ULTRASTRUCTURAL 3D INVESTIGATION OF OVARIAN STRUCTURES IN A BOVINE MODEL BY SYNCHROTRON CONTRAST-ENHANCED X-RAY IMAGING AND MICROSCOPY

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Abstract Body

An improved understanding of ovary's structures is highly desirable to support advances in folliculogenesis knowledge and reproductive technology. Although up to date the golden standard for structural analysis is provided by combining histological sections, staining, and visible 2D microscopic inspection, synchrotron radiation phase-contrast microtomography is becoming a new challenge for three-dimensional studies at micrometric resolution (1). To this aim, the proper use of contrast agents can improve the visualisation of internal structures in ovary tissues, which normally present a low radiopacity. In this study we report a comparison of four staining protocols, based on Iodine or Tungsten containing agents, applied to bovine ovarian tissues fixed in Bouin's solution. The microCT analyses at two synchrotron facilities (ESRF and Elettra) under different set-ups were performed at different energies in order to maximise the image contrast. While Tungsten based agent allows well identifying large structures, Iodine ones better highlight smaller features, especially when acquired above the K-edge energy of the specific metal (69 and 33 keV, respectively), both at 2.2 micron pixel size resolution. Further scans were performed at lower energy at Syrmep of Elettra, where the set up was optimised for overall quality and sensitivity from phase-contrast, still provided highly resolved visualitation of follicular and intrafollicular structures at different maturation stages with a sub-micron resolution. A The analyses were complemented by X-ray Fluorescence microscopy on 2D sections, showing that the tungsten-based agent has a higher penetration compared to Iodine in this type of tissues. It is expected that this type of advanced tridimensionality ultrastructural analyses may found applicability to monitor damages from cryopreservation protocols or in the more futuristic attempts like the artificial ovary.

(1)Pascolo, L.; Sena, G.; Gianoncelli, A.; Cernogoraz, A.; Kourousias; et al.. Journal of Synchrotron Radiation 2019, 26, 1329.