Establishment of a fetal rat Leydig cell culture to investigate effects of chemicals on steroidogenesis

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Introduction

Endocrine disrupting chemicals (EDCs) represent a growing global health burden and their assessment still relies heavily on animal studies. This is in part due to the complexity of endocrine signaling pathways and feedback systems *in vivo*, which can be difficult to reproduce *in vitro*. Exposure during pregnancy is particularly concerning, as male sexual development is tightly regulated by androgenic steroids; a more comprehensive understanding of steroidogenic disruption is therefore needed for the development of predictive non-animal toxicological assays. In this project, the aim is to better characterize rat fetal Leydig cells (FLCs), which are considered the primary site of androgen production in the testis. To do this, we intend to develop an isolation and primary cell culture method of fetal Leydig cells (FLCs).

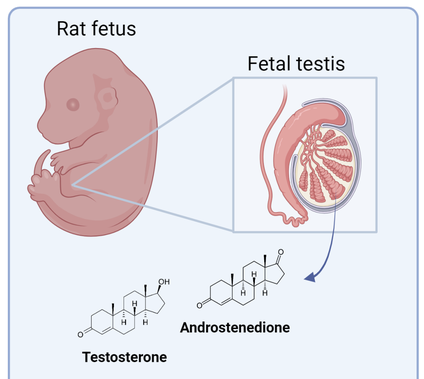

Figure 1: Androgens produced in the fetal testis are essential for male sexual development.

Development of a fetal Leydig cell isolation and culture protocol

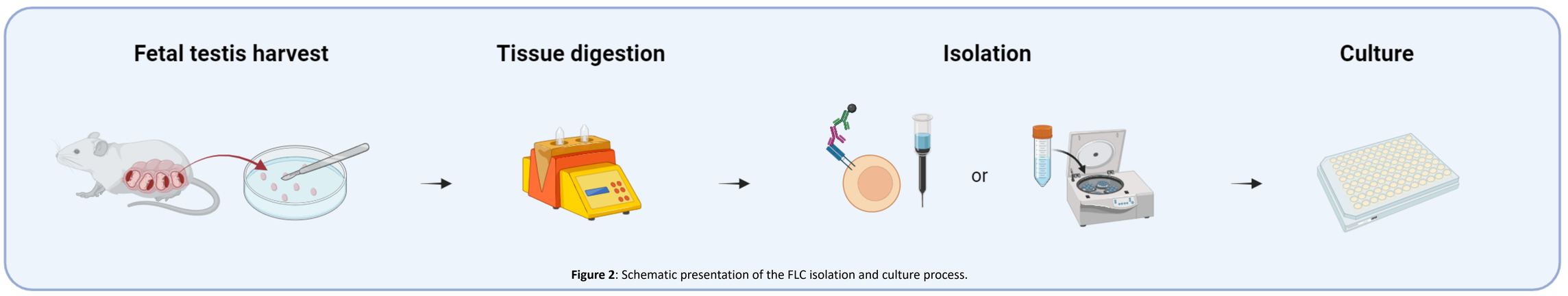

Figure 2: Schematic presentation of the FLC isolation and culture process.

Fetal testes will be collected from pregnant dams and cut into smaller pieces, before undergoing enzymatic and mechanical digestion (i.e., using a gentleMACS™ dissociator). For FLC isolation, two approaches will be considered: magnetic-assisted cell sorting (MACS) and density gradient centrifugation. The first method entails labelling cells with a Leydig cell specific antibody which is subsequently bound to a secondary, magnetic antibody and specifically retained on a magnetic column. The second method consists of separating cells based on their different buoyant densities in a gradient medium (i.e., Percoll). Successful isolation will be determined by immunostaining for a specific Leydig cell marker. Finally, we intend to optimize culture conditions: this will include testing different coating agents (such as collagen, Poly-D-Lysine and Matrigel) to promote cell attachment and cell viability. Our ultimate goal is to achieve a high yield of FLCs which can be sustained in a homogenous and highly viable culture for up to 72h. Lastly, the cells should also exhibit detectable steroidogenic activity.

Fetal Leydig cell characterization and comparison to established cell line

Successfully isolated cells will be cultured with and without EDCs to determine changes in androgens levels and key enzymes, both at gene and protein expression level. This steroidogenic profile can then be compared to established models such as the H295R adrenocarcinoma cell line, commonly used as an *in vitro* EDC screening tool. Establishing key differences and similarities between primary FLC and H295R culture will contribute towards our understanding of rat fetal androgen steroidogenesis and its corresponding extrapolation to humans. Should the established cell line be able to accurately reproduce steroidogenic mechanisms of fetal testicular cells, animal and primary cell use could potentially be reduced in future toxicological EDC assessments.

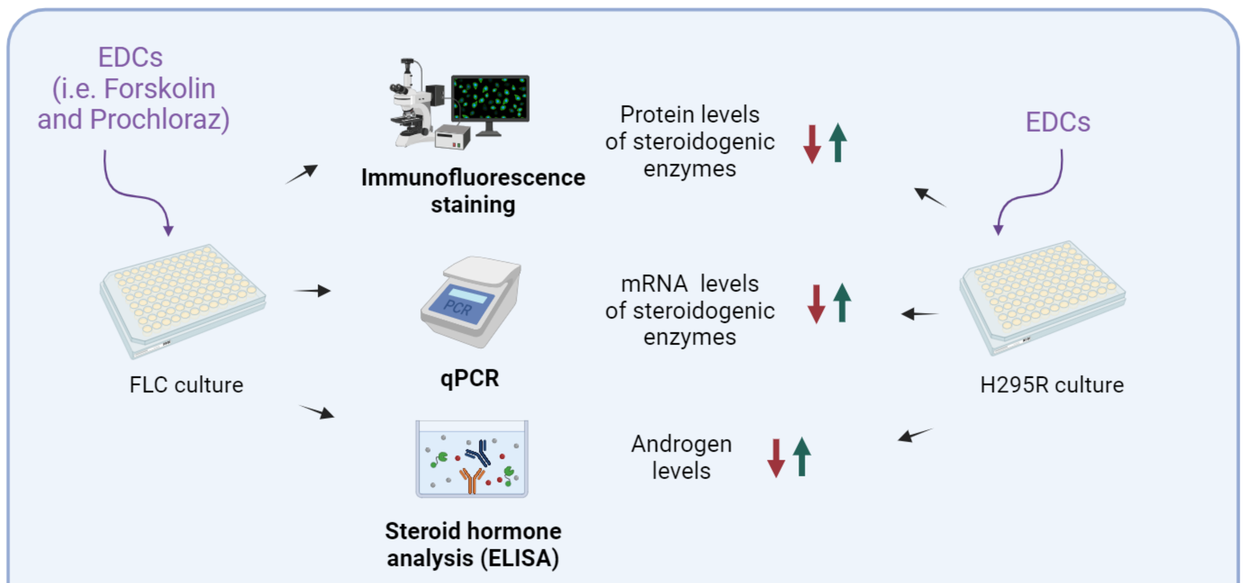

Figure 3: FLC characterization and comparison to established steroidogenic cell line, such as H295R.

Conclusions

- In order to align reproductive toxicology research with 3R principles, development of predictive EDC screening tools is essential.
- Characterizing fetal Leydig cells is a crucial step toward achieving a more complete understanding of fetal steroidogenic disruption.
- Comparing rat FLCs' steroidogenic profile to established cell lines can potentially reduce the need for animal studies and primary cells models in the long-term.

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