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HFSP CASE STUDY

Longterm Fellowship: Unlocking the Molecular Key to Fertilization

Molecular and physical mechanisms of gamete binding and fusion in zebrafish

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Client
Human Frontier Science Program Organization (HFSPO)

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Project Title	**Molecular and physical mechanisms of gamete binding and fusion in zebrafish**
Starting Year	2020
Principal Investigator	Victoria Deneke
Host institution	Research Institute of Molecular Pathology
Host country	Austria
Supervisor	Andrea Pauli
Disciplines	Cell Biology, Molecular Biology, Chemical Engineering, Developmental Biology
Task	Investigate the molecular mechanisms by which sperm and egg recognize and bind each other during vertebrate fertilization,
Key output	Identification of a conserved three-protein sperm complex required for egg recognition across vertebrates

In addition to the cited documents, interviews with the researchers and HFSP data were used as sources. Furthermore, quantitative analyses were carried out using the Dimensions Database.

1 Overview

The union of sperm and egg is the most fundamental encounter in sexually reproducing life. Despite its relevance, the molecular mechanisms and processes that make it possible have remained remarkably poorly understood. Scientists knew that specific proteins on the surface of sperm and egg cells were required for fertilization, but no one had been able to explain how those proteins worked together, or even whether they did. Fertilization research faced a structural problem: the proteins involved reside in lipid membranes, making them technically difficult to study with classical biochemical approaches. As a result, researchers had spent decades cataloguing individual molecular players without being able to assemble them into a coherent picture of the process.

This HFSP-funded project set out to change that. Led by a postdoctoral fellow with an engineering background who brought a deliberately cross-disciplinary perspective to the problem, and supported by the Research Institute of Molecular Pathology, the project aimed to go beyond single molecules and understand how the components of fertilization work as a system. The timing of a transformative new technology changed both the approach and the scale of what became possible. The resulting discovery identified a conserved three-protein complex on sperm that functions as a molecular key to the egg, present across vertebrates from fish to humans, and attracted international press attention within days of publication.

2 The Project

The project's research question emerged from a persistent gap in fundamental biological knowledge. Despite fertilization being essential for sexual reproduction across all vertebrates, the molecular mechanisms enabling sperm-egg recognition and fusion remained largely mysterious. Previous research had identified individual proteins important for the process, for instance, the sperm protein Izumo1 and the egg receptor Juno in mammals. However, scientists were finding single "puzzle pieces" without understanding how they fit together or functioned as an integrated system.

The HFSP project aimed to tackle this fundamental question using multidisciplinary approaches. Trained as a chemical engineer and holding a PhD in molecular biology, the fellow brought a broad background into the project and joined the laboratory at the Research Institute of Molecular Biology, which had been working on the topic.

Zebrafish served as the primary model organism. These vertebrates share many fertilization molecules with mammals, including humans, but release their eggs and sperm into water, making them more experimentally tractable than mammalian systems while maintaining biological relevance.

The project required methodological innovation and adaptability. During the first year, a game-changing technology emerged: AlphaFold. This AI-powered program from Google DeepMind could predict three-dimensional protein structures from amino acid sequences and, crucially, model how multiple proteins might assemble into complexes. Recognizing its potential, she became an early adopter, feeding the program the known sperm surface proteins into the program and screening them against a library of approximately 1,400 proteins found on zebrafish testis cell surfaces to look for predicted interaction partners.

This technological pivot represented the kind of adaptive, multidisciplinary approach HFSP funding enables. While the core research question remained unchanged, the methodology shifted fundamentally to leverage this breakthrough. The HFSP work, together with related activities in the lab, combined:

- Computational predictions to identify candidate protein complexes;

- Genetic validation using CRISPR/Cas9 to knock out genes and test their necessity for fertilization;
- Biochemical experiments to confirm predicted interactions in biological samples;
- Cross-species verification in zebrafish, mice, and human cells to establish evolutionary conservation.

The team used CRISPR/Cas9 gene editing to knock out the gene for Tmem81 in zebrafish. The male fish produced sperm that swam normally – but could not fuse with an egg. They were infertile. The same result was obtained in mice. Biochemical pull-down experiments then confirmed that the three proteins physically associated with one another in zebrafish and human sperm, forming the complex AlphaFold had predicted.

3 Outcomes and Impact

The research, published in Cell in October 2024, revealed a fundamental three-protein complex on sperm that acts as a universal key for fertilization across vertebrates. The complex consists of Izumo1 (previously known), Spaca6 (previously known) and Tmem81 (newly discovered). Next to the discovery of the new protein, the study provided the first evidence that multiple sperm proteins must come together as a complex, in the right structural configuration, to recognize and bind the egg. Without any of the three components, fertilization fails. The research furthermore revealed striking evolutionary patterns: The three-protein sperm complex has remained virtually unchanged across millions of years of vertebrate evolution, from fish to mammals. This complex exists in nearly all sexually reproducing male vertebrates studied.

The conceptual shift this represents is, in the fellow's framing, a move from single molecules to systems: the field now asks how proteins work together, not merely which proteins matter individually. The initial discovery of three proteins has proven to be just the beginning, as the research team has now identified seven in fish and ten in humans. Remarkably, eight of these ten human proteins had been previously identified by other researchers over the past 20-30 years, but their function remained unclear. The findings of the fellowship project and the adjacent research group hence delivered explanations.

While the work represents fundamental research, it opens pathways for clinically relevant advances in research on infertility and contraception. Screening for mutations in the newly identified complex components could help explain cases of infertility that currently have no molecular diagnosis. At the same time, ff the sperm proteins must assemble into a complex to recognize the egg, a molecule that prevents assembly – or blocks the binding interface – could in principle prevent fertilization without hormonal intervention.

The work attracted significant attention as it was featured in featured in major media outlets and scientific venues including The New York Times, Nature, Science, and GEO magazine. The developers of the AlphaFoldAI technologies used in the HFSP project to predict protein structures were awarded the 2024 chemistry Nobel prize.

4 Role of HFSP and Conclusion

This project exemplifies HFSP's mission to enable frontier research that fundamentally reshapes biological understanding. The project has contributed to revealing how fertilization proteins work as integrated molecular complexes rather than isolated components opening new directions in reproductive biology. Beyond advancing basic knowledge, it creates pathways toward addressing human infertility and developing non-hormonal contraceptive approaches in the long run, demonstrating how fundamental research ultimately serves societal needs.

The project also showcases HFSP's ability to adapt to rapidly evolving scientific landscapes. The emergence of AlphaFold mid-fellowship – a technology that would earn its developers the 2024 Nobel Prize in Chemistry – required exactly the kind of adaptive flexibility that HFSP's trust-based funding model enables. By focusing on ambitious goals and innovative thinking rather than prescriptive methods, HFSP allowed the fellow to pivot toward breakthrough technology while maintaining her fundamental research question. The case demonstrates the value in supporting early-career researchers with interdisciplinary backgrounds to tackle ambitious questions with fresh approaches; while providing the time, flexibility, and community to pursue their vision.

5 Sources

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