A Multiscale Integration of In Vitro and In Vivo Ovarian and Endocrine Dynamics, via Computational Modeling, to Predict Toxicant Effects on Female Reproduction

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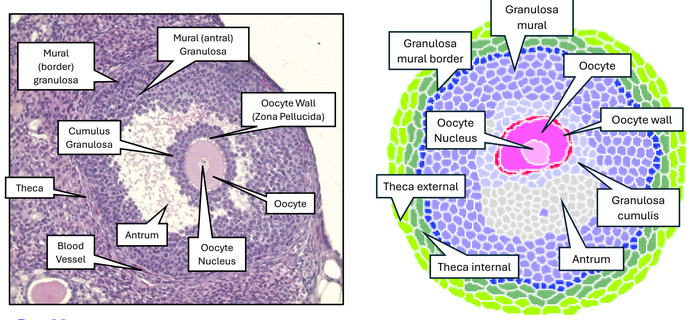
Simulation Methods Supplement

We have developed, in CompuCell3D (**CC3D**)[1], a spatial model representing a growing ovarian follicle with multiple interacting cell types and a dynamic antrum. Cell behaviors—including adhesion, growth, secretion, and proliferation—drive structural organization and expansion over a 10-day in vitro simulation.

Ovarian histology section → CompuCell3D Cell Layout



Cell types:

- Theca cells: **external and internal**
- Granulosa cells: **mural, mural border, and cumulus**
- Oocyte: **nucleus, cytosol, and wall (zona pellucida)**
- **Antrum ECM:** proteoglycan-rich matrix

Antrum formation:

- Cumulus and some mural granulosa cells secrete **proteoglycans ("org-salt")**
- Org-salt forms a **diffusing field confined to antrum**
- This field drives **water influx** and antrum expansion

Growth and proliferation:

- Mural border granulosa and theca cells proliferate to maintain proper layer structure during expansion

Tissue organization:

- **Cell–cell adhesion** largely maintains spatial layering
- Additional constraints **link oocyte to cumulus cells**

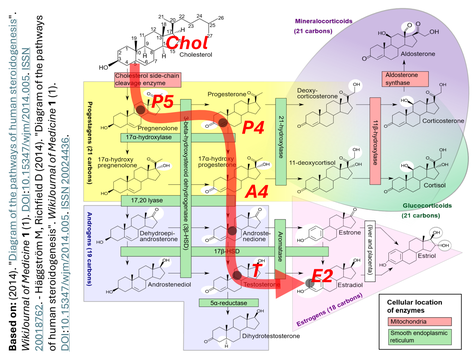
Morphogen Fields:

- **Growth Differentiation Factor 9 (GDF9)** is secreted by the oocyte and stimulates differentiation and growth of granulosa cells. [2]
- **WNT5α** is secreted by the Theca internal cells and stimulates differentiation and growth of granulosa cells. [3]
- GDF9 and WNT are used to differentiate granulosa into mural and cumulus.
- Testosterone and Estradiol are assumed to freely, and rapidly diffuse

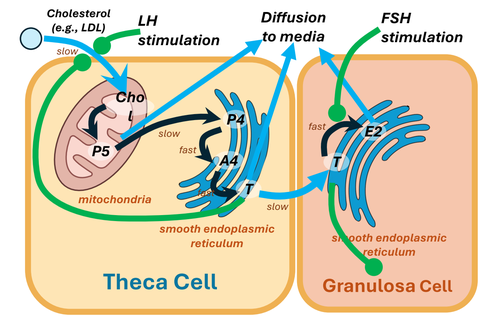
Simulation details:

- Duration: **10 simulated days**
- Resolution: **1 μm/pixel**
- Time scale: **1 simulation step = 1 min**
- The simulation includes removal of 50% of the culture media once a day.

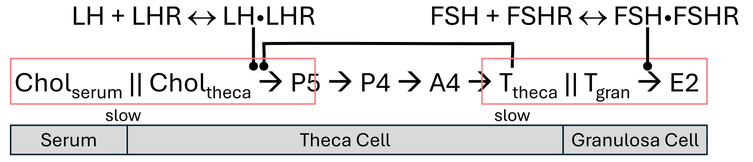
Steroidogenesis Pathways



Based on: (2014). "Diagram of the pathways of human steroidogenesis". *WikiJournal of Medicine* **1** (1). DOI:10.15347/wjm/2014.005. ISSN 20018762. - Häggström M, Richfield D (2014). "Diagram of the pathways of human steroidogenesis". *WikiJournal of Medicine* **1** (1). DOI:10.15347/wjm/2014.005. ISSN 20018762.



Not shown: Steroid transfer facilitated by LDL receptor on theca cells, and sterol carrier proteins in both theca and granulosa cells.



Steroidogenesis:

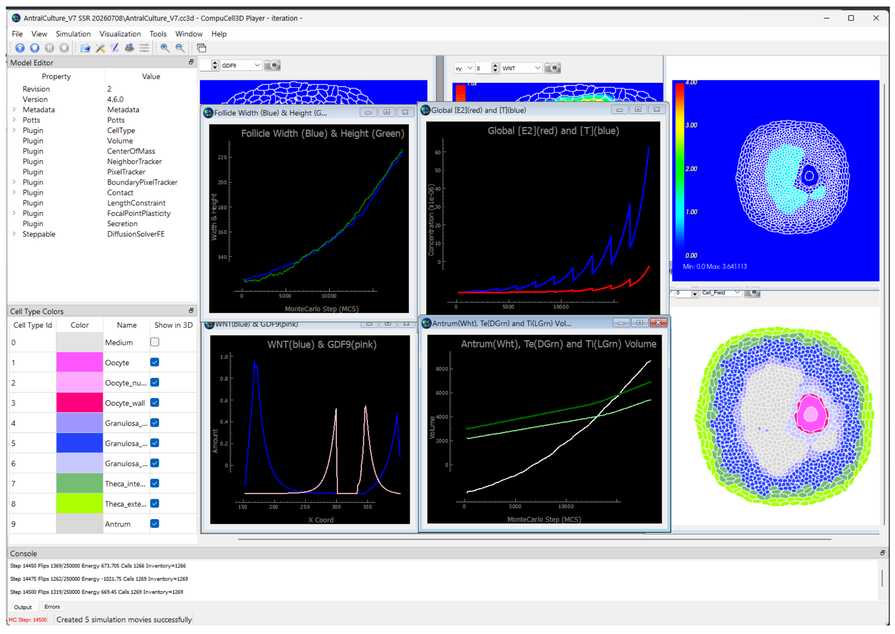
- **Theca interna cells:** Each cell has an ODE model simulating steroidogenesis:
  cholesterol (Chol) → pregnenolone (P5) → progesterone (P4) → androstenedione (A4) → testosterone (T)
- **Mural Granulosa cells:** Each cell has an ODE model simulating conversion of
  testosterone (T) → estradiol (E2)
- **Rate-limiting assumption:** Steps involving intracellular transport or intercellular diffusion are treated as slow, with potential loss of substrate to the media.
- **Cholesterol → pregnenolone (Chol → P5):** Modeled as a single slow step, reflecting transport of cholesterol from the media or within theca cells.
- **Testosterone transport:** T is produced in theca cells and diffuses to granulosa cells; this is assumed to be slow, with partial loss to the media.
- **Granulosa (T → E2):** Diffusion of T into granulosa cells and conversion to E2 are combined into a single process.

Feedback Pathways:

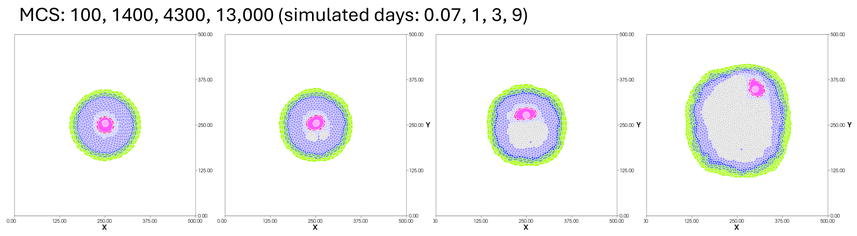
- Testosterone stimulates cholesterol transfer in theca cells and mural granulosa cell proliferation

Simulation Results

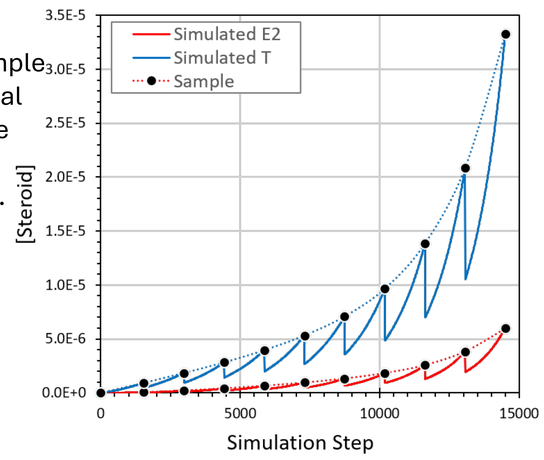
CompuCell3D simulation window. Subwindows show the cell field (lower right), proteoglycan osmotic pressure (upper right), follicle height and width (upper left), testosterone and estradiol levels, (upper middle), Wnt and GDF9 fields along a horizontal line through the follicle (bottom left) and antrum and theca cell "volumes" (areas in this 2D simulation) (bottom center).



Typical results for the follicle growth simulation. Cell field after 10 simulated days. The follicle diameter increases by 50%. Cell maintain reasonable type-specific locations.



The in vitro system includes removal of 50% of the well volume once daily and replacement with fresh media. The removed sample is used for hormone measurements. This significant loss of material in the media needs to be accounted for in the simulation. Here, the total T and E2 concentrations in the **simulation** is plotted versus time where one "Simulation Step" is one minute of simulated time. The in vitro samples correspond to the top of the peaks in the curves, connected by the dashed lines. The in vitro sampling not only creates concentration fluctuations but also reduces the measured total amount of T and E2 secreted by the follicle in the in vitro data.



Acknowledgments

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