3D analysis of the ovarian folliculogenesis

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Aims

One of the great scientific challenges of these times is the functional reconstruction of an entire organ or organism in a three-dimensional (3D) manner. To achieve this goal, various methodologies are being developed using different imaging techniques alone or in multimodal combination.
In the field of reproduction, these aspects of 3D reconstruction are of particular importance. Here we present a study of 3D mapping and quantification of mouse ovarian follicles, and the identification of the major vascular branches of the organ. We optimized a soft tissue contrast protocol for micro-CT imaging to provide a spatial atlas of the functional units of the ovary without affecting its three-dimensional integrity.

Method

Ovaries of 8-week-old CD1 female mice females were isolated and individually fixed in 4% Paraformaldehyde (PFA; 4 gr Paraformaldehyde in 100 mL 1X PBS) overnight at 4°C. Research on mice was conducted with permission from the Ministry of Health (No. 1100/2016-PR) in accordance with the guiding principles of European (No. 2010/63/UE) and Italian (No. 26/2014) laws protecting animals used for scientific research.
Following fixation, ovaries were individually treated at room temperature with 25% Lugol's solution and then washed out with its solvent.
Following contrast treatment, ovaries were placed in a 0.5 mL Eppendorf tubes filled with distilled water to avoid organ dehydration and shrinkage. Then, samples were scanned by microtomographic system Skyscan 1172 (Bruker MicroCT, Kontich, Belgium). The scanner X-ray source was set at a voltage of 60 kV, 165 mA current. We used a 0.5 mm aluminum filter between source and object and we set the pixel size at 5 µm. Samples were rotated to 180°with a rotation step of 0.4°. Additional three ovaries were scanned with same source setting but at 1.5 µm/pixel for nearly 5 h each. Samples were rotated to 180° with a rotation step of 0.1°. The scanning dataset consisted of about 2000 images in 16-bit tiff format. The final microCT sections were reconstructed using the NRecon software (Bruker MicroCT, Kontich, Belgium) in 8-bit jpg format (2000 X 2000 pixels). Sections were visualized with both Fiji ImageJ (NIH) and DataViewer (Bruker MicroCT, Kontich, Belgium) in order to identify follicles and blood vessels. 3D rendering of the follicular and vascular components was also obtained.
Moreover, microCT results were compared with histology performed on the same sample to validate the method.
Follicular structures identified in the microCT sections of each ovary, based on a combination of morphological parameters and the size in diameter, were individually counted and assigned to a specific follicle type (T4, T5, T6, T7, or T8) or *corpus luteum*.

Results

Through our procedure, it was possible to identify the main functional compartments of the growing follicle: granulosa, antrum, cumulus cells, zona pellucida and oocyte with its nucleus (Figure 1). A quantitative analysis of the distribution of the various follicle types (from T4 to T8) was carried out taking into account the different dorsal and ventral regions of the adult ovary. The data led to the hypothesis of a homogeneous recruitment with respect to the ovarian surface.

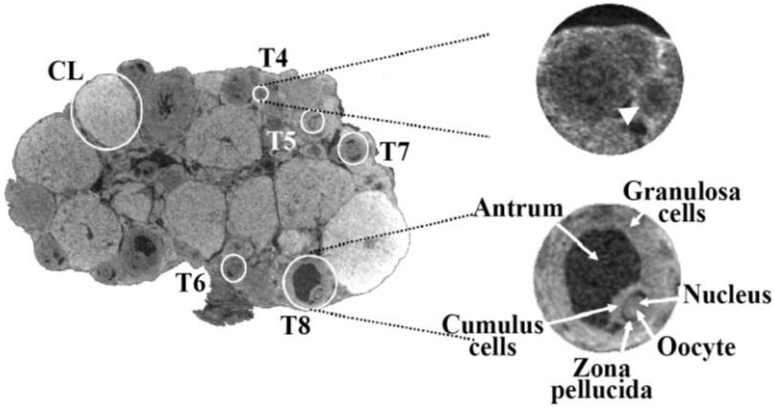

Figure 1. A representative micro-CT section highlighting follicles from T4 to T8 and *corpora lutea* (CL). On the right-hand side (top), an enlargement of a secondary T4 follicle (arrow); on the right-hand side (bottom), an enlargement of a fully grown T8 follicle, in which the different cytological components (granulosa cells, antrum, cumulus cells, zona pellucida space, oocyte with its nucleus) are clearly visible (Adapted from Ref. [2])

Conclusion

This study lays the groundwork for further investigations in the field of reproductive biology during the differentiation phases of the ovary, for the study of infertility pathologies such as that found in polycystic ovary and for the development of new therapeutic approaches *via* hormones or drugs administration of [1]. The highly inter- and multi-disciplinary aspect of this type of research has the potential to contribute significantly to our understanding of the dynamics of folliculogenesis.

References:

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