# Advancing Male Reproductive Toxicology with Innovative 3D hiPSCs-based Models of Testicular Steroidogenesis

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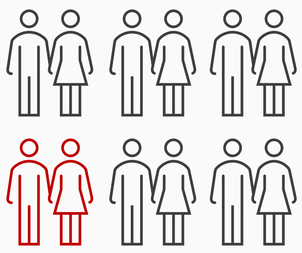
## BACKGROUND

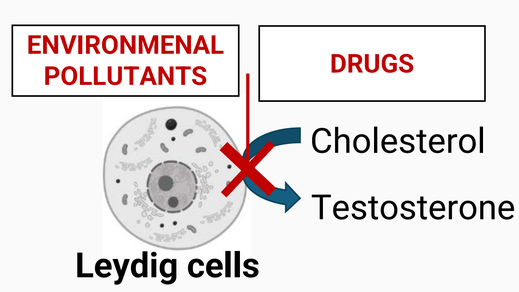


Approximately **1 out of 6 couple** experience **INFERTILITY** in their lifetime [1]

World Health Organization

♂ In male, **androgens** (e.g. testosterone) play a pivotal role not only in **reproduction health** but also in development, behaviour, gender identity and many other processes.

- **Endocrine disruptors** (e.g. drugs, pollutants) can disrupt **testicular steroidogenesis** (converting cholesterol into androgens) → **severe consequences for human health**




## AIM OF THE STUDY

**To develop an in vitro model for testicular steroidogenesis for both toxicological purposes and biomedicine research,** characterized by:

- Based on human **induced pluripotent stem cells** (hiPSCs)
- Emphasis on mimicking the natural development of Leydig cells (LCs), focusing on **critical developmental windows**
- Replication of physiological conditions through **3D cell culture**
- Implementation of **hormonally controlled conditions**, facilitating the study of men's individual hormone ratios, daily variations, and age-related changes.

## CURRENT IN VITRO MODELS

Conducted a comprehensive review of current in vitro models for testicular steroidogenesis (**Figure 1**), revealing limitations:

- One OECD validated test employs **H295R cell line**, derived from adrenal carcinoma (female patient) → possibly **lacking gonadal relevance.**
- Majority of models rely on **rodent (74%), cancer (75%) cell lines** → Species differences and lack of key enzymes → **lacking human relevance.**
- **Predominantly cultured in 2D** (94%) → ignoring the physiological **micro-environment.**

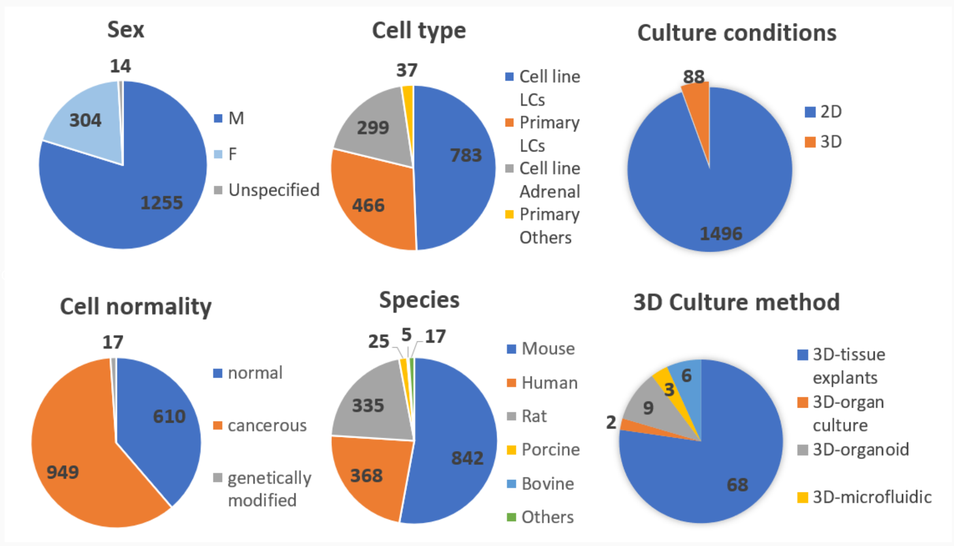
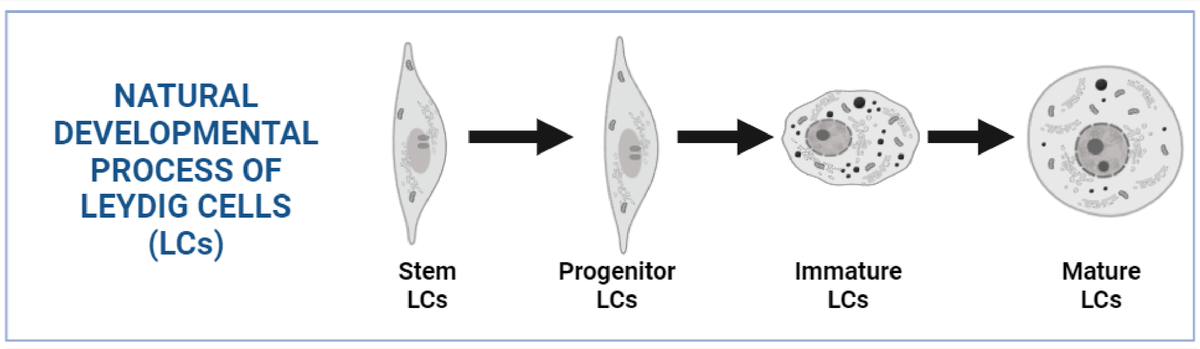
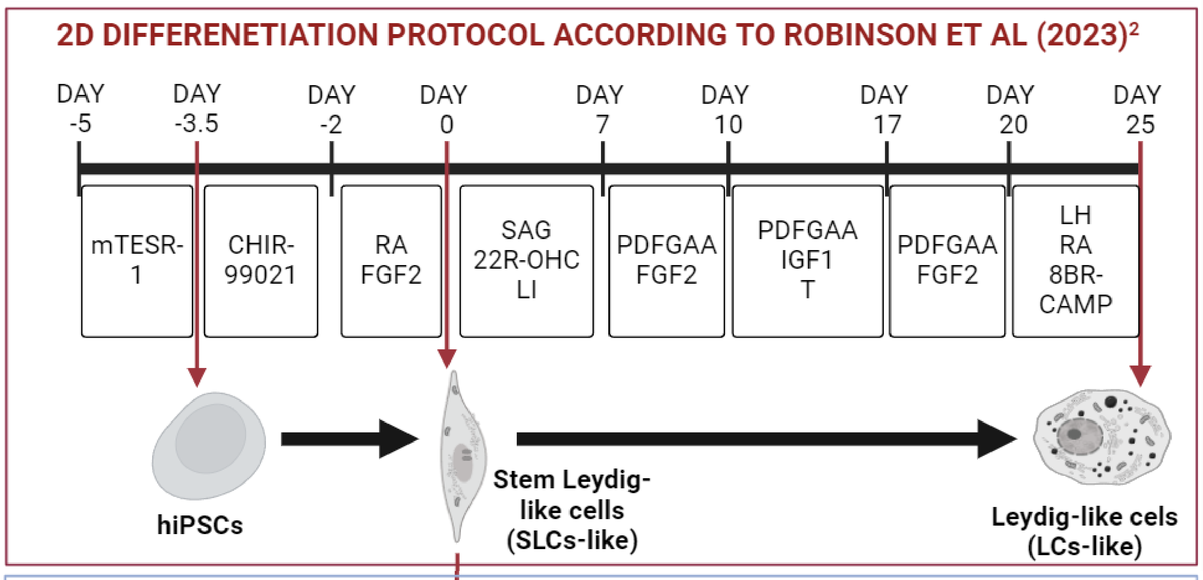
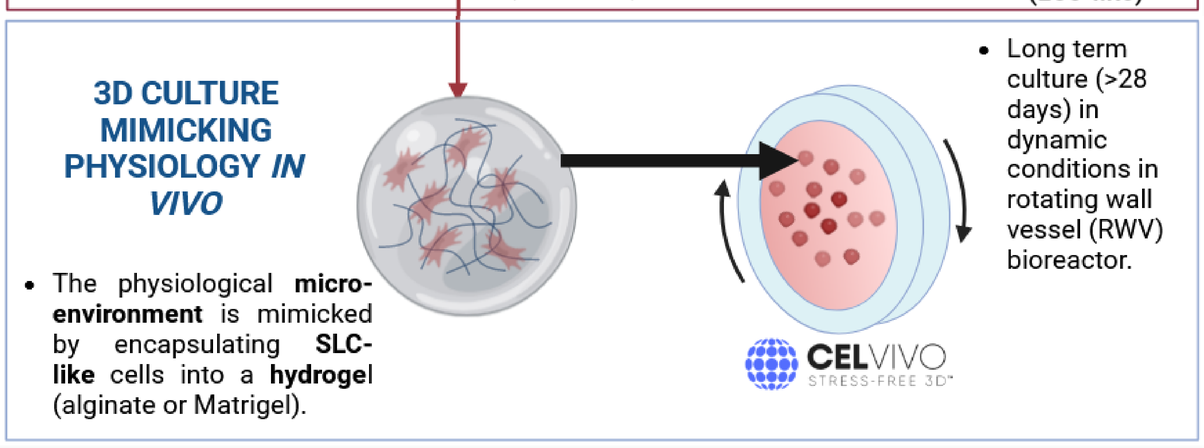



**Figure 1:** Overview of characteristics of currently utilized in vitro testicular steroidogenesis models. F, female; LCs, Leydig cells, M, male.

## METHODOLOGY




## RESULTS 2D CONDITION

**Successful adaptation of differentiation protocol:**

- ✓ The presence of lipid droplets typical for LCs present in LCs-like cells (**Figure 2**).
- ✓ Decrease of pluripotency markers (*Nanog*).
- ✓ Expression of NR5A1 and LHCGR – driving testicular steroidogenesis.
- ✓ Expression of steroidogenic enzymes (*STAR, HSD3B2, HSD3B1, CYP17A1, CYP11A1*).

SLCs-like cells (D0) exhibit mitotic activity while showing markers indicating a transition toward LCs differentiation (**Figure 3 & 4**) → optimal phase for transfer to 3D conditions.

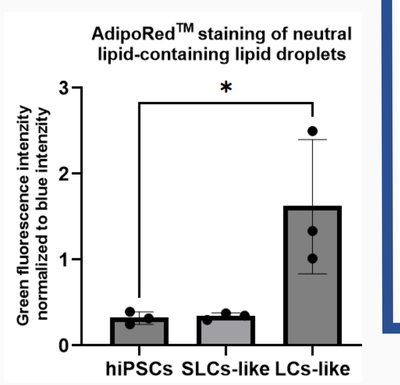



**Figure 2:** Accumulation of neutral lipid-containing lipid droplets, blue: nucleus by DAPI; green: lipid droplets by Adipored™.

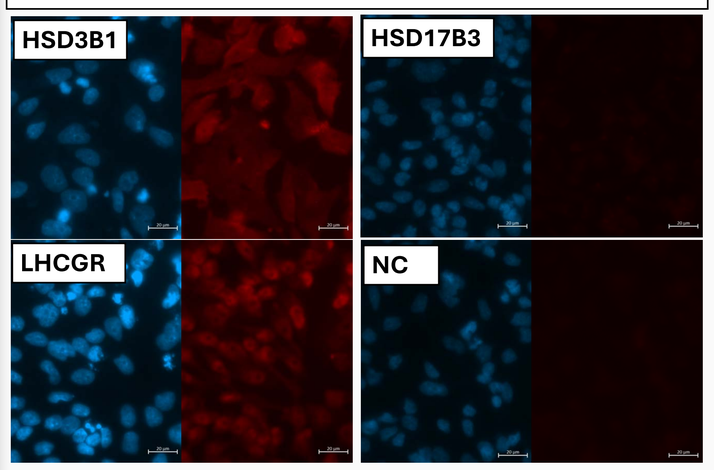



**Figure 3:** Immunostaining analysis of HSD3B1, LHCGR, HSD17B3, and NC (negative control) in SLCs-like cells (D0). Red: Target protein; Blue: DAPI for nuclear staining.

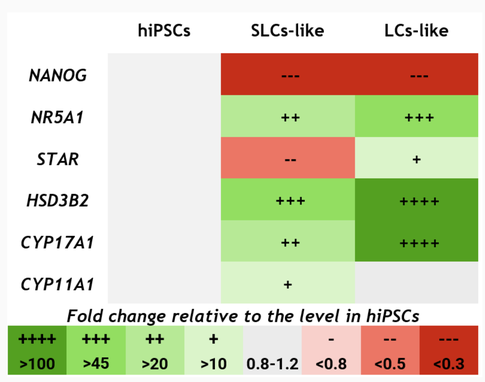



**Figure 4:** qPCR results, fold change relative to the level in hiPSCs.

## RESULTS 3D CONDITION

- Mouse cell line **TM3 was used for the optimization** process.
- Encapsulation into **Matrigel® Matrix unsuitable** for dynamic culture → hydrogel breakdown.
- **Successful optimization** of encapsulation of TM3 cells into **alginate** and **long-term culture** in dynamic conditions in RWV.

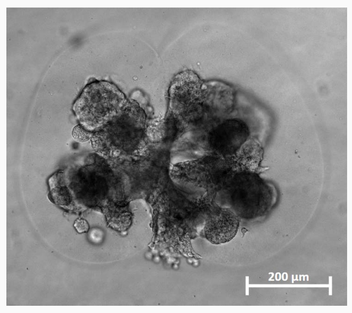



**Figure 6**: TM3 cells encapsulated in Matrigel® Matrix under static culture

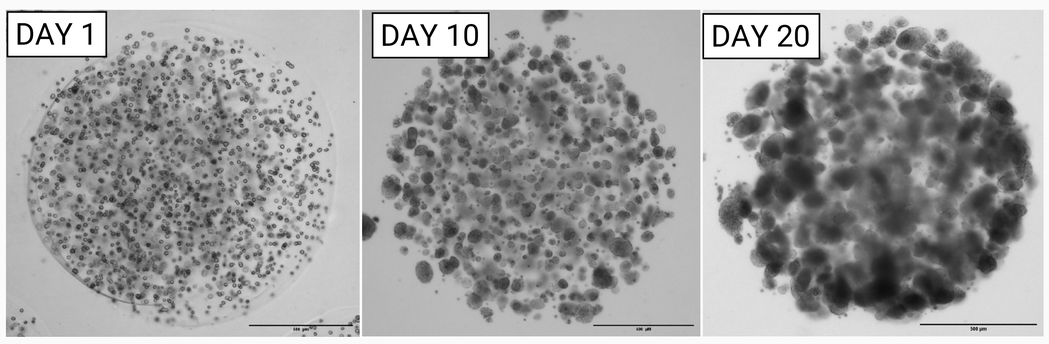



**Figure 5:** TM3 cells encapsulated in alginate, culture under dynamic cultivation in RWV

## REFERENCE

[1] World Health Organization. (Licence: CC BY-NC-SA 3.0 IGO., 2023)

[2] Robinson, M. et al. Adv Biol 7, (2023)

## ACKNOWLEDGMENT

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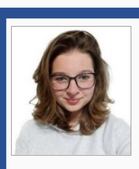

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