

Structural diversity of the spermatozoon end piece

Yui Yee Chow¹, Flavia Bieczynski¹, Davide Zabeo¹, Jacob T. Croft^{1,2}, Johanna L. Höög¹

¹Department of Chemistry and Molecular Biology, University of Gothenburg, Gothenburg, Sweden;

²Department of Medicinal Chemistry, University of Washington, Seattle, USA

Flagella (cilia) are conserved microtubule-based organelles that play essential roles in motility and sensing across eukaryotes. In mammalian spermatozoa, the flagellum drives propulsion, and defects in its structure are linked to disorders such as primary ciliary dyskinesia. The axoneme, characterised by a conserved “9+2” microtubule arrangement, forms the structural core of motile flagella. However, spermatozoa exhibit species-specific differences in the composition and organisation of proteins associated with these microtubules [1].

The distal end piece, where the symmetry of the canonical 9+2 axonemal structure is progressively lost, remains understudied. Current understanding of this region is largely inferred from a limited number of model organisms and often assumed to extend across species, including human spermatozoa. However, recent studies show that the human spermatozoa end piece deviates from these expectations. Variability in doublet termination and singlet microtubule persistence suggests that distal flagellar architecture is more heterogeneous than previously appreciated [2].

Despite this, the end piece is frequently treated as a passive or inactive region in biophysical models of spermatozoa motility. This assumption is challenged by recent work demonstrating that a distal inactive segment enhances propulsive thrust and swimming efficiency compared to a fully active flagellum of equal length, indicating a functional role for this region [3].

Here, we present a comparative meta-analysis of spermatozoa flagellar dimensions across four vertebrate species, combined with ultrastructural characterisation of end piece microtubule organisation using cryo-electron microscopy and tomography. We model distal microtubule arrangements, revealing variation in end piece morphology, including differences in microtubule number and doublet-to-singlet transition patterns.

Together, these findings challenge the assumption that distal axonemal organisation can be generalised from model organisms and highlight the end piece as a structurally diverse and functionally significant component of the spermatozoon flagellum.

- [1] M. R. Leung, *et al.*, “The multi-scale architecture of mammalian sperm flagella and implications for ciliary motility,” *EMBO J.*, vol. 40, no. 7, pp. EMBJ2020107410, Mar. 2021, doi: 10.15252/embj.2020107410.
- [2] D. Zabeo, J. T. Croft, and J. L. Höög, “Axonemal doublet microtubules can split into two complete singlets in human sperm flagellum tips,” *FEBS Lett.*, vol. 593, no. 9, pp. 892–902, May 2019, doi: 10.1002/1873-3468.13379.
- [3] C. V. Neal, A. L. Hall-McNair, J. Kirkman-Brown, D. J. Smith, and M. T. Gallagher, “Doing more with less: The flagellar end piece enhances the propulsive effectiveness of human spermatozoa,” *Phys. Rev. Fluids*, vol. 5, no. 7, pp. 073101, Jul. 2020, doi: 10.1103/PhysRevFluids.5.073101.