POSTERS

# 3D MORPHO-FUNCTIONAL RECONSTRUCTION OF THE PREPUBERTAL MOUSE OVARY COMBINING NANO-COMPUTED TOMOGRAPHY AND CONFOCAL IMAGING

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Since most of our knowledge of mammalian folliculogenesis derives from studies that disrupt the 3D integrity or do not preserve a complete view of organ morphology, we are combining imaging methods to investigate ovarian morpho-functional features while maintaining 3D organization.
In this study, 3 ovaries from prepubertal (25-day-old) mice were analysed by nano-Computed Tomography (nanoCT) to classify and map all follicle types within the volume, from primary Type 3 (T3) to preovulatory T8, distinguishing healthy and atretic follicles based on morphological hallmarks. The same ovaries were then sectioned at 20 µm thickness, stained with DAPI, and analysed by confocal microscopy. This staining allowed oocyte classification based on nuclear chromatin configuration: SN (surrounded nucleolus) oocytes, transcriptionally inactive, and characterized by a ring of condensed chromatin around the nucleolus, and NSN (not surrounded nucleolus) oocytes, transcriptionally active and with a more dispersed chromatin.
A manual co-registration approach was then used to align nanoCT and confocal datasets, integrating information on follicle type, health status, and oocyte transcriptional state into a single dataset.
25-day-old ovaries contained on average 247.7±42.9 T3-T8 follicles, of which 21.8±5.5% corresponded to highly disorganized atretic follicles that could not be classified as SN or NSN. The remaining 191±38.2 follicles were classified as 75.1±6.2% NSN (78.3±6.0% healthy; 21.7±6.0% atretic) and 24.9±6.2% SN (21.7±6.1% healthy; 78.3±6.1% atretic).
NSN oocytes were mainly found in healthy T3-T6 follicles, where transcriptional activity supports growth. SN organization displays a dual role: transcriptional inactivity is associated with atresia in T4-T6 follicles, whereas in preovulatory T8 follicles it reflects the chromatin reorganisation required for meiotic resumption and acquisition of preimplantation developmental competence.