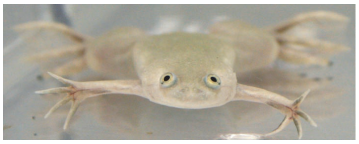
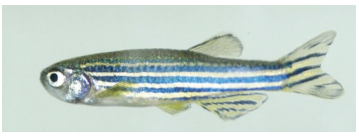
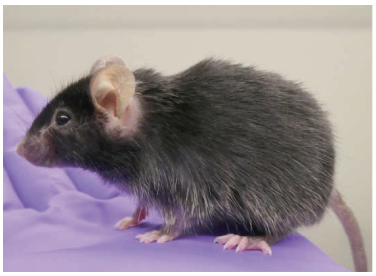
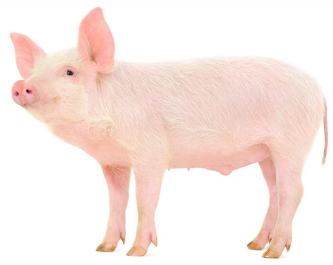
FSHD model organisms: Overview (Pt 1)

The drive to develop treatments and a cure for FSHD requires the ethical use of animals and FSHD-like models in research.









Animal research, including using frogs, zebrafish, mice, pigs, and non-human primates (for toxicology) is vital to developing and getting an experimental FSHD therapy safely to the clinic and ultimately to patients.

We understand that this can be a sensitive issue. Unfortunately, there is a lot of misinformation out there. We will not find a cure for FSHD without the limited use of appropriate animal models and systems.

All animal research in the USA is governed by the U.S. Department of Agriculture and Animal Welfare Act, which sets high standards of care for research animals.

The U.S. Public Health Service (PHS) Act requires that all institutions receiving federal funding from the National Institutes of Health (NIH), the Food and Drug Administration (FDA), or the Centers for Disease Control and Prevention (CDC) adhere to the standards set forth in the Guide for the Care and Use of Laboratory Animals.

Animal research is overseen locally at each PHS funded institution by an Institutional Animal Care and Use Committee (IACUC). IACUCs include a veterinarian and an unaffiliated community representative. IACUCs require researchers to justify the need for animals and to use the lowest possible numbers to answer a specific question. IACUCs can reject inadequate proposals.

Scientists are mandated to adhere to the 3Rs: Reduction, Replacement, and Refinement to reduce the overall number of animals used in a study.

Animal research must be conducted ethically at all times, and only when alternatives to obtaining the required information in a whole living system are not available.

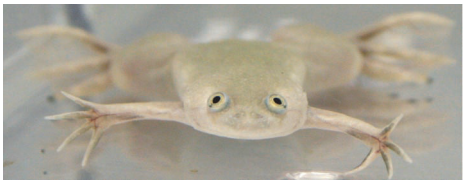
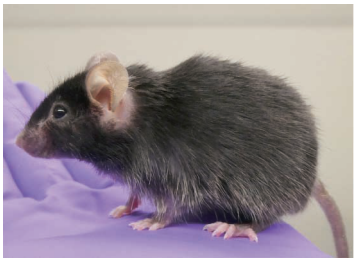
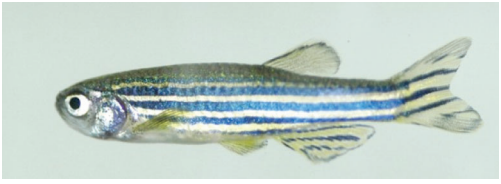
For more information on the necessity and benefit of using animals in biomedical research, please visit the Foundation for Biomedical Research (https://fbresearch.org/)

In addition, see “Animal Research Perceptions vs Reality: A Digital Flipbook

https://www.flipsnack.com/FBResearch/animal-research-perceptions-vs-reality.html

FSHD model organisms: Overview (Pt 2)

The drive to develop treatments and a cure for FSHD requires the ethical use of animals and FSHD-like models in research.

FSHD research has used *Drosophila melanogaster* (fruit fly), *Xenopus laevis* (African clawed frog), *Danio rerio* (zebrafish), and *Mus musculus* (mouse) to better understand FSHD pathogenic mechanisms, model disease, and perform preclinical testing of potential FSHD therapeutics.

Many muscular dystrophies and neuromuscular diseases use natural or induced animal models of the disease caused by mutations in the endogenous animal (mouse or dog) genome giving rise to the disease in that model animal system.

Alternatively, the endogenous disease-causing genes can be studied for their normal and pathogenic functions using mice or dogs since their genes are very similar to the human gene.

However, while the DUX family of genes is found in all mammals, the *DUX4* gene that causes FSHD is specific to old world primates (i.e. humans, gorillas, chimpanzees, etc.), none of which are used in biomedical research anymore and will not be used in FSHD research.

Thus, in order to generate FSHD in a model organism, one must express the human *DUX4* gene in the desired system. Fortunately, since FSHD is a dominant gain-of-function disease, there are many techniques available to do this; however, it is technically challenging since DUX4 is very toxic.

Approaches for studying DUX4 expression and preclinical testing FSHD therapeutics in animal models:

- Inject DUX4 mRNA into developing embryos.
  - *Xenopus laevis* (Wuebbles *et al*. 2010. *Int. J. Exp. Pathol* 3:388-400.)
  - Zebrafish (Mitsuhashi *et al*. 2013. *Human Molecular Genetics* 22:568-77.)
- Inject virus expressing DUX4 into muscle (Wallace *et al*. 2011. *Ann Neurology* 69:540-52.)
- Electroporate DUX4 plasmid into muscle (Derenne *et al*. 2020. *Scientific Reports* 10:11301.)
- Make transgenic mouse with the D4Z4 region. (Krom *et al*. 2013. *PLoS Genetics* 9:e1003415.)
- Make transgenic animals expressing DUX4.
  - Fly (Jones *et al*. 2016. *PLoS One* 11:e0150938.)
  - Mouse (Bosnakovski *et al*. 2017 *Nature Communications* 8:550.)
    - (Jones *et al*. 2018. *PLoS One* 13:e0192657.)
    - (Giesige *et al*. 2018. *JCI Insight* 3:e123538.)
  - Zebrafish (Pakula et al. 2019. *Human Molecular Genetics* 28:320-331.)
- Make human xenografts of FSHD muscle in mice (Mueller *et al*. 2019. *Exp Neurol* 320:113011.)