Correlative live cell imaging and serial section scanning electron microscopy of Arabidopsis ovules during fertilization

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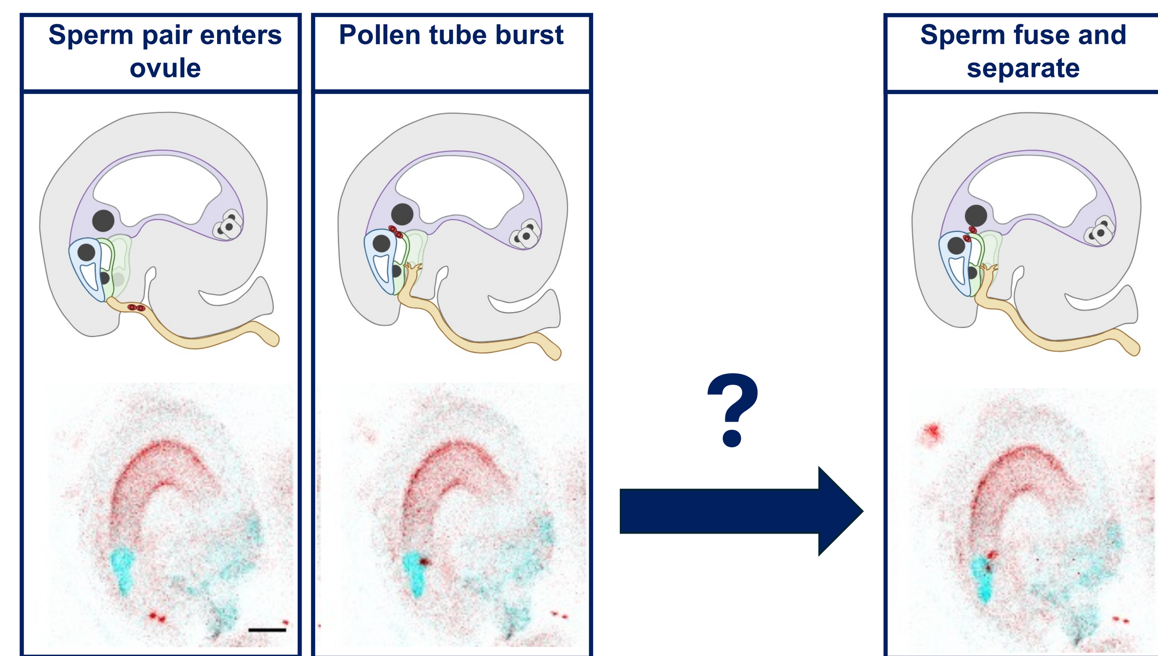
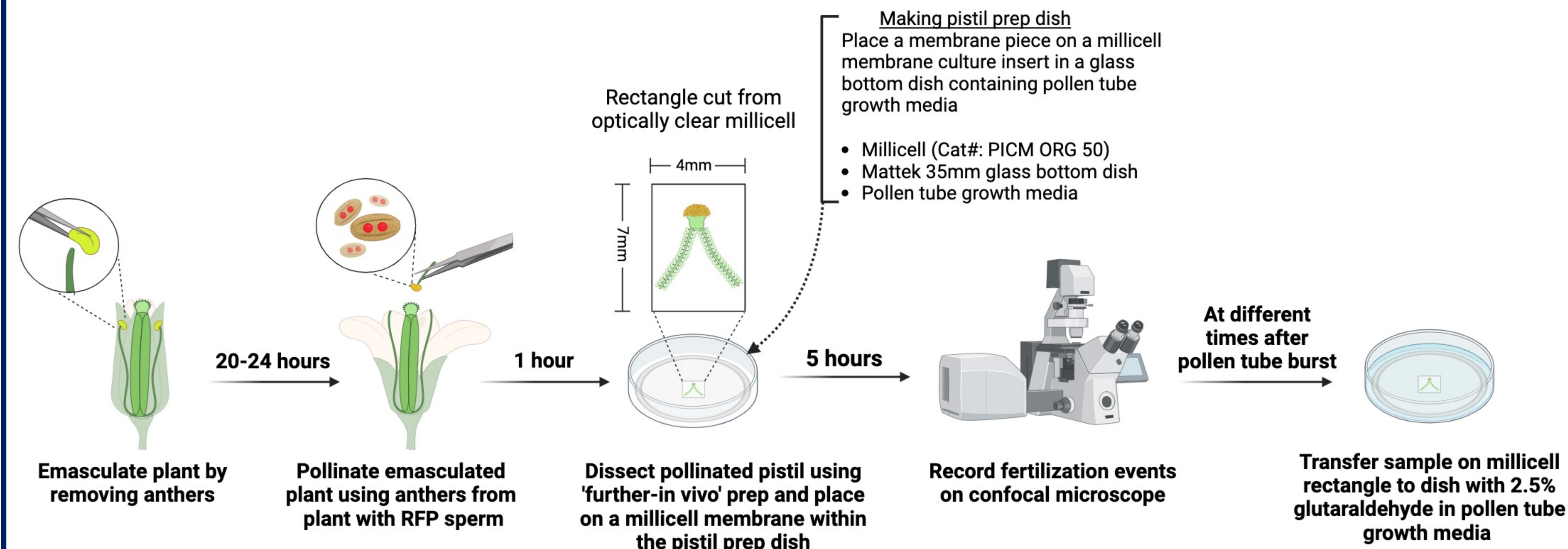
Introduction

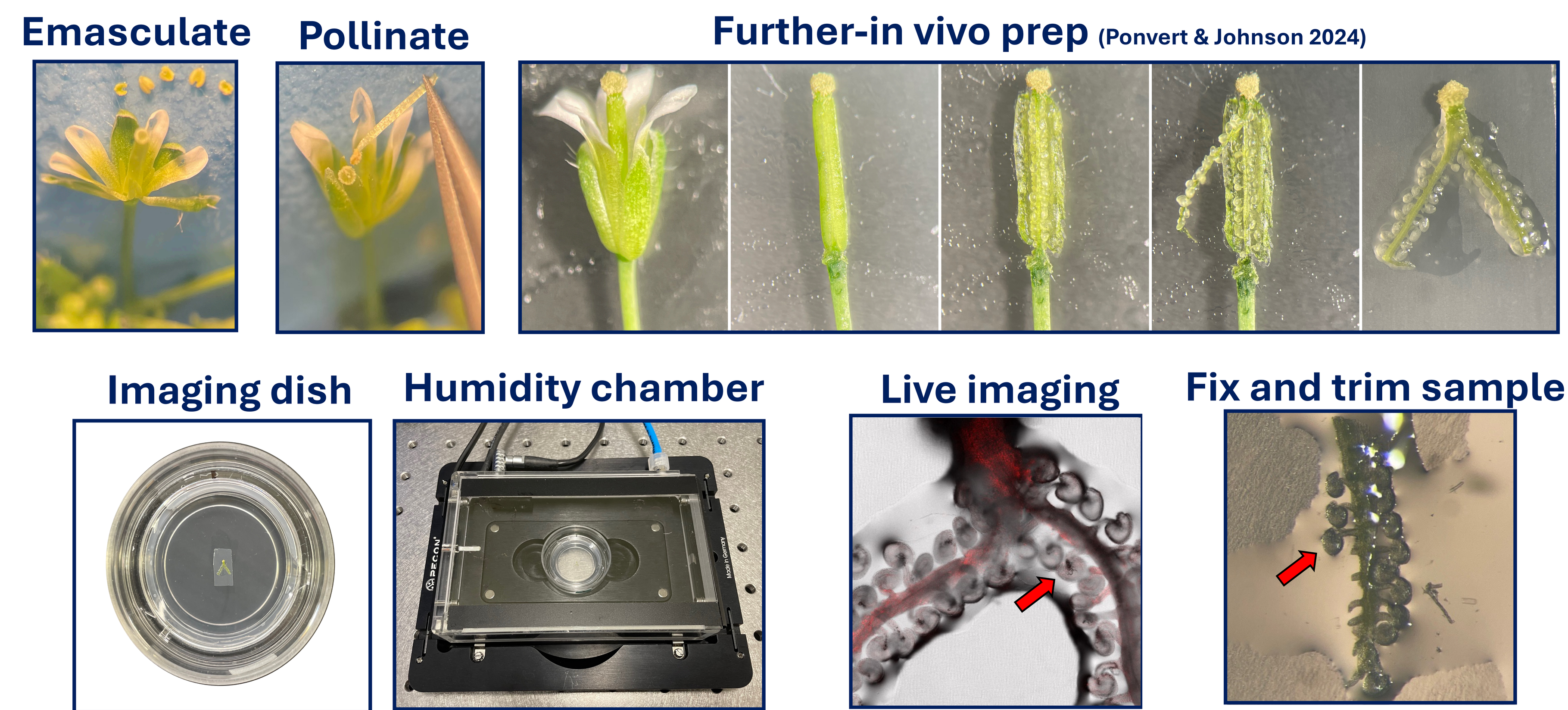
Live cell imaging of Arabidopsis ovules during fertilization has provided valuable insights (Hamamura et al., 2011), but ultrastructural information is limited. Here we describe a method for live cell imaging of pistils to identify the time of pollen tube bursting, based on Ponvert and Johnson, 2024, for subsequent preparation of the same ovule for electron microscopy. This workflow will be useful for investigating sperm interactions with the egg and central cell, and for comparing wildtype sperm with sperm lacking the fusogen HAP2.



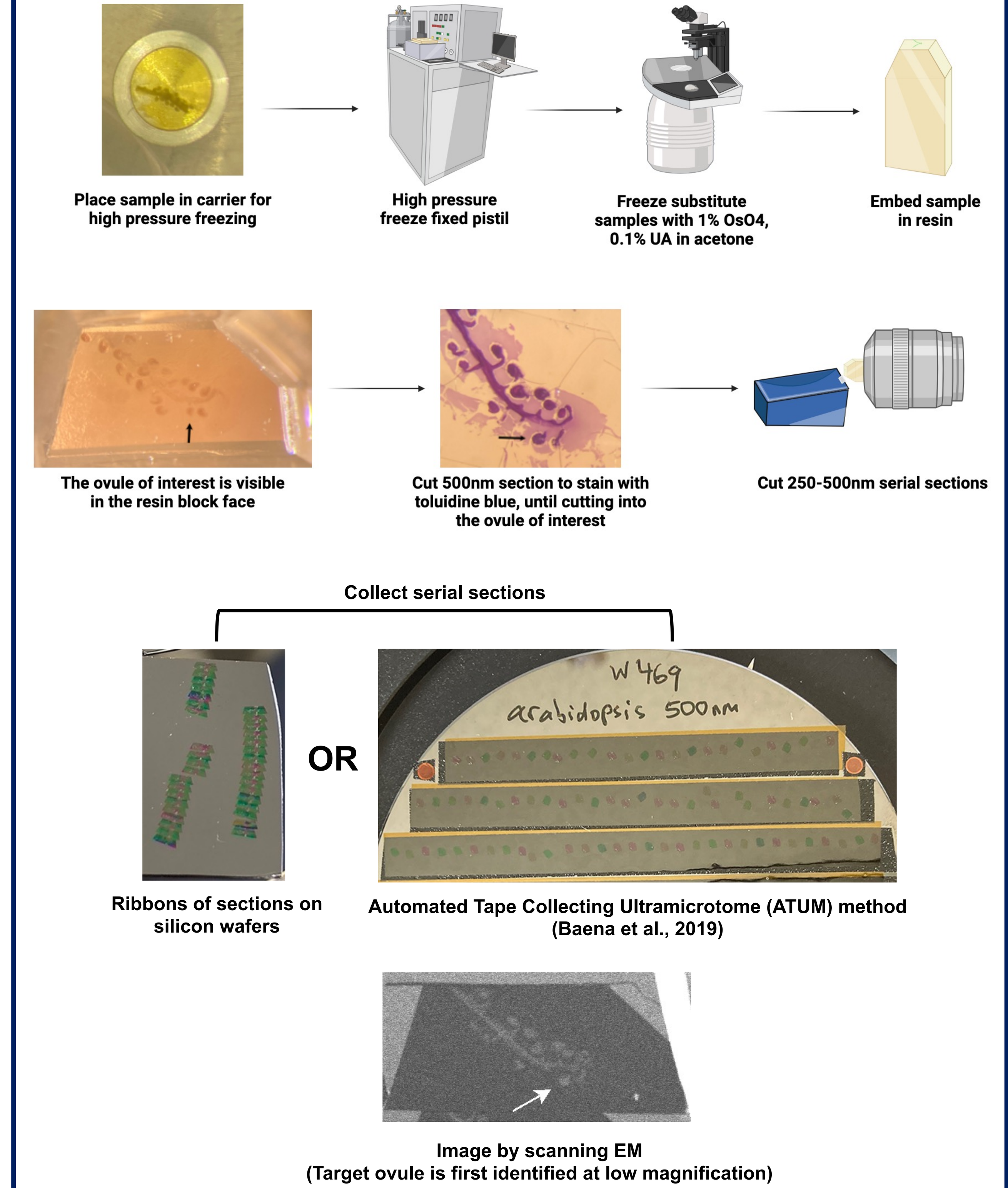
Main question: What events occur between the time the pollen tube bursts, releasing the sperm pair, and the time the sperm fuse and nuclei begin to separate?

Pistil prep and live cell imaging





EM processing



Examples of images by scanning electron microscopy

Identifying structures in ovule

Unpollinated ovule: Central cell, Egg, Synergid cell, Filiform apparatus, 5μm

Ovule 10 minutes after pollen tube burst: Pollen tube, Degenerated synergid cell, Sperm pair, 10μm, 5μm

Electron microscopy images of ovules without and with pollen tube burst:
The unpollinated ovule consist of the egg, central cell, and intact synergid cells. Image of the ovule after pollen tube burst shows pollen tube and sperm pair within the degenerated synergid cell. Serial sections allowed us to confirm that the sperm pair was within close proximity to the egg (not shown here).

Wildtype ovule X HTR10:RFP sperm

Confocal microscopy — Pollen tube burst: Before; 2 minutes after (25μm)

Electron microscopy (Zoom-in): Egg, Sperm, 10μm, 5μm, 2μm

Zoomed-in high resolution images of an ovule and sperm:
Serial sections allow for the sperm and egg to be located within the sample.

HAP2 knockout (self-pollinated)

Egg, Sperm, 10μm; Sperm 1, 3μm; Serial section; Sperm 2, Sperm 1, Degenerated synergid cell, 5μm; Sperm 2, 5μm

Zoomed-in high resolution images of serial sectioned sperm and egg:
Serial sections allow for both sperm cells to be identified. These images offer insight on where the sperm are localized in relation to the egg after the pollen tube bursts.

Conclusions

1. Millicell inserts allow for live imaging of fertilization and a means for fixing pistil tissue.
2. High pressure freezing after fixing the tissue yielded the best morphology.
3. Collecting serial sections for scanning EM imaging ensures we can locate gametes within ovules and will allow high resolution imaging of sperm contact sites with the egg and central cell.

Future Directions

- Examine more time points between pollen tube bursting and sperm nuclei separation.
- Compare the appearance of sperm from Hap2(-/-) pollen at defined times after pollen tube bursting.

References

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