Luteinizing hormone stimulates ingression of granulosa cells within the mouse preovulatory follicle

Poster #255

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Background

Mammalian oocytes are contained in the ovary within individual ovarian follicles (Figure 1). In preovulatory follicles, oocytes are surrounded by somatic cumulus cells, a fluid filled antrum, and ~5-15 layers of mural granulosa cells. These are contained within a basal lamina, with theca cells, consisting of steroidogenic cells and myofibroblasts, and vasculature encompassing the structure.

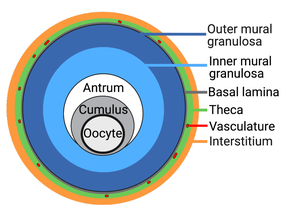


Figure 1. Structure of a mammalian ovarian preovulatory follicle.

Each reproductive cycle, one or more oocytes (depending on the species) are released from the follicle at ovulation. Most work on ovulation has focused on the actions of cells **outside** the basal lamina, and how those cells contract to force the oocyte out of the follicle (Ko et al., 2022, Figure 2).

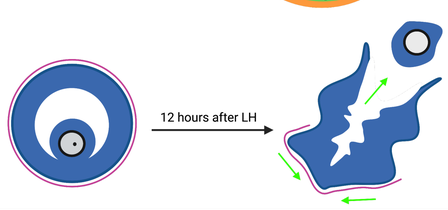


Figure 2. Current understanding of how the follicle forces the ooycte out during ovulation. Myofibroblasts (pink) contract in the final moments leading to ovulation, resulting in oocyte release and basal lamina rupture.

Hypothesis

In this project, we questioned if forces **inside** of the basal lamina could contribute to the process of follicle rupture (Figure 3). LH has previously been reported to cause cell shape changes and migration in isolated granulosa cells (Karllson et al., 2010, Bianco et al., 2018).

Since cell migration can regulate tissue morphology in other systems, we investigated whether granulosa cells migrate in vivo, and if so, how it could contribute to the ovulatory process.

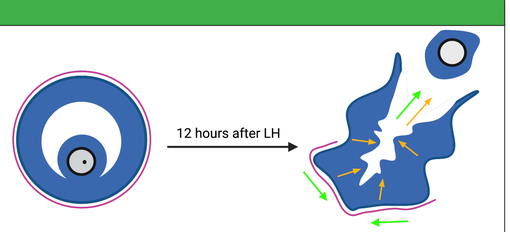


Figure 3. Hypothesis for forces **inside** and **outside** of the basal lamina contributing to ovulation and follicle rupture.

Methods and Experimental Design

- Previously, we generated a mouse model with a hemagluttinin tag on the endogenous LH receptor (HA-LHR). This allows visualization of the receptor at cellular and subcellular resolution using a commercially available anti HA antibody (Baena et al., 2020).
- Intraperitoneal injection of kisspeptin-54 leads to a surge-like release of endogenous LH with a peak at 90 min after injection, and the release is similar in amplitude and duration to the endogenous LH surge (Owen et al., 2021, Figure 4).
- Using these two advances, we generated the following experimental design to investigate the localization of LH receptor expressing cells (Figure 5).

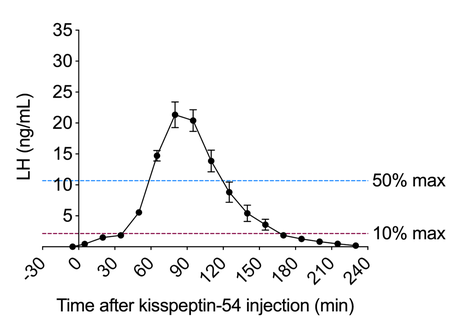


Figure 4. Kisspeptin-54 injection induces surge-like release of LH that peaks 90 min after injection. From Owen et al., 2021.

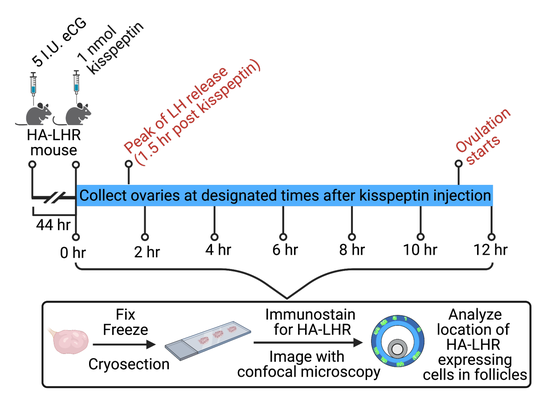


Figure 5. Experimental design to investigate changes in mural granulosa cell localization during the periovulatory period.

LH induces rapid ingression of LH receptor expressing cells

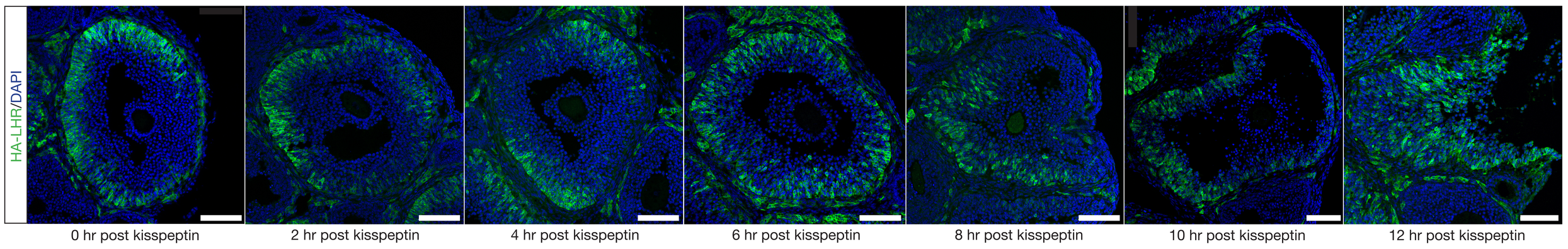


Figure 6. Time course of HA-LHR expressing cell localization during kisspeptin-induced ovulation. HA-LHR = green, DAPI = blue, scale bars = 100 µm.

LH induces ingression of LH receptor expressing cells and an epithelial-to-mesenchymal-like transition

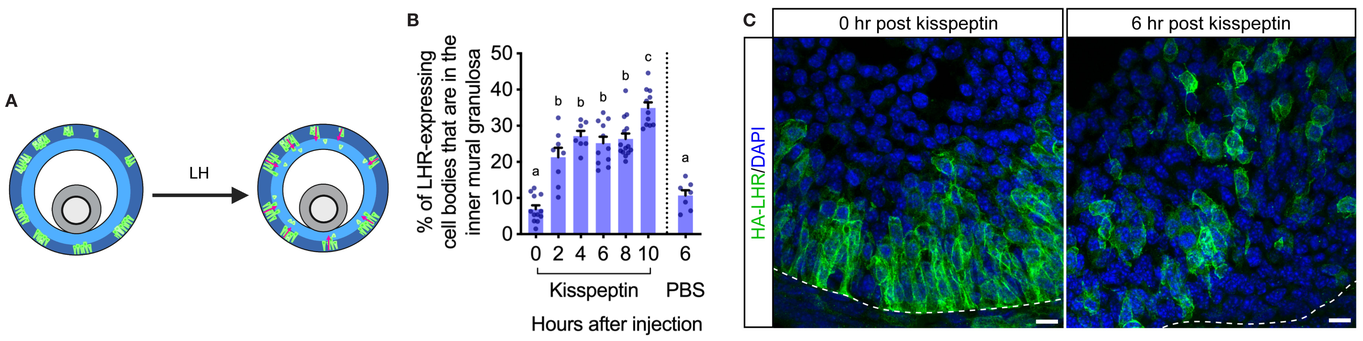


Figure 7. LH induces rapid ingression of HA-LHR expressing cells. **A)** Model for inward migration. **B)** The percent of total HA-LHR expressing cell bodies that are in the inner mural at different times after kisspeptin injection. Different letters represent significantly different values ($P<0.05$). **C)** HA-LHR expressing cells undergo morphological changes in response to LH. HA-LHR = green, DAPI = blue, scale bars = 10 µm.

LH induced ingression occurs in naturally cycling adult mice

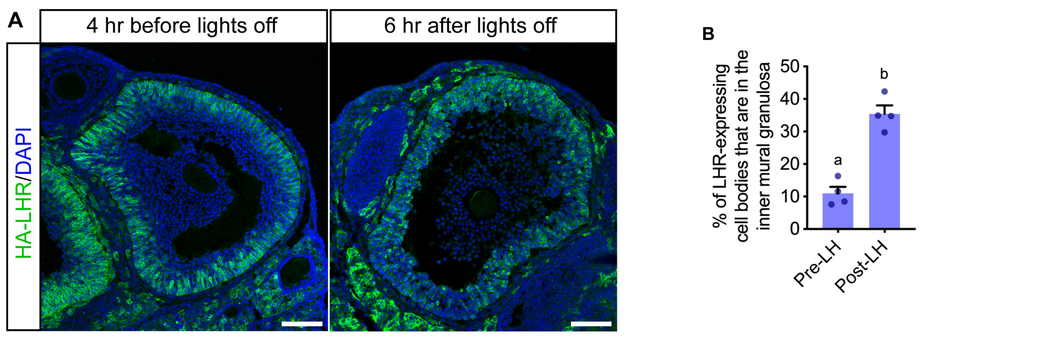


Figure 8. LH induces rapid ingression of HA-LHR expressing cells in naturally cycling adult mice. **A)** Example follicles from HA-LHR mice on the day of proestrus either 4 hours before lights off (left) or 6 hours after lights off (right). The LH surge begins at lights off in cycling females (Czieselsky et al., 2016). HA-LHR = green, DAPI = blue, scale bars = 100 µm. **B)** The percent of total HA-LHR expressing cell bodies that are in the inner mural. Different letters represent significantly different values ($P<0.05$).

Structural changes potentially mediated by ingression

Basal lamina invaginations and constrictions on the basolateral sides of the follicle

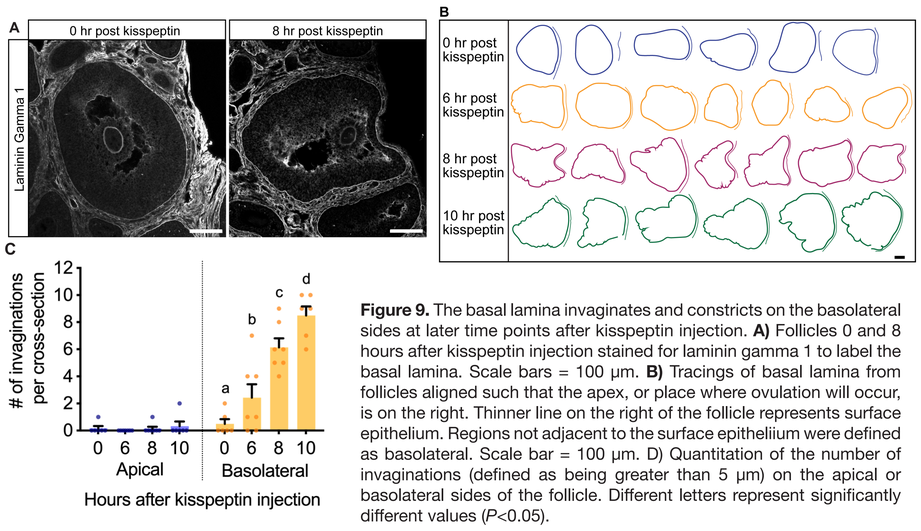


Figure 9. The basal lamina invaginates and constricts on the basolateral sides at later time points after kisspeptin injection. **A)** Follicles 0 and 8 hours after kisspeptin injection stained for laminin gamma 1 to label the basal lamina. Scale bars = 100 µm. **B)** Tracings of basal lamina from follicles aligned such that the apex, or place where ovulation will occur, is on the right. Thinner line on the right of the follicle represents surface epithelium. Regions not adjacent to the surface epitheliium were defined as basolateral. Scale bar = 100 µm. D) Quantitation of the number of invaginations (defined as being greater than 5 µm) on the apical or basolateral sides of the follicle. Different letters represent significantly different values ($P<0.05$).

Mural granulosa layer thins on the apical side where ovulation will occur

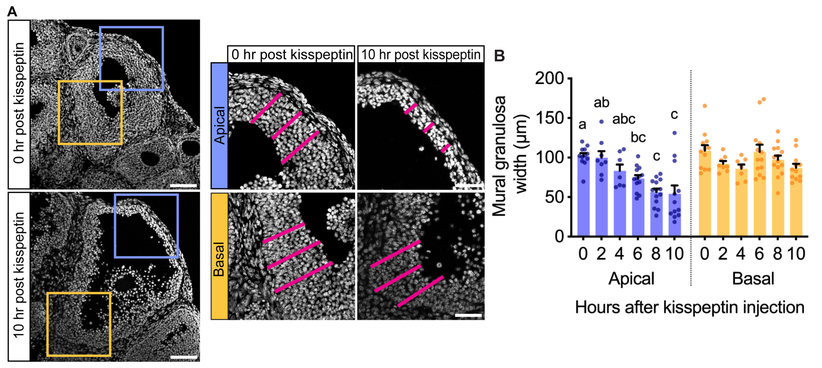
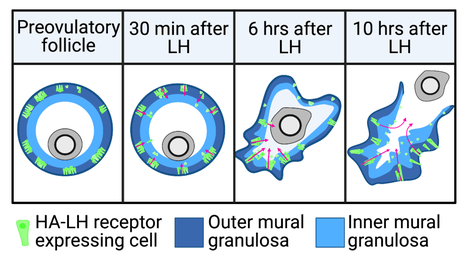


Figure 10. The mural granulosa layer in the apical, but not the basal, region thins in response to LH. **A)** Follicles at 0 and 10 hours post kisspeptin stained for DAPI (gray). The apical (blue) and basal (orange) sides of the follicle are highlighted by boxes and shown magnified on the right. The pink lines represent the width measured in three places 50 µm apart. Scale bars = 100 µm for images on left, 50 µm for images on right. **B)** Quantification of the width of the mural region at the apical (blue) or basal (orange) region. Each dot represents the average of the three measurements for an individual follicle. Different letters represent significantly different values ($P<0.05$).

Conclusions

- LH stimulates rapid ingression of cells expressing its receptor.
- The follicle undergoes dramatic structural changes starting about 6 hours after LH stimulation.
- Ovulation occurs ~10 hrs post LH peak.



Future Directions

- **What is the mechanism of LH induced migration?**
  - Cofilin is a promising candidate - dephosphorylated, and thus activated, in response to LH and responsible for changes in motility in isolated granulosa cells in vitro (Karllson et al., 2010).
- **What role does ingression play in structural changes in the follicle and ovulation?**
  - We are making a mouse in which cofilin cannot be dephosphorylated and activated in granulosa cells. We will investigate ingression, structural changes in the follicle, and ovulation.

References:
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