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P-600 Development of human ovarian spheroids as an innovating 3D model to study female hormone-related disorders

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Study question: Can spheroids of theca and granulosa cells serve as a new model to explore the cellular and molecular mechanisms involved in female hormone-related disorders?

Summary answer: Layered theca-granulosa cell spheroids can be successfully assembled with preserved cellular viability, β -estradiol production, and significant upregulation of extracellular matrix (ECM) components.

What is known already: Spheroid models can revolutionize reproductive biology research, faithfully replicating the ovarian microenvironment for insights into hormonal regulation, cell-cell interactions, and pathologies like polycystic ovarian syndrome (PCOS) and premature ovarian insufficiency (POI). These models can replicate the spatial configuration of the theca cells (TCs) and granulosa cells (GCs), which are the primary components of the follicle's somatic compartment. Studies using rodent spheroids demonstrate improved estradiol and progesterone secretion, suggesting potential applicability. Though challenging to obtain, recent studies have successfully derived TCs through the differentiation of stromal cells (SCs), introducing new possibilities for spheroid reproductive research in the context of human models.

Study design, size, duration: The study included GCs from around 15 IVF patients and SCs from postmortem ovaries of 3 postmenopausal women were used to be differentiated into TCs.

Participants/materials, setting, methods: TCs and GCs were used to construct three spheroid types: layered theca-collagen-granulosa (TCG), layered theca-granulosa (TG), and non-layered. In TCG, GCs formed granulosa-spheroids, which were coated with collagen-I and combined with TCs. TG-spheroids were produced similarly without the collagen layer. Non-layered spheroids were assembled using isolated GCs and TCs. All embedded in PEGylated fibrin hydrogel. Media estradiol synthesis was measured via enzyme-linked immune assay (ELISA), and immunofluorescence was employed to characterize cellular functionality and ECM.

Main results and the role of chance: In GCs, positive expressions for AMHR, CYP19A1, and FHSR were observed, while following differentiation, TCs exhibited positivity for CYP17A1, LHR and CD13. A notable distinction emerged when comparing TCs within the spheroids to those in 2D cultures, as the former demonstrated an improvement in longevity. Layered TG-spheroids exhibited significant increases in collagen-IV, EMILIN-I, and collagen type I compared to G-spheroids. Interestingly, TCG-spheroids exhibited a distinct decrease in collagen type IV. Across all spheroid types, collagen type VI and laminin were notably absent from their ECM. Moreover, all spheroids had a significant increase in β -estradiol production. Non-layered spheroids showed a significant viability decrease inside the PEGylated fibrin over 30 days, in contrast to the sustained viability observed in the layered TG- and TCG-spheroids.

Limitations, reasons for caution: Utilizing primary cells, particularly specific patient-derived cells, poses a limitation due to their short availability, which could impact the study's size.

Wider implications of the findings: Theca-granulosa spheroids deepen our understanding of reproductive health, offering insights into conditions such as POI and menopausal symptoms. By investigating the nuanced endocrine interplay, this 3D model paves the way for new toxicity studies, provides insights into PCOS, and hints at potential applications in hormone replacement therapy (HRT).

Trial registration number: Not applicable