

Assessment of Microgravity-Induced Alterations in Ovarian Follicles Using an Organ-on-a-Chip System

Melis Emanet

Smart Bio-interfaces, Italian Institute of Technology, Pontedera, Italy

Introduction

Gravity is a key physical force shaping living systems, from cytoskeletal organization and intracellular transport to cell proliferation, differentiation, polarity, and communication¹. Because ovarian follicles rely on tightly coordinated local and endocrine signals, ovarian function is especially vulnerable to physical stressors such as altered gravity². To assess potential reproductive risks during spaceflight, we investigated the effects of simulated microgravity (S- μ G), generated by a random positioning machine, on KGN granulosa spheroids and H295R theca-like cultures, developing 3D human ovarian follicle models. Follicular activity was supported with follicle-stimulating hormone (FSH) and luteinizing hormone (LH). We then examined how S- μ G affected paracrine signaling, cellular stress responses, and cell death compared with earth-gravity controls.

Materials and Methods

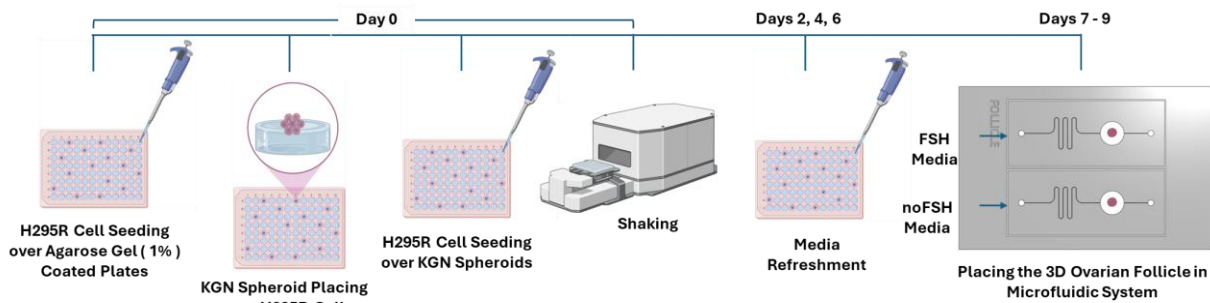


Figure 1. Schematic illustration of the 3D ovarian follicle model development procedure

Results

The 3D ovarian follicle model was structurally characterized by confocal microscopy following staining of nucleic acids with Hoechst and F-actin with phalloidin-FITC (**Figure 2A-C**). The distinct nuclear morphology of KGN and H295R cells enabled rough spatial discrimination within the construct, with KGN cells organized into a central spheroid surrounded by an outer layer of H295R cells. This arrangement was further confirmed by histological evaluation of sectioned samples stained with hematoxylin and eosin, which supported the confocal observations (**Figure 2D**). Functional analyses showed that FSH or LH modulated steroidogenic enzyme expression under both earth gravity and S- μ G (**Figure 2E and F**). In KGN cells, FSH treatment significantly increased CYP19A1 expression regardless of gravitational status. In contrast, CYP17A1 expression in H295R cells was significantly reduced under S- μ G, although LH treatment partially restored its levels. S- μ G also induced a significant ROS production in both cell types (**Figures 2G and H**), and KGN cells exhibited altered necrosis and late apoptosis under S- μ G without FSH treatment (**Figures 2I and J**).

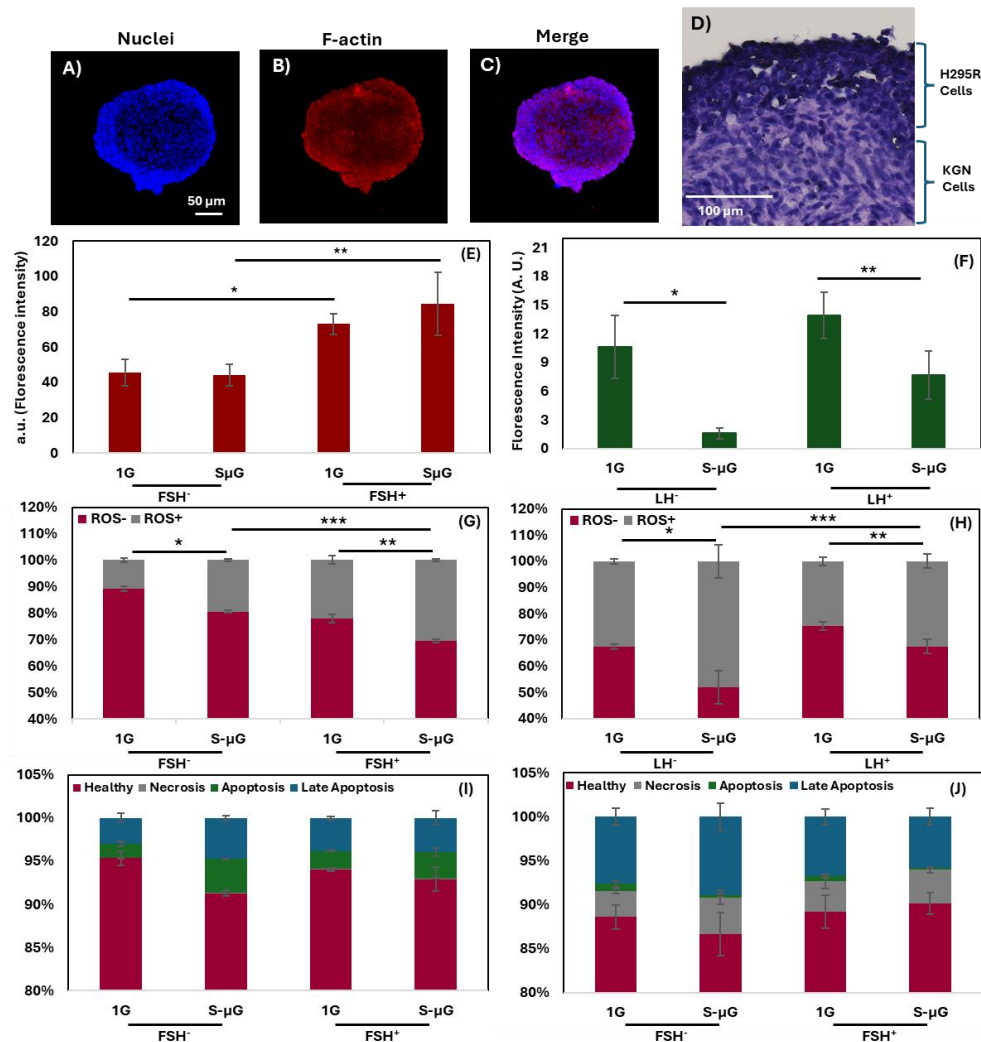


Figure 2. Representative confocal microscopy images of the nuclei (A), f-actin (B), and their merging (C); histological images (D) of the 3D ovarian follicle model. Quantitative analysis of CYP19A1 and CYP17A1 confocal microscopy images (E and F), ROS (G and H), and apoptosis and necrosis (I and J) performed on KGN and H295R cells, respectively.

Conclusion

The 3D ovarian follicle model successfully recapitulated follicular architecture, with KGN spheroids centrally enclosed by H295R cells, as confirmed by confocal and histological analyses. Functionally, gonadotropin signaling partly preserved steroidogenic competence under S-μG: FSH sustained CYP19A1 expression, whereas CYP17A1 declined despite partial LH rescue. S-μG also increased oxidative stress and selectively promoted KGN apoptotic/necrotic vulnerability in this model.

Acknowledgements

This research has received funding from the European Space Agency (4000145476).

References

- ¹H Winkler, L. Human Physiological Limitations to Long-Term Spaceflight and Living in Space, *Aerospace Medicine and Human Performance* (2023) 94:444.
- ²Liu, M. Ultrastructure of isolated mouse ovarian follicles cultured in vitro, *Reproductive Biology and Endocrinology* (2011) 9:1.