

Digital analysis of ovarian tissue: generating a standardized method of follicle classification

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Ovarian follicles develop through a series of growth stages that correspond with morphological changes in the oocyte as well as the surrounding granulosa and theca cells. Classical follicular stages assessed in histological sections of ovarian tissue include primordial, primary, secondary, and antral stages. However, follicles transitioning between primordial and primary stages display key molecular changes that define activation and determine their subsequent development, and these transitional stages are not currently classified. This is due, in part, to the lack of standardized methods for classifying, counting and morphologically analyzing follicles. QuPath is a user-friendly open-source software extensively used in digital pathology. In addition to its multiple functions for image analysis, it supports collaboration by enabling sharing of exported annotated data and scripts between users. We hypothesize that establishing an automated pipeline of analysis to classify follicles based on key histomorphometric parameters using QuPath will support a more robust, rigorous and reproducible follicle classification method, ultimately defining early healthy follicles stages as they are activated within human ovary sections. Hence, this project aimed to develop a pipeline that requires minimum input from the user and allows automatic detection of principal elements of the ovarian follicle in digitized H&E-stained whole slide images. We used samples from healthy human ovarian biopsies. By using a supervised approach, follicles were selected and classified by expert ovarian morphologists. Then, segmentation of the whole follicle area, individual granulosa cell nuclei and oocyte was done using manual and automatic QuPath annotation tools. Feature extraction regarding size and shape and distribution analyses were performed to determine cut-off values that represented each follicle stage. Then, a tunable script was generated using Groovy programming language to automatically classify follicles. Finally, validation of follicle classification was performed. We were able to establish the morphometric parameters to classify follicles into primordial, transitional, primary, secondary and antral stages. Granulosa cell eccentricity was shown to be an accurate parameter to distinguish flat versus cuboidal granulosa cells in primordial and transitional stages, and granulosa cell number and follicle area were sufficient to distinguish all follicle stages. Future studies will focus on color deconvolution normalization of H&E staining to minimize inter-stain differences and define intensity parameters of the oocyte and granulosa cells as potential markers of oocyte degeneration and granulosa cell death. Altogether, in-depth digital analyses of ovarian follicle features may be useful for the assessment of morphometrical follicle normality, with the end goal of reaching a more transparent, unbiased and standardized method to assess different diseases or exogenous conditions that may affect the ovary against healthy folliculogenesis.

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