Decreased fertility in female cystic fibrosis patients: deciphering the role of the endometrium using organoid models

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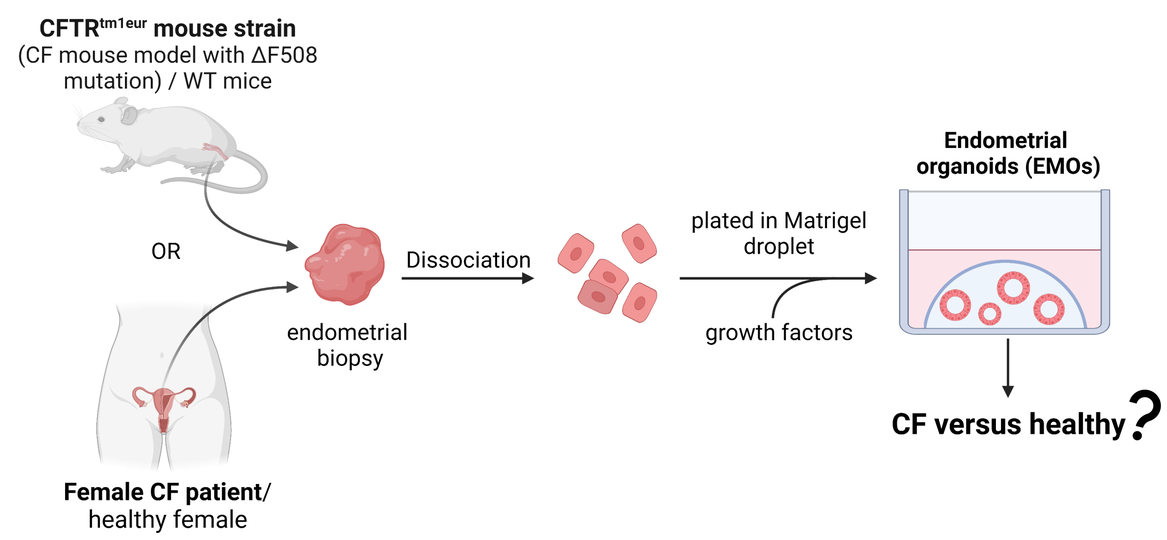
Background

Cystic fibrosis (CF) is the most common recessive **genetic disease** in Caucasians. CF is caused by mutations in the gene encoding CF transmembrane conductance regulator (**CFTR**), **a Cl^-/HCO_3^- channel** which plays a key role in the secretion of electrolytes and fluid in epithelial cells. Due to substantial increase in life expectancy over the past decade, CF is now considered a chronic disease of adults. Therefore, people with CF become increasingly interested in starting families. However, **female CF patients** suffer from **decreased fertility**. The position of the **endometrium** (i.e., the inner mucosal lining of the uterus) in this defective reproductive condition has only poorly been investigated, largely due to a **lack of reliable and flexible study models**.

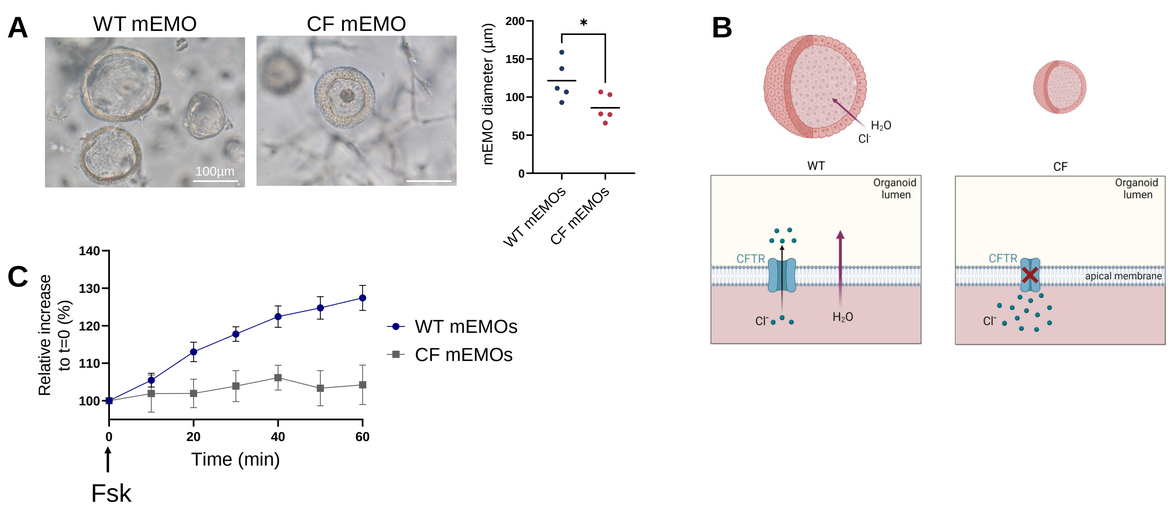
Goal and methodology

Deciphering role of the endometrium in decreased fertility of female CF patients



Results

Endometrial organoids from CF mouse model



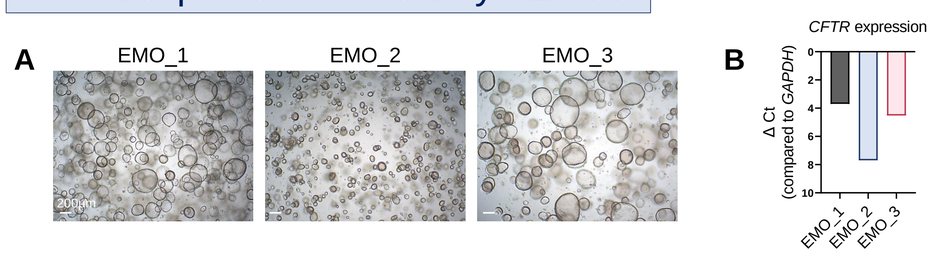
A) CF mouse (m) EMOs have a smaller organoid diameter and lumen compared to wildtype (WT) mEMOs, which is indicative of CFTR dysfunction (see model in **B**)
C) Loss of swelling in CF mEMOs was confirmed using the forskolin (Fsk)-induced swelling assay.

Conclusions

- Endometrial organoids provide a new and powerful research model to unravel the role of the endometrium in CF-associated sub-/infertility.
- WT and CF mEMO show morphological and functional differences, supporting the ion channel defect in CF.
- CFTR is expressed and functional in healthy hEMOs. CFTR inhibition abrogated organoid formation.

Results

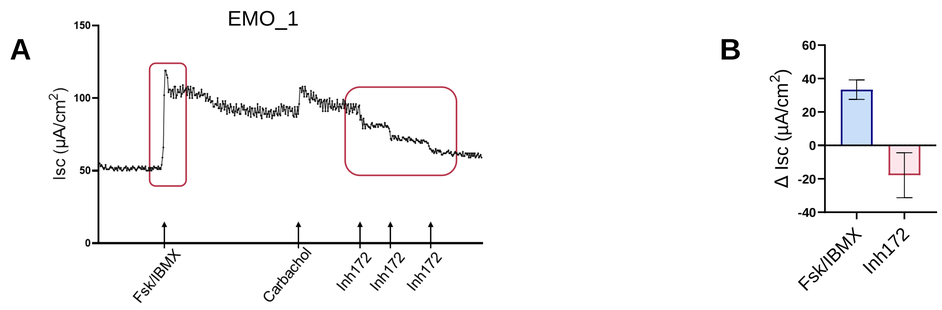
CFTR expression in healthy hEMOs



A) Different healthy human (h) EMO lines showed different morphologies.
B) CFTR expression seemed correlated with the morphology of the hEMOs i.e., higher in the case of more cystic organoids (EMO_1 & EMO_3).

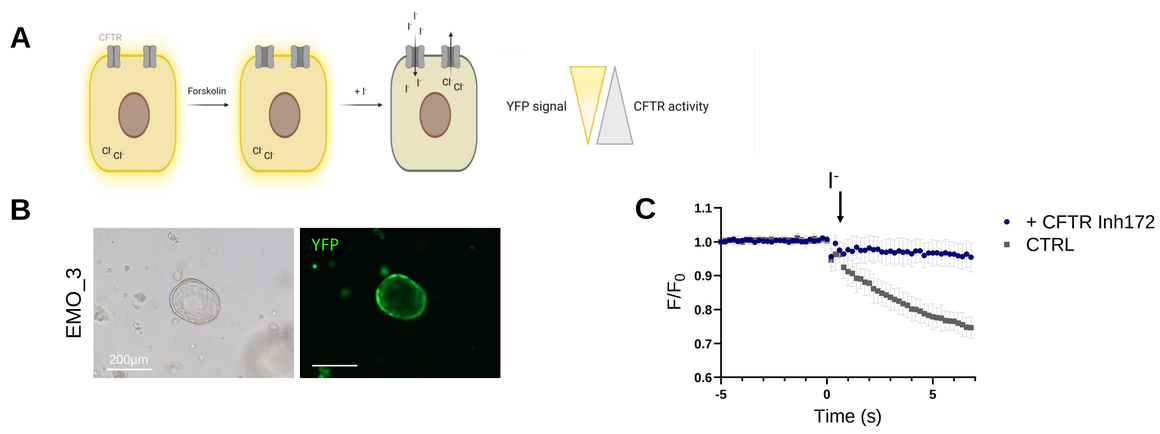
Assessing CFTR functionality in healthy hEMOs: two ways

I. with Ussing chamber assay



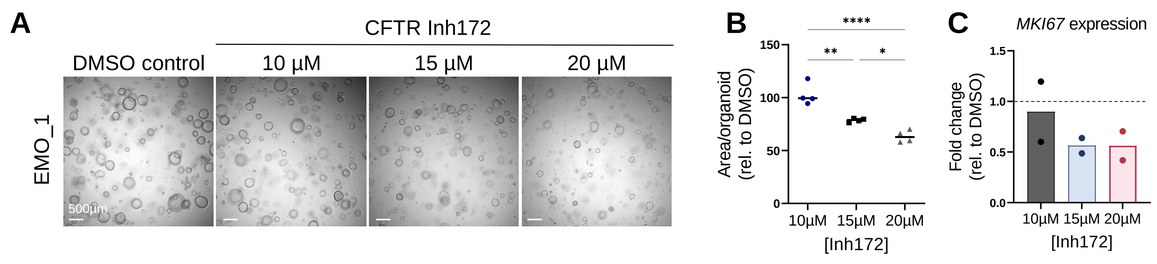
A,B) CFTR activators (Fsk/IBMX) induced an increase in short circuit current (Δ Isc-Fsk/IBMX) when added to hEMOs. Addition of the specific CFTR inhibitor (Inh) 172 induced a decrease in Isc (Δ $Isc-_{Inh172}$).

II. with halide sensitive-yellow fluorescent protein (HS-YFP) quenching assay



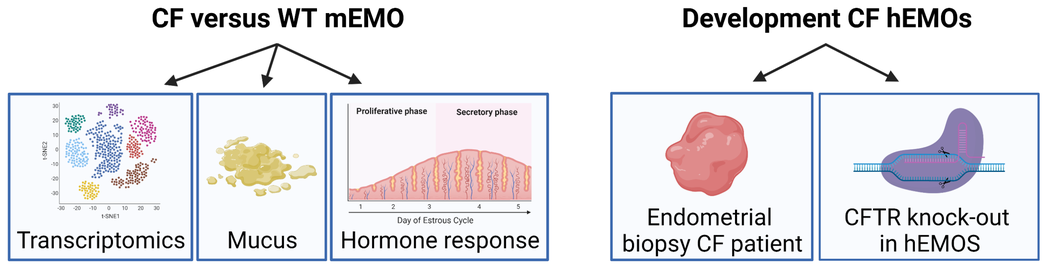
A) Principle of HS-YFP assay.
B) Healthy hEMOs are amenable to lentiviral transduction with HS-YFP.
C) YFP quenching was observed in healthy hEMOs upon iodide addition. Pre-incubation with CFTR Inh172 prevented YFP quenching.

CFTR inhibition in healthy hEMOs



A) Addition of CFTR Inh172 to hEMO cultures visually reduced organoid formation.
B) Surface area/organoid decreased significantly with increasing CFTR Inh172 concentration.
C) CFTR Inh172 treatment decreased proliferation in hEMOs at 15 and 20µM concentration.

Future perspectives



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