הכינוס השנתי ה-67 של
האגודה הישראלית לחקר הפוריות (איל"ה)
12 במאי 2025, מלון דיוויד אינטרקונטיננטל, תל אביב
האגודה הישראלית לחקר הפוריות (איל"ה)
THE ISRAEL FERTILITY ASSOCIATION (IFA)
الجمعية الإسرائيلية لأبحاث الخصوبة

DIALOGUE ON THE FLY: DROSOPHILA AS A MODEL FOR INTER-SEX AND INTER-ORGAN COMMUNICATION IN THE FEMALE REPRODUCTIVE TRACT

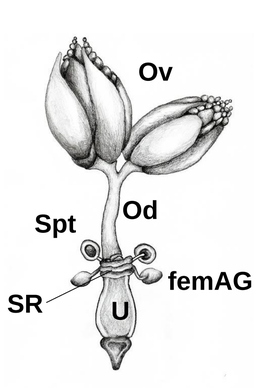
Michael Belenky, Javier Arturo Sanchez-Lopez, Shani Chen Kornhaeuser, Amalia Gothilf, Ido Apel, Yaara Itzhak, Yael Heifetz

Department of Entomology, The Robert H. Smith Faculty of Agriculture, Food and Environment, Hebrew University, Rehovot

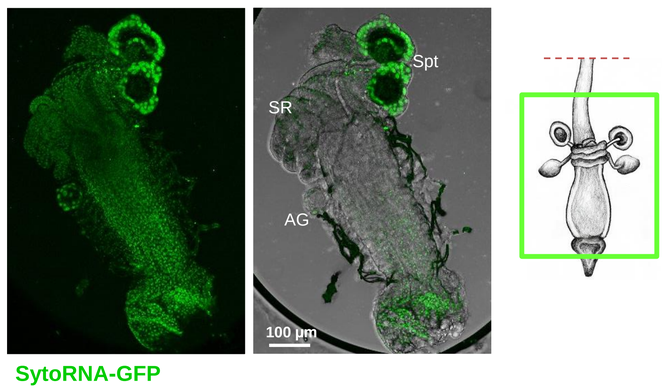
INTRODUCTION

Intercellular communication within the female reproductive tract is essential for reproductive success in internally reproduce organisms. Investigating communication inside the reproductive tract is challenging in higher organisms, such as humans, however, many processes are conserved throughout the animal kingdom. In *Drosophila melanogaster*, reproductive communication involves complex signaling between male and female tissues, with combined regulation of female fertility by both male and female-derived components. Successful reproduction requires maintenance of a crosstalk between the seminal fluid (SF) and the products of the two reproductive endocrine glands – the female accessory glands (femAG) and the spermathecae, and other secretory cells along the tract. Our study shows that this crosstalk orchestrates various processes, influencing sperm storage and release, oogenesis and egg laying, and therefore exemplifies a sophisticated level of inter-organ an inter-sex coordination. However, a comprehensive understanding of the crosstalk is lacking. Specifically, the dynamics of the cross-talk, its landscape, and how different levels of crosstalk can affect the overall function of the system. Here, we developed a systematic approach to identify potential interactions and deepen understanding of the potential link and roles of this crosstalk between and within the reproductive tracts of *Drosophila*. This study contributes to a deeper understanding of how reproductive communication supports fertility and may provide insights relevant to broader biological and biomedical contexts.

1. Seminal fluid reaches every organ of the female RT



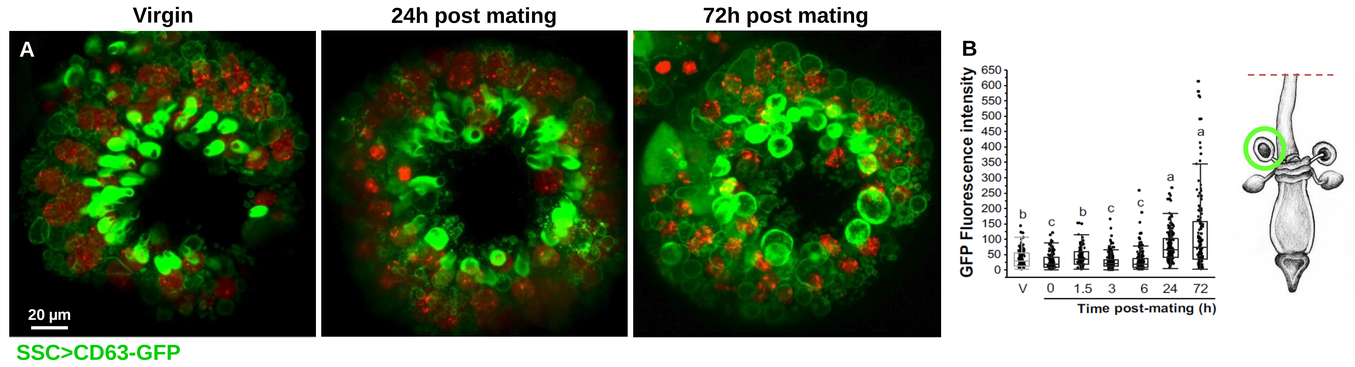
Schematic of the *Drosophila* female reproductive tract: Ov – ovaries, Od – oviduct, Spt – spermathecae, femAG – accessory glands, SR – seminal receptacle, U - uterus



SytoRNA-GFP

Uptake of seminal fluid-derived RNA by all female reproductive organs and tissues, with high concentration in the spermathecae. Representative confocal maximum projection image of a female RT after incubation *ex vivo* with SytoRNA-stained male RT secretion.

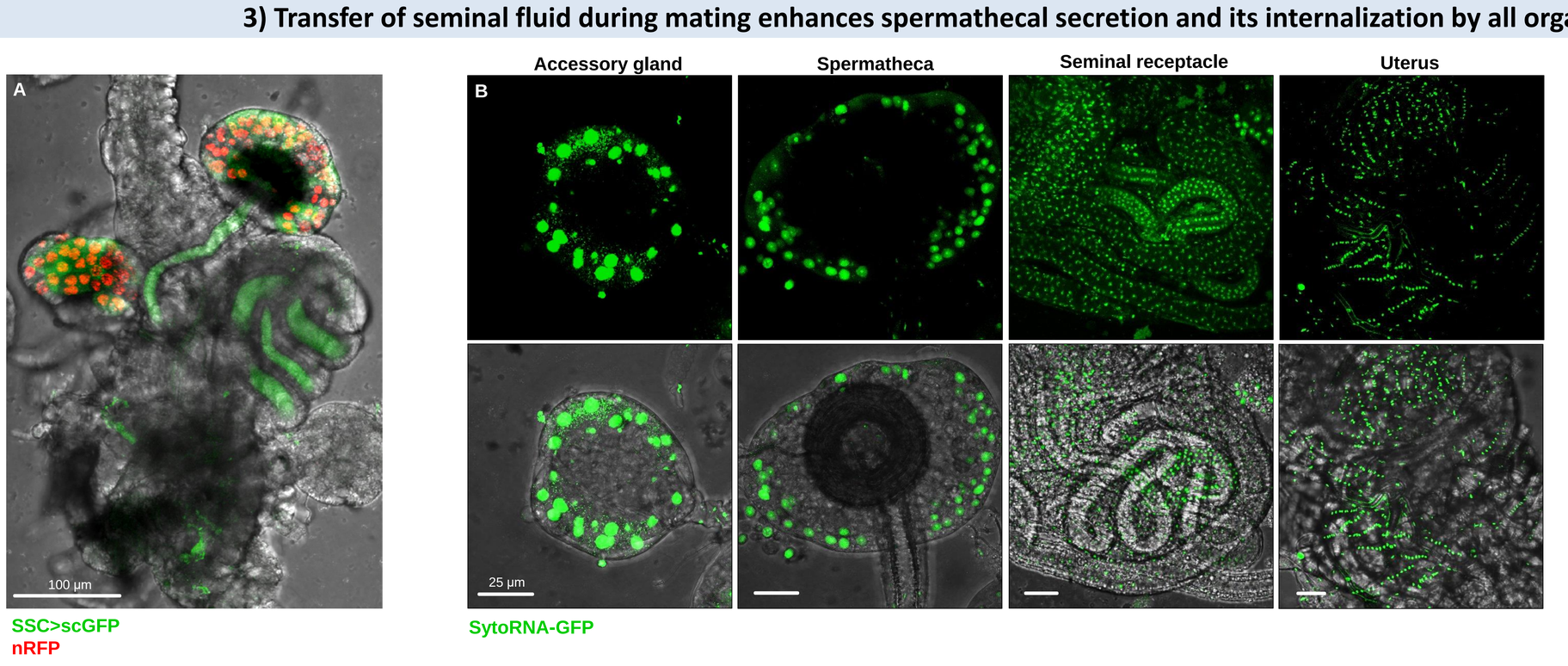
2. Seminal fluid modulates the physiology of the female secreting glands



SSC>CD63-GFP
nRFP

Mating induces morphological and functional changes in the secretory cells of the spermathecae. A. Representative confocal maximum projection z-stack images of CD63-GFP expressed in the spermathecal secretory cells, showing a gradual change in secretory cell morphology and secretory activity following mating. **B.** Time-dependent change in secretion-dependent fluorescence in spermathecal secretory cells following mating. Letters denote significant difference (one-way ANOVA, with multiple comparison post-hoc test, $p<0.05$).

3) Transfer of seminal fluid during mating enhances spermathecal secretion and its internalization by all organs of the female RT

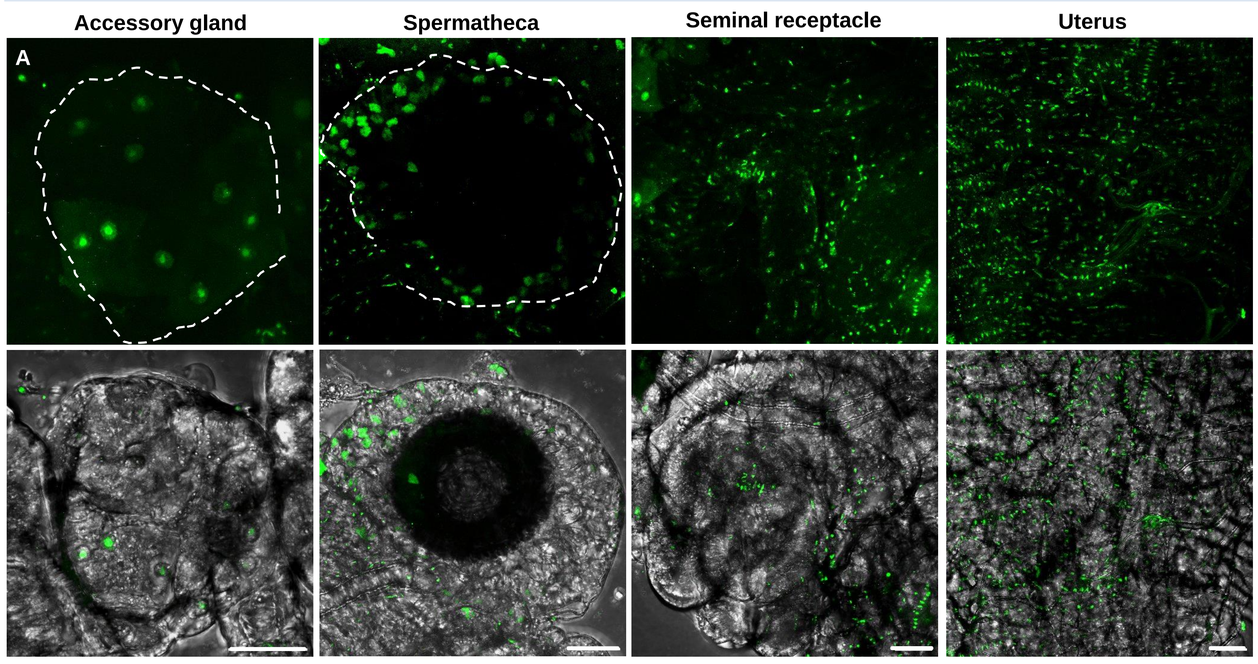


SSC>scGFP
nRFP

SytoRNA-GFP

Spermathecal secretion and its uptake by the female RT are increased following mating. A. Confocal maximum projection z-stack image of the female RT expressing secreted GFP in the spermathecae showing its diffusion throughout the system. **B.** Representative confocal maximum projection z-stack images of the major organs of the female RT after *ex vivo* incubation with spermathecal secretions stained with SytoRNA showing uptake by all major organs of the female RT. **C.** Particle distribution in spermathecal secretions of virgin vs. mated females. **D)** Fluorescence intensity comparison between organs of virgin and mated female RT after incubation with respective spermathecal secretions stained with SytoRNA, *p>0.05

4) Spermathecal extracellular vesicles are involved in intra-organ communication within the female RT



SytoRNA-GFP

SSC>Myr-RFP

Extracellular vesicles play a role in the communication of the spermathecae with the rest of the female RT. A. Representative confocal maximum projection z-stack images of the major organs of the female RT after ex vivo incubation with spermathecal extracellular vesicles (i.e., exosomes), isolated using a SmartSecTM exosome isolation system, stained with SytoRNA, are taken up by all major organs of the female RT. **B.** Fluorescence intensity of the different female RT organs after incubation with either whole or exosome-enriched SytoRNA-stained spermathecal secretions, showing significant (*$p<0.05$) decrease in fluorescence only in spermathecae, but not in other organs. This suggests that extracellular vesicles play a major role in communication with these organs but less so between the spermathecae themselves. **C.** Representative confocal maximum projection z-stack image of a spermatheca expressing myristoylated(myr)-RFP in the secretory cells showing myr-RFP-positive exosomes being secreted into the spermathecal lumen.

CONCLUSION

Inter-sex and inter-tissue communication is an essential part of reproduction. Here we show that uptake of seminal fluid is systemic and occurs in every organ of the female RT. In addition, we show how mating enhances secretion of the endocrine glands and the uptake of these secretions by other organs. Moreover, we provide evidence for involvement of extracellular vesicles (EVs) in the communication of the endocrine glands with the female RT. Both spermathecal and femAG-derived EVs are internalized by all other organs, delivering their RNA cargo to the nuclei of the recipient cells (data not shown), suggesting their regulatory roles in physiological processes throughout the female RT. Our next step will be to confirm release of distinct populations of EVs targeted for specific organs and the dynamics of their release and uptake. EVs have lately been of great interest in in reproduction. Here we use *Drosophila* as a model organism for EV-based reproductive communication. Lessons learned from *Drosophila* could be translated into human and livestock models, with the potential of being implemented into diagnostic and therapeutic tools in the future.