviours and tissue-scale phase transitions. Together, OvulAtlas will provide the first direct, quantitative description of ovulation at the cellular scale and link single-cell dynamics to the phase transitions we have defined at the tissue level.

Objective 1 – Molecular-to-spatial mapping of follicular cell types during ovulation

The ovarian follicle is the multicellular functional unit of the ovary, each containing an oocyte—an immature egg prior to ovulation. The ovulatory follicle is highly compartmentalised, with key cell types distributed between inner and outer regions separated by a basement membrane (Fig. 2). Despite a broad understanding of follicular cell types, **we still lack a cell-resolved understanding of how this architecture is built and remodelled during ovulation**—how boundaries are defined, how populations reposition and interact, and how these dynamics generate directionality. Critically, although the follicle is roughly spherical, it is strongly polarised: one side remains embedded within the ovary, while the other protrudes and becomes the apical rupture site. How cellular organisation and compartmental asymmetry establish and reinforce this polarity to drive directional rupture and oocyte release is a central unanswered question.

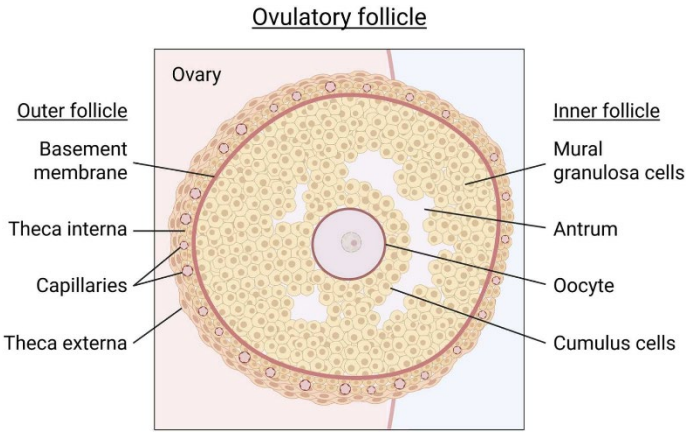

Figure 2. Cellular anatomy of the ovulatory follicle. Schematic diagram of a mature, pre-ovulatory follicle, highlighting its compartmentalised structure (1).

We previously performed scRNA-seq across the entire ovulatory time course in vivo and ex vivo, generating high-quality transcriptomes for 84,755 cells and identifying **15 major follicular cell types** with robust markers (Fig. 3a, b). However, we have **almost no information on how these cell types are arranged in 3D**—whether they occupy distinct niches, form gradients along the basal–apical axis, or reorganise during the phase transitions that culminate in rupture. Objective 1 will convert this transcriptomic "parts list" into a **quantitative spatial atlas** by mapping cell types and subtypes back into intact follicles across ovulation and measuring how organisation and polarity change over time.

Experimental plan. We will select a **minimal, multiplexable marker panel** from the scRNA-seq atlas to label major compartments and key subpopulations. We will then perform **whole-mount immunofluorescence on intact ovulatory follicles** collected at 0, 3, 6, 9 and 12 h post hCG, combined with tissue clearing and deep imaging (confocal and two-photon) to capture the full follicle volume without sectioning. Next, we will quantify organisation and polarity using established analysis approaches: nuclei-based cell identification for counts/densities, compartment boundary reconstruction, and spatial statistics describing cell-type distributions along the basal–apical axis, with standard corrections for depth-dependent signal and channel cross-talk.

Readouts will include **(i)** a 3D map of where each cell type and subtype sits at each timepoint; **(ii)** density and packing across compartments; **(iii)** quantitative polarity metrics (enrichment/depletion towards the future rupture site versus the basal side); and **(iv)** time-resolved changes in compartment geometry

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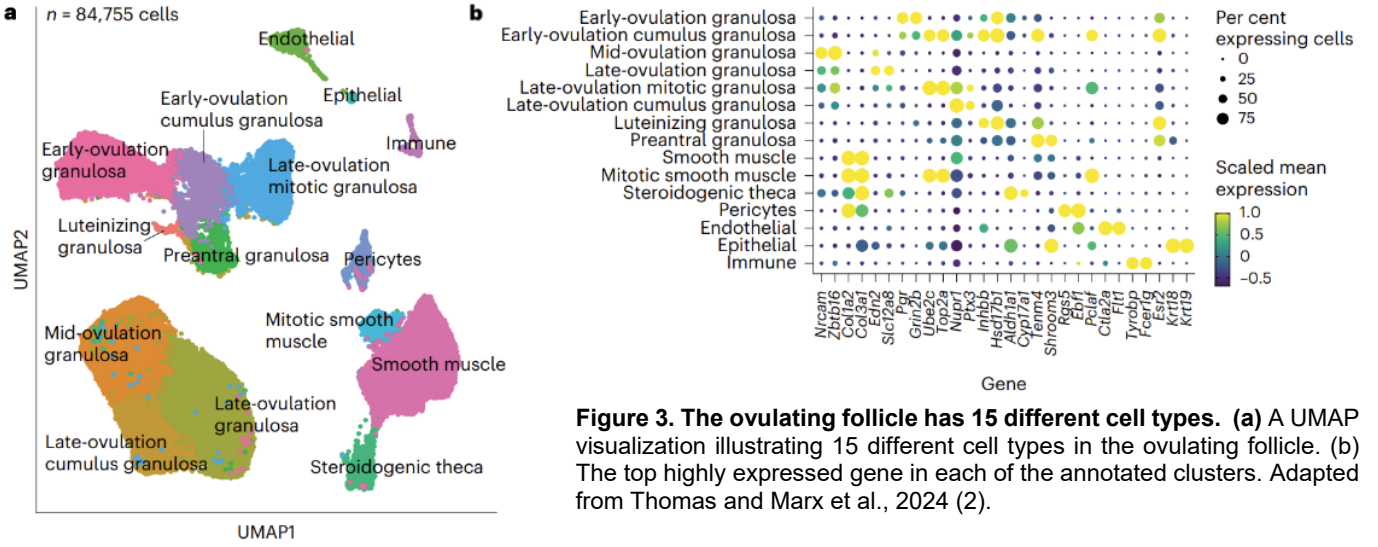


Figure 3. The ovulating follicle has 15 different cell types. (a) A UMAP visualization illustrating 15 different cell types in the ovulating follicle. (b) The top highly expressed gene in each of the annotated clusters. Adapted from Thomas and Marx et al., 2024 (2).

aligned to the tissue-scale phases of ovulation. To capture variability and obtain robust statistics, we will analyse ≥20 follicles per timepoint.

Finally, key spatial findings will be validated in vivo using time-matched whole-ovary cryosections, ensuring patterns reflect native follicle context and ovarian polarity.

Together, Objective 1 will deliver **the first quantitative, cell-resolved description of follicle organisation and polar remodelling during ovulation**, providing a mechanistic framework for understanding directional rupture and oocyte release.

Objective 2 – Single-cell trajectory tracking to quantify cellular dynamics across ovulation

Objective 1 will define where cell types sit in 3D and how organisation and polarity change across ovulation. Objective 2 adds the missing dynamic layer: **how individual cells move, divide, change shape and rearrange in real time as the follicle transitions from expansion to contraction to rupture**. We will quantify these behaviours within the spatial framework from Objective 1 to identify the cellular programmes that generate directionality and culminate in oocyte release.

Experimental plan. Live imaging and single-cell tracking in intact follicles. Using our established ex vivo ovulation system, we will image isolated antral follicles from CAG-TAG mice (H2B-GFP nuclei; Myr-TdTomato membranes) for global tracking to resolve **single-cell behaviours in dense compartments**, following proven imaging strategies that enable long-term cellular tracking.

We will test whether granulosa cells execute collective behaviours during follicle contraction and rupture, consistent with late scRNA-seq upregulation of cytoskeletal and contractility genes. We will quantify coordinated velocity fields, neighbour rearrangements, cell-shape dynamics, and spatiotemporal patterns of actomyosin cytoskeleton enrichment. We will link these dynamics to polarity and rupture-site formation by analysing all metrics with respect to the basal–apical axis.

Quantification with established workflows. We will apply established segmentation (Figure 4a, b) and long-term tracking methods to reconstruct single-cell trajectories, cell divisions and tissue-scale motion fields, inspired by

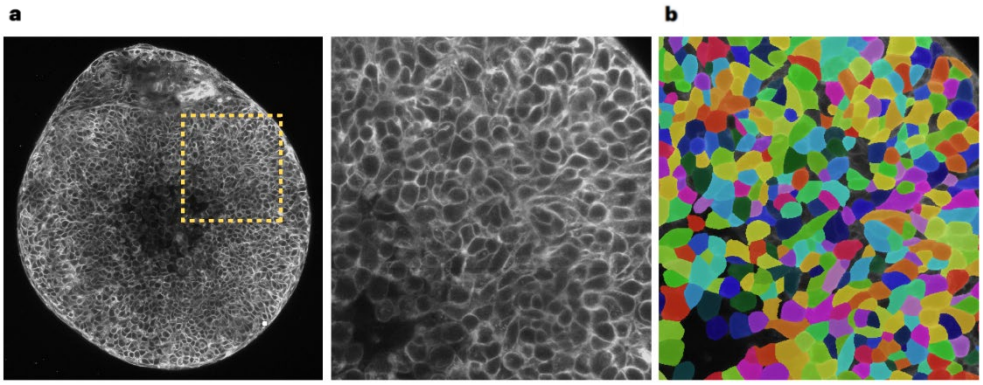


Figure 4. Cellular segmentation of mural granulosa cells in ovulating follicles. (a) Representative confocal image of an ovulating follicle (left). The dotted yellow square indicates the region of inset (right). Cell membranes (Myr-TdTomato) are shown in white. **(b)** Cellular segmentation of mural granulosa cells from inset using Cellpose-SAM (6).

whole-embryo imaging approaches that extract conserved morphodynamic programmes from long time-course recordings(4–6).

Readouts will include: **(i)** single-cell trajectories (speed, persistence, directionality) for defined populations; **(ii)** cell division timing and localisation; **(iii)** collective motion fields aligned to the basal–apical axis; **(iv)** cell-shape and packing changes; and **(v)** event timing relative to the phase transitions of ovulation.

A statistical dynamic atlas. Finally, we will register follicles into a common or average coordinate system defined by geometry and polarity to quantify stereotypy versus variability across individuals. We will image and analyse ≥100 follicles, enabling robust statistics on variability in the cellular dynamics that precede follicle rupture and egg release.

Together, Objective 2 will convert the static spatial atlas from Objective 1 into a **quantitative, time-resolved map of cell behaviours**—identifying which populations move, when they move, and how collective dynamics couple to polarity, contraction and rupture. This will deliver the **first mechanistic, cell-dynamic description of ovulation**.

Objective 3 – Functional validation of ovulatory cell dynamics in Adelaide, Australia

Building on the static spatial atlas (Objective 1) and live single-cell trajectory maps (Objective 2), Objective 3 will **functionally test the cellular behaviours and phase-transition signatures** we identify by deploying our imaging and analysis workflow in the lab of **Rebecca Robker (University of Adelaide, Australia)**. We will perturb **progesterone receptor (PGR) signalling**—using both acute molecular inhibition and PGR knockout mouse lines established in the Robker and Russell labs—to determine how progesterone reshapes follicle phase transitions and cellular dynamics(7).

PGR is a particularly powerful lever because it sits **upstream of multiple late ovulatory programmes**, including the induction of actin/cell-motility genes in granulosa cells, and protease-driven follicle rupture(7,8). By quantifying single-cell trajectories and collective motion fields under PGR perturbation, we will directly test whether progesterone controls not only rupture-associated remodelling but also tissue dynamics during the contraction-to-rupture transition. Together, Objective 3 will **validate atlas-derived predictions, establish causality for progesterone-dependent cell behaviours, and demonstrate portability of our quantitative framework across laboratories**.

1.2. Interdisciplinary and intersectoral dimension of the project

This interdisciplinary project integrates reproductive cell biology and advanced quantitative imaging and analysis to investigate the mechanism of ovulation.

How follicle cell dynamics drive ovulation has never been possible to study directly before. Our unique model system and ex vivo imaging approach allow us to answer fundamental questions that represent **completely new ground in our understanding of female reproductive biology**. The live and fixed imaging experiments will be done under the supervision of **Dr. Christopher Thomas** (IBDM), an expert in reproductive cell biology and advanced microscopy.

By combining this approach with advanced image analysis, we will provide the first cell-dynamic description of ovulation. This aspect of the project will be supervised by **Dr. Leo Guignard** (IBDM), an expert in computational approaches for analysing morphogenesis.

Together, this **pioneering and synergistic approach** will bring ovarian biology into the modern era in the same way that organoid models have revolutionised the way in which we can study the function of the brain, kidney, lung, and many other organs in vitro. This fusion of ex vivo follicle culture, quantitative microscopy, and advanced image analysis will not only advance the state of the art but also provide an invaluable resource for the reproductive biology field.

Throughout the project, the student will gain intersectoral experience through biannual mentorship sessions with **Dr Vedran Vasic, Senior Scientist at Roche Pharma Research and Early Development (pRED; Munich, Germany) and Technical Project Leader in protein engineering and antibody development**. As part of this experience, they will discuss how antibodies such as those used in Objective 1 are developed and brought to market. This exchange will provide insight into careers beyond academia, helping the student explore potential future career paths, including roles in industry.

2. IMPACT (2 pages max)

This PhD will provide the candidate with high-level, interdisciplinary training at the interface of reproductive biology, advanced quantitative microscopy, and computational image analysis. By constructing a cell-resolved atlas of the ovulating follicle—combining whole-mount 3D mapping, long-term live imaging, single-cell tracking, and quantification of collective motion—the candidate will acquire a rare and highly transferable skill set spanning experimental design, high-content imaging, and quantitative interpretation of complex dynamic tissues.

Training will be delivered through close day-to-day supervision within IBDM. Under Dr Christopher Thomas, the candidate will gain deep expertise in ovarian cell biology, follicle culture, and advanced microscopy, including optimisation of acquisition strategies for dense, moving 3D samples. In parallel, Dr Leo Guignard will provide continuous input on quantitative analysis for both fixed and live datasets, reflecting a central practical challenge of the project: robustly extracting cell identities, spatial organisation, and dynamic behaviours from follicles containing >100,000 cells. Together, this dual supervision will ensure the candidate becomes fluent in the full workflow from sample preparation and imaging design through segmentation, tracking, statistical analysis, and the development of reproducible analysis pipelines.

The project also includes an international secondment in the laboratory of Prof. Rebecca Robker at the University of Adelaide, an internationally-recognised leader in reproductive biology, molecular endocrinology, and ovulation research. There, the candidate will apply the project's imaging and analysis framework to functional experiments perturbing progesterone receptor signalling using established genetic models and acute inhibition approaches. This experience will expand the candidate's scientific perspective, strengthen their ability to connect cellular mechanisms to endocrine control, and broaden their international professional network.

Finally, the intersectoral component—biannual mentorship with Dr Vedran Vasic, Senior Scientist at Roche pRED (Munich) and Technical Project Leader in protein engineering and antibody development—will provide direct exposure to industrial approaches to reagent development, validation, and translational decision-making. By drawing explicit parallels between antibody performance and quantitative immunofluorescence/whole-mount imaging readouts, the candidate will gain a practical understanding of how reagent quality, specificity, and validation standards shape the reliability and interpretability of biological conclusions. Collectively, these academic, international, and intersectoral experiences will position the candidate strongly for careers in academia and for quantitatively oriented roles in imaging, computational biology, and biotechnology/pharmaceutical R&D.

2.2. Expected impact for the thematic axis

Ovulation is the decisive event that enables natural conception: a precisely timed, hormone-triggered tissue remodelling programme that culminates in follicle rupture and egg release. Yet, despite decades of research, the field still lacks a cell-resolved, quantitative understanding of how distinct follicular cell populations coordinate their behaviours to drive the expansion–contraction–rupture sequence and to specify a directional rupture site. OvulAtlas directly addresses this gap by combining whole-mount 3D mapping with long-term live imaging and single-cell/collective-dynamics quantification. The resulting dynamic cellular atlas will provide a new reference framework for the thematic axis by transforming ovulation from a largely descriptive process into a measurable, mechanistic sequence of state transitions with defined cellular readouts.

Beyond its immediate relevance to reproductive biology, the project contributes broadly to themes centred on dynamic tissue remodelling, morphogenesis, and endocrine control of organ function. The adult ovary is a rare example of an organ that undergoes repeated, stereotyped, large-scale structural reorganisation across the lifespan, making it a powerful system for studying how hormonal cues are converted into

coordinated cell behaviours, force generation, and controlled rupture in a living tissue. By quantifying polarity, compartment remodelling, single-cell trajectories, and motion fields at scale, OvulAtlas will also provide generalisable concepts and analysis approaches applicable to other contexts of rapid, regulated tissue remodelling.

The project also has clear translational relevance. Many common forms of female infertility involve disrupted ovulation (e.g., failures in timing, follicle rupture, or endocrine responsiveness), and current clinical assessment often relies on indirect or endpoint measures that cannot resolve underlying cellular mechanisms(9). A quantitative, cell-resolved framework for ovulation will help bridge this gap by defining measurable cellular phenotypes that can be linked to molecular pathways and endocrine perturbations. In particular, functional testing of progesterone receptor signalling will establish causal connections between a clinically important endocrine axis and specific, quantifiable cell and tissue dynamics, providing a principled basis for interpreting ovulatory defects.

Finally, by delivering an annotated atlas, benchmark imaging datasets, and reusable analysis workflows, OvulAtlas will create enabling infrastructure for the community. This resource will accelerate mechanistic studies of ovulation, support more reproducible comparisons across laboratories and perturbations, and provide a foundation for future work aimed at improving fertility treatments and informing non-hormonal contraceptive strategies that target ovulatory tissue remodelling with greater precision.

2.3. Dissemination, exploitation and communication activities planned

The results of this project will be disseminated primarily through peer-reviewed publications and presentations at leading international conferences spanning reproductive biology, morphogenesis, and quantitative imaging. Target meetings will include the Society for the Study of Reproduction (SSR) Annual Meeting, relevant EMBO workshops/conferences, and Gordon Research Conferences aligned with reproductive biology, tissue remodelling, and quantitative cell dynamics. The PhD student will play a central role in preparing manuscripts, presenting findings, and engaging with the international community through conference participation and targeted networking.

To reach audiences beyond academia, we will pursue proactive science communication through press releases, institutional channels, and media outreach. This strategy has been highly effective in the past, as demonstrated by the widespread media coverage of our work on ovulation, which was reported by 32 news outlets globally. The PhD student will contribute to these outreach efforts by producing accessible summaries of key results for non-specialist audiences and participating in local and national engagement activities.

A key aspect of this communication strategy will be the integration of art and science to convey complex dynamics in an intuitive and compelling way. This approach is exemplified by the artwork Ovulation in Bubbles and Ink, which was selected as the cover piece of the issue of Nature Cell Biology featuring our work on ovulation. Such creative formats can enhance public understanding of reproductive biology by translating complex processes into accessible visual narratives.

The project will also generate resources with clear reuse potential: an annotated cellular atlas, benchmark imaging datasets, and analysis workflows that can be adopted by other laboratories to study ovulation and hormone-triggered tissue remodelling. These outputs will be disseminated through open scientific channels (e.g., appropriate repositories and project webpages) to maximise reproducibility and community uptake. In parallel, we will support uptake through knowledge transfer activities such as seminars, institute events, and cross-laboratory exchanges.

3. IMPLEMENTATION (2 pages max)

Objective 1 – Molecular-to-spatial mapping of follicular cell types during ovulation

In Objective 1, the student will convert our existing scRNA-seq data into a quantitative 3D spatial atlas of the ovulating follicle. Using robust markers from the 15 annotated follicle cell types, the student will design a minimal, multiplexable immunostaining panel and apply whole-mount immunofluorescence to intact mouse antral follicles throughout ovulation. Follicles will be imaged using tissue clearing and deep confocal/two-photon microscopy to capture complete follicle geometry and compartment organisation.

This objective requires method optimisation in Year 1 to ensure consistent follicle handling, fixation, staining, clearing, and imaging across timepoints, including controls for depth-dependent signal loss and channel cross-talk. The student will then generate the main spatial dataset during Years 1–2, analysing ≥20 follicles per timepoint to quantify (i) 3D localisation of each cell type/subtype; (ii) densities and packing across compartments; (iii) compartment boundaries and geometry; and (iv) polarity metrics describing enrichment/depletion along the basal–apical axis. Key spatial findings will be validated in vivo using time-matched whole-ovary cryosections to ensure mapped patterns reflect native follicle context and ovarian polarity.

Objective 2 – Single-cell trajectory tracking to quantify cellular dynamics across ovulation

Using our established live ovulation system, the student will image isolated antral follicles from CAG-TAG mice (H2B-GFP nuclei; Myr-TdTomato membranes) over the full 12-hour ovulatory programme. A combined confocal/two-photon strategy will be used to balance cellular resolution with tissue-scale coverage and long-term viability. The main technical and analytical challenge is scale: a single follicle contains >100,000 cells undergoing rapid 3D remodelling. The student will therefore implement and refine segmentation and long-term tracking pipelines to reconstruct single-cell trajectories and tissue-scale motion fields, applying established morphogenesis-analysis approaches adapted to the follicle. Readouts will include (i) trajectory features (speed, persistence, directionality) for defined populations; (ii) spatiotemporal patterns of collective motion aligned to the embedded–apical axis; (iii) neighbour rearrangements and packing changes; (iv) cell-shape dynamics; and (v) event timing relative to phase transitions.

Across Years 2–3, the student will build a statistical dynamic atlas by registering follicles into a common, polarity-defined coordinate system, enabling direct comparison across individuals and quantification of stereotypy versus variability. This objective will include imaging and analysis of ≥100 follicles to provide robust statistics for the cellular programmes that precede rupture and oocyte release.

Objective 3 – Functional validation of ovulatory cell dynamics through endocrine perturbation in Adelaide

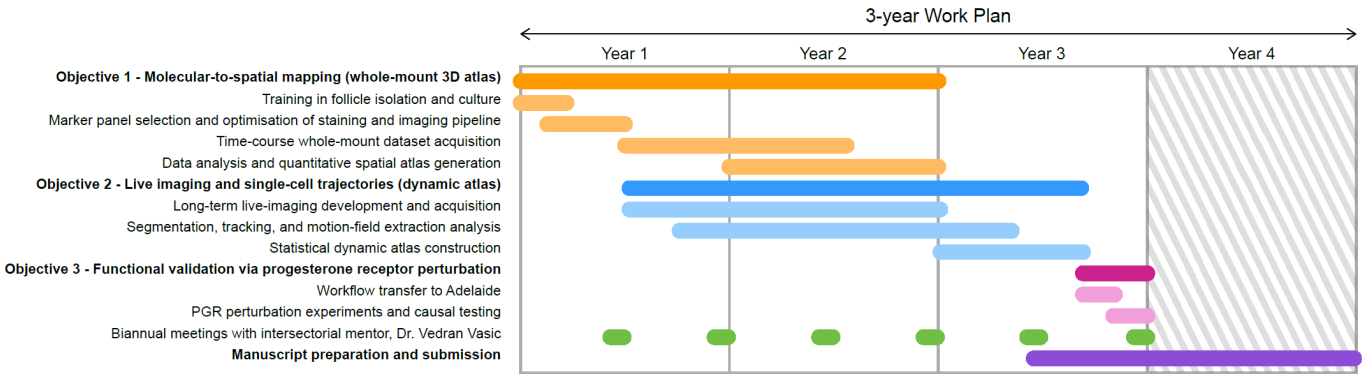
In Objective 3, the student will functionally test atlas-derived predictions by perturbing progesterone receptor (PGR) signalling during an international secondment in the laboratory of Prof. Rebecca Robker at the University of Adelaide (Australia). During a two-month exchange in the third year of the PhD, the student will deploy the project's quantitative imaging and analysis workflow in Adelaide and use both acute molecular inhibition and established genetic models (including PGR-deficient lines) to determine how progesterone reshapes follicle phase transitions and cellular dynamics.

PGR is a powerful experimental lever because it sits upstream of late ovulatory programmes, including induction of actin/cell-motility genes in granulosa cells and protease-driven rupture. By quantifying single-cell trajectories and collective motion fields under PGR perturbation, the student will directly test whether progesterone controls not only rupture-associated remodelling but also tissue dynamics during the

contraction-to-rupture transition. Objective 3 will therefore establish causality for progesterone-dependent cell behaviours, validate key signatures identified in Objectives 1–2, and demonstrate portability and robustness of the quantitative framework across laboratories.

Overall timeline and integration

Year 1 will focus on establishing and validating the whole-mount mapping workflow (Objective 1) and implementing core analysis pipelines for fixed datasets, while initiating pilot live imaging for Objective 2. Year 2 will generate the bulk of the spatial atlas and expand long-term live imaging to build trajectory and motion-field datasets, with analysis iterating in parallel. Year 3 will consolidate the statistical dynamic atlas, perform functional tests during the Adelaide secondment (Objective 3), and integrate outputs into a unified atlas resource and publication package.



Feasibility and Resources

Throughout the project, the student will receive sustained support from the two co-supervisors through frequent technical training, regular 1-to-1 meetings, and joint discussions to troubleshoot challenges and adjust experimental plans when needed. Both co-supervisors have strong experience supervising and training PhD students. Clear milestones and a detailed 36-month timeline have been defined to ensure that the research and training objectives are achieved on schedule. By the end of the work plan, all objectives are expected to be completed and integrated into a coherent publication package ready for submission to high-impact, peer-reviewed journals.

The project is well supported by existing infrastructure and resources. Key requirements include access to mouse ovaries and advanced confocal and two-photon microscopy for long-term live imaging and deep whole-mount acquisition. The required transgenic mouse lines for cell tracking are already established in the IBDM animal facility. Imaging will be performed within the PiCsL–IBDM imaging facility, which provides state-of-the-art light microscopy instrumentation, including Zeiss LSM 880 confocal systems with two-photon capability, together with the computing environment and software needed for high-content image processing and analysis.

Feasibility is further underpinned by strong preliminary data and established protocols demonstrating robust long-term culture and cellular-resolution imaging of ovulation, as well as clear readouts aligned with the project objectives. The complementary expertise of the co-supervisors—spanning ovarian cell biology, quantitative microscopy, and computational analysis of dense 3D datasets—places the team in a strong position to deliver a quantitative spatial and dynamic atlas of ovulation within the proposed timeframe. The student will also benefit from the wider scientific ecosystem at IBDM and the Luminy campus, with access to local expertise in tissue morphogenesis, quantitative imaging, and mechanics, providing additional support where specialised input is required.

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4. ETHICS SELF-ASSEMENT

SECTION 5 - ANIMALS

1) Details on the numbers of animals to be used, nature of the experiments, procedures and techniques to be used.

The experiments using mouse ovarian follicles will last for the 3-year duration of the SHADOC project, during which the student will perform on average 2 experiments per week. For each experiment, we will need to sacrifice 2 transgenic females. We will therefore use 580 transgenic females for experiments during the project. As females only represent 50% of each litter, this requires the birth of 1,160 animals. Of these, all females will be used either for experiments or for mating and 100 males (+/p) will be used for mating. All animals will be sexed after birth and genotyped using a UV light for detecting and visualising green fluorescent protein (GFP) in the skin of positive animals. Any male animals that will not be used for mating will be sacrificed immediately by decapitation. To generate the required number of animals for our experiments, we will keep a colony of 20 breeding pairs at a given time. As breeding animals are typically retired at 6-8 months to ensure consistent litter size, we will use 100 transgenic CAGTAG males (+/p) and 100 wild type (C57BL6/J) females for mating throughout the project.

2) Details on species and rationale for their use.

The objectives of OvulAtlas and the significance of its findings are highly relevant to human reproduction and fertility, making it essential to use a model system that is both experimentally tractable and biologically informative. In this project, all objectives will be addressed in the mouse, using isolated antral follicles in a controlled culture system that supports long-term live imaging and quantitative perturbation of the ovulatory programme.

Mouse follicles provide a uniquely powerful platform for this work because they combine reproducibility, throughput, and genetic accessibility with well-established relevance to conserved mechanisms of ovulation. In particular, the CAG-TAG transgenic line enables in vivo labelling of nuclei (H2B-GFP) and cell membranes (Myr-TdTomato), providing the signal quality required for dense 3D segmentation, single-cell tracking, and quantitative reconstruction of collective tissue dynamics. This genetic labelling is central to Objectives 2–3, where the project requires robust long-term tracking in follicles containing >100,000 cells undergoing rapid remodelling.

Although humans and mice differ in ovulation number, the endocrine logic and core cellular programmes driving ovulation are strongly conserved across mammals, including progesterone receptor signalling and tissue remodelling pathways. By combining a quantitative spatial atlas (whole-mount mapping) with a dynamic atlas (live single-cell trajectories) and causal endocrine perturbation, OvulAtlas will establish general principles of ovulatory tissue remodelling and provide mechanistic readouts that can guide future comparative and translational studies in human-relevant contexts.

3) Details on procedures to ensure animal welfare.

Mice will be maintained under standard housing conditions (22°C, 40% humidity, 12 h light cycles, and free access to food and water). No experiments will be done on living animals. All mice used for experiments will receive no other treatment than sacrifice by decapitation, after which the ovaries will be collected.

4) Details on implementation of the 3Rs Principle.

As emphasised in section 2, it is essential that we use transgenic mice as a model system for this project, given the requirement for fluorescent reporters for the live microscopy experiments. In all cases, we will use statistical testing to determine the minimal number of animals required to give significant results for each experiment type. We will ensure minimal suffering, distress and harm to the animals.

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SECTION 6 – NON-EU COUNTRIES

In the final year of the project, the student will undertake a secondment in Adelaide, Australia. During this period, they will work in the laboratory of Prof. Rebecca Robker at the University of Adelaide, performing experiments using mouse ovary samples under all relevant local ethical approvals.

5. REFERENCES

1. Thomas C. Ovulation: A cellular symphony in three movements. Vol. 174, Seminars in Cell and Developmental Biology. Elsevier Ltd; 2025.
2. Thomas C, Marx TL, Penir SM, Schuh M. Ex vivo imaging reveals the spatiotemporal control of ovulation. Nat Cell Biol [Internet]. 2024;26(11):1997–2008.
3. Trichas G, Begbie J, Srinivas S. Use of the viral 2A peptide for bicistronic expression in transgenic mice. BMC Biol. 2008;6:1–13.
4. McDole K, Guignard L, Amat F, Berger A, Malandain G, Royer LA, et al. In Toto Imaging and Reconstruction of Post-Implantation Mouse Development at the Single-Cell Level. Cell [Internet]. 2018;175(3):859-876.e33.
5. Guignard L, Fiúza UM, Leggio B, Laussu J, Faure E, Michelin G, et al. Contact area–dependent cell communication and the morphological invariance of ascidian embryogenesis. Science (1979) [Internet]. 2020 Jul 10;369(6500):eaar5663.
6. Pachitariu M, Rariden M, Stringer C. Cellpose-SAM: superhuman generalization for cellular segmentation. bioRxiv [Internet]. 2025 Jan 1;2025.04.28.651001.
7. Robker RL, Russell DL, Espey LL, Lydon JP, O'Malley BW, Richards JS. Progesterone-regulated genes in the ovulation process: ADAMTS-1 and cathepsin L proteases. Proc Natl Acad Sci U S A. 2000;97(9):4689–94.
8. Smith KM, Dinh DT, Akison LK, Nicholls M, Dunning KR, Morimoto A, et al. Intraovarian, Isoform-Specific Transcriptional Roles of Progesterone Receptor in Ovulation. Cells. 2022;
9. Thonneau P, Marchand S, Tallec A, Ferial ML, Ducot B, Lansac J, et al. Incidence and main causes of infertility in a resident population (1 850 000) of three French regions (1988–1989)*. Human Reproduction [Internet]. 1991 Jul 1;6(6):811–6.