FINAL REPORT SEED GRANT PROJECT:

"Avatar mouse model of a new genetic variant of ARSACS detected in Uruguay."

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The first clinical case of ARSACS in Uruguay was reported in 2023 in a three-year-old girl (Raggio et al., 2025). Genetic testing (exome sequencing) revealed two mutations in the SACS gene, both of which were found in exon 10. One of these mutations was already known to be harmful; it causes a frameshift that leads to a premature stop in the gene (p.His1915Argfs*19). The second mutation (p.Pro2369Leu) was predicted to be damaging based on computer analysis.

The aim of this project was to create an ARSACS mouse model using CRISPR/Cas gene-editing technology. This **'avatar mouse'** would carry the same two mutations as the girl. To achieve this, two separate mouse lines were required, one for each of the girl's mutations.

Using our own expertise and resources, we edited mouse embryos (zygotes) from the C57BL/6J strain and implanted them in female mice. This resulted in the birth of 'founder' mice:

- For the paternal mutation (frameshift), three out of seventeen pups were successfully edited (17.6%).
- For the maternal mutation (missense), two out of four pups were successfully edited (50%).

These founder mice were then bred with regular C57BL/6J mice to create the next generations (N1 and N2). The breeding was successful, and the mice produced litters of the expected size. After weaning, the pups were genotyped to select the most suitable individuals for establishing a stable colony. So far, neither the edited mice nor their offspring have shown any behavioural issues. The next step will be to cryopreserve the genetic lines to prevent any future genetic drift.

To create the full ARSACS model, the two mouse lines carrying the frameshift and missense mutations will be crossed. Plans for the future include running behavioural tests, studying brain tissue using immunohistochemistry and measuring Sacs protein levels using Western blot analysis.