

Assessing toxicology in ovaries using a high-throughput 3D imaging and AI-based follicle segmentation and categorization platform

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All authors declare no conflict of interest

Background & Aim

Two-dimensional (2D) histopathology is the current gold-standard for assessing toxicology in ovaries. However, it can be a time-consuming process limited by section-to section variability, subjective interpretation and sampling bias.

To overcome these limitations, we have developed a 3D imaging platform that allows for comprehensive analysis of the whole ovary, combining high-throughput light-sheet microscopy with deep-learning AI segmentation, providing unbiased assessment of multiple ovary endpoints across different species.

Methods

Whole mouse ovaries were processed using iDISCO whole-organ immunohistochemistry targeting CD31 (vasculature), MVH (oocytes), AMH (granulosa cells), and CC3 (apoptosis), followed by optical clearing and light-sheet fluorescence microscopy. Deep-learning AI segmentation models for mouse ovaries were developed to compute ovary endpoints in vehicle and cyclophosphamide (150 mg/kg) treated mice.

Conclusion

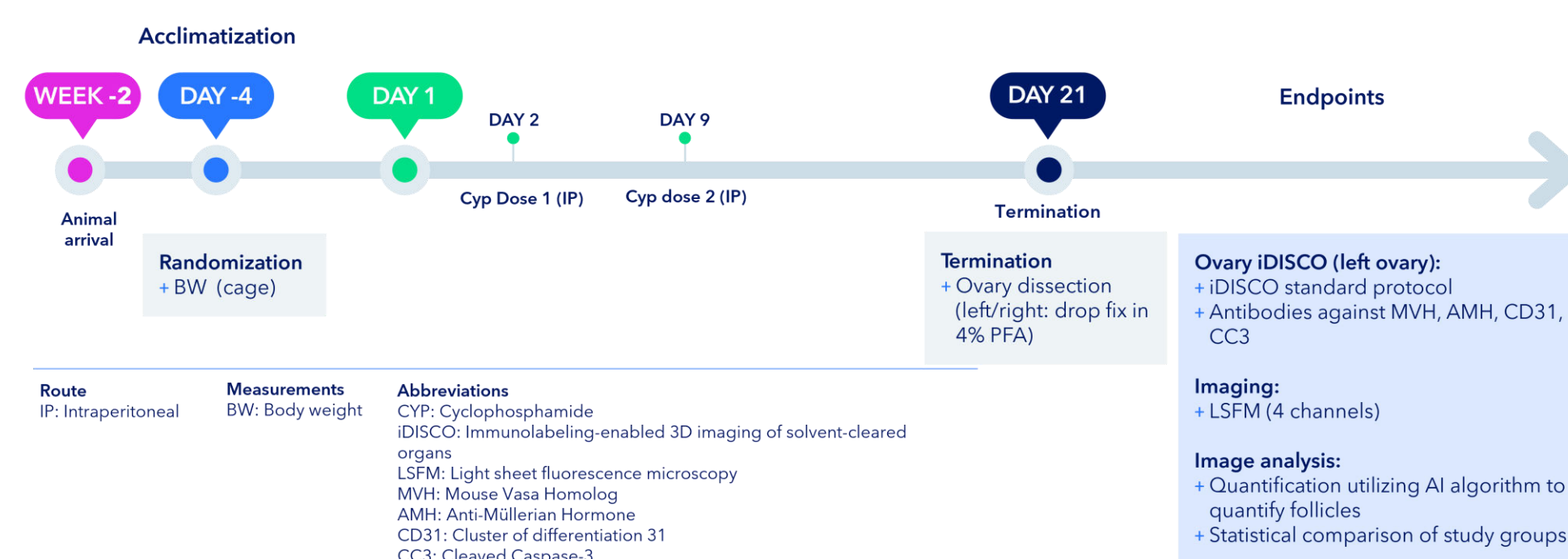
Our 3D imaging platform provides accurate, unbiased and high-throughput quantification of ovarian endpoints, with scalability across multiple species. The approach has the potential to complement and enhance conventional histopathological assessment in reproductive toxicology studies.

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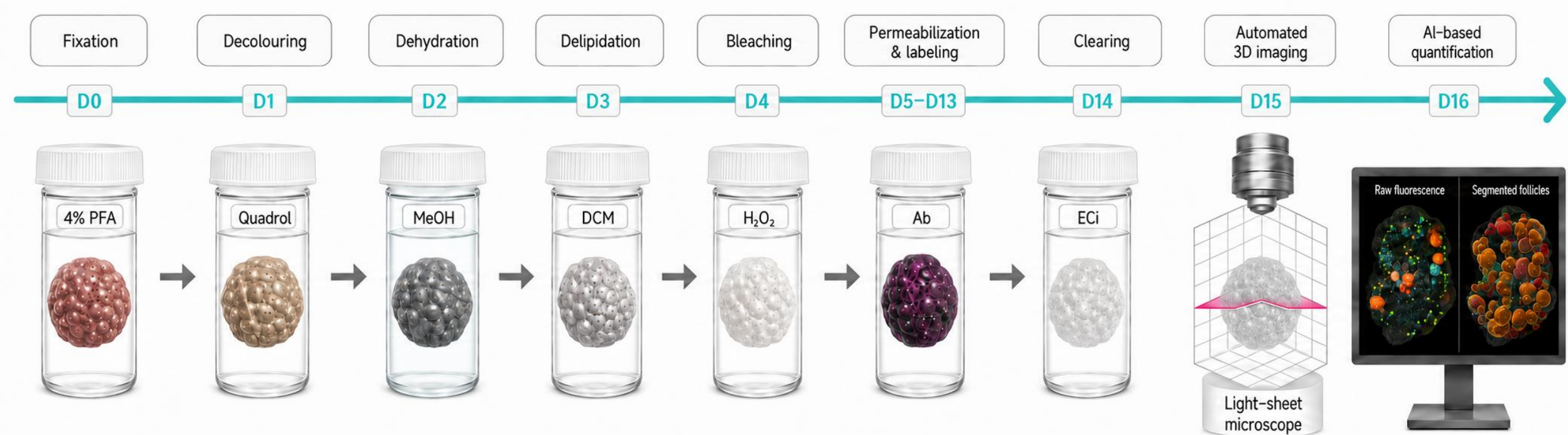
1 Study outline and group overview



#	Group name	n in group	Animal model	Dose [mg/kg]	Route of administration	Dosing frequency
1	Control	6	Control	NA	IP	Study day 2 and day 9
2	Cyclophosphamide (CYP)	6	CYP-POI	10	IP	Study day 2 and day 9

Figure 1. Study outline and group summary. CYP-POI: Cyclophosphamide-induced primary ovarian insufficiency. Control was vehicle-treated.

2 3D imaging platform and follicle categorization



3 Ovary staining

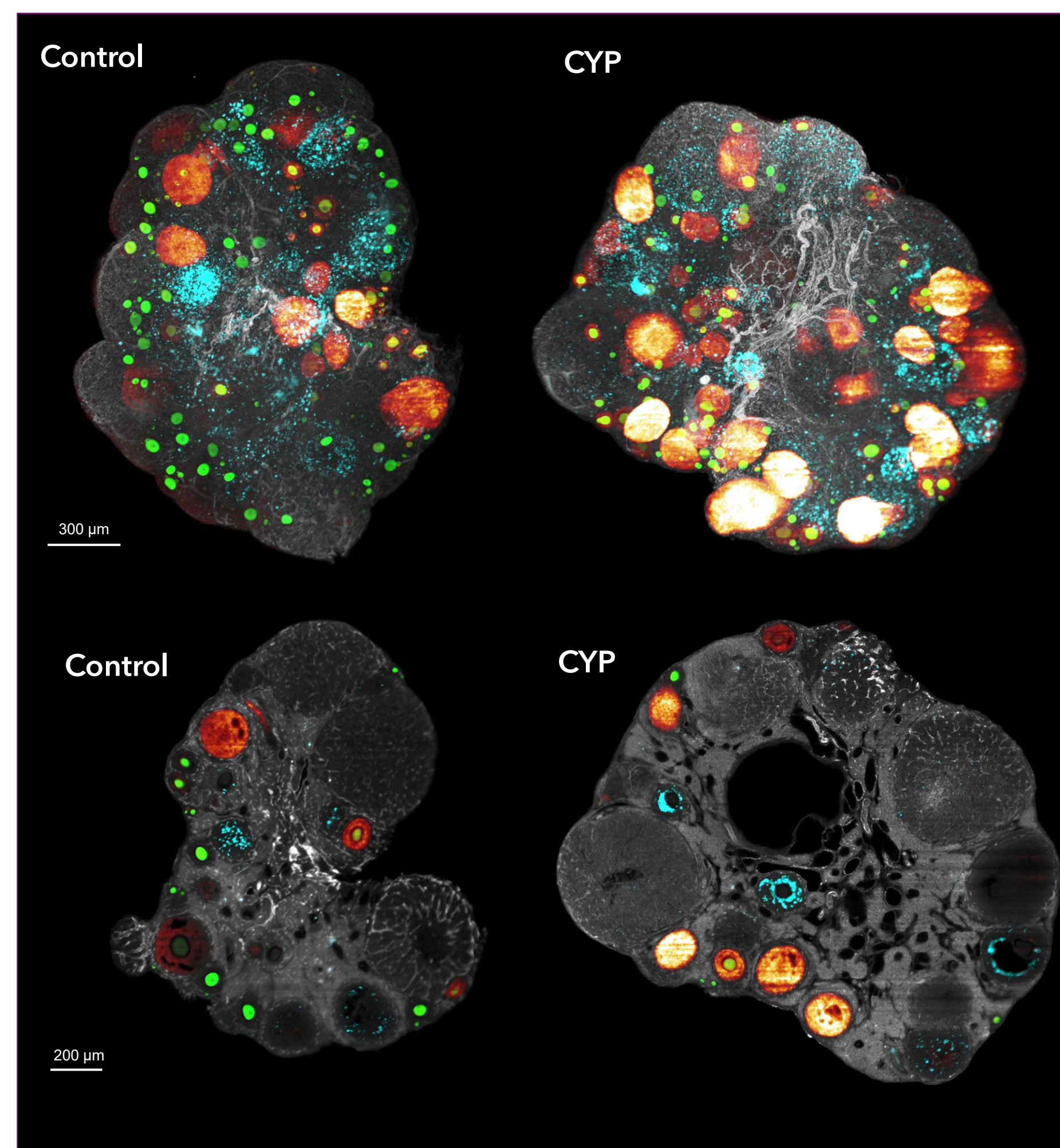
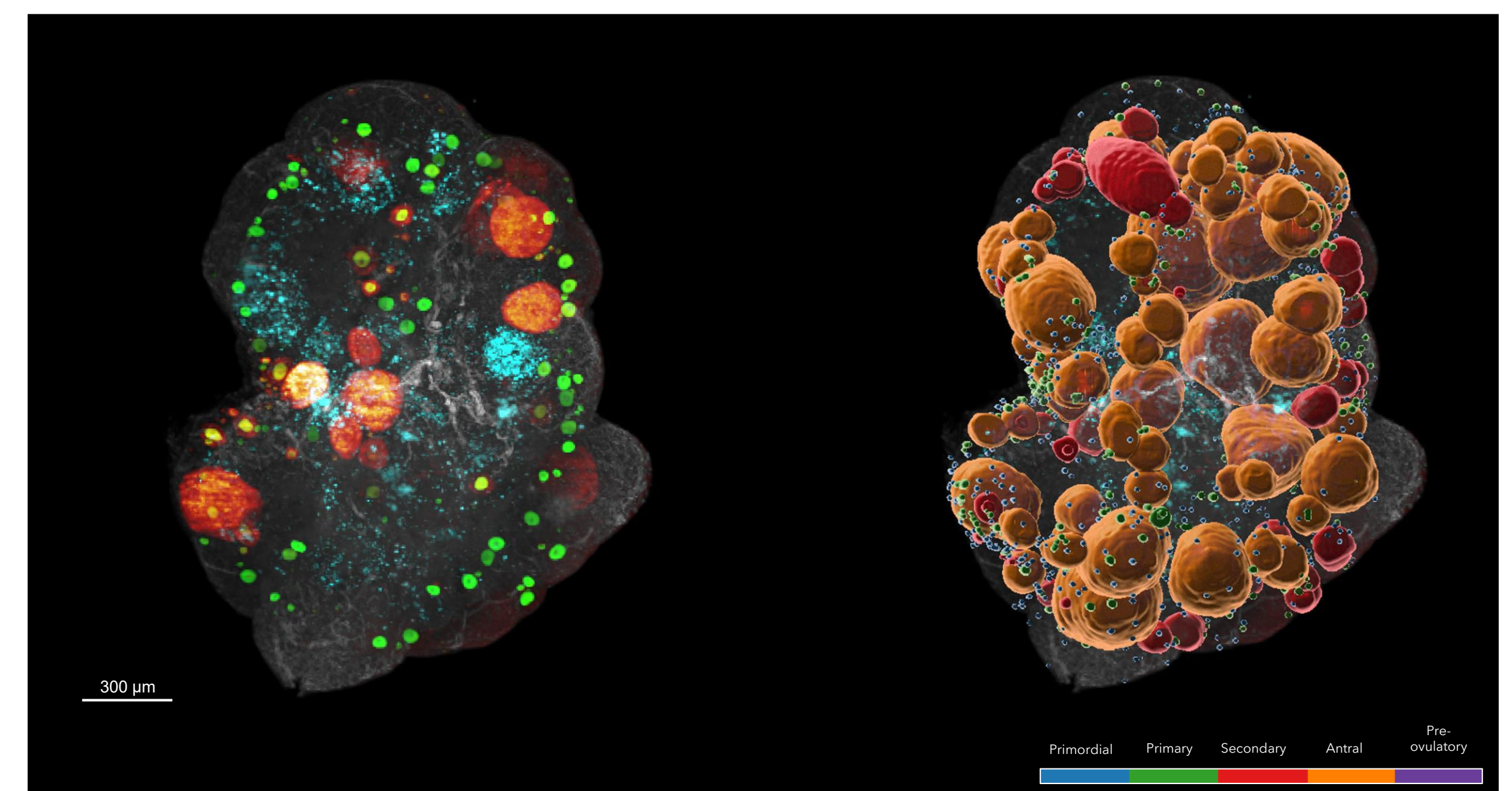


Figure 3. Representative images of mouse ovary staining MVH (oocytes, green), AMH (granulosa cells, glow), CC3 (apoptosis, cyan) and CD31 (vasculature, grey). Top panel - 3D maximum intensity projection of ovaries from control and CYP groups. Scale bar = 300 μ m. Bottom panel - 2D section (3 μ m) of corresponding whole 3D ovary scan from control and CYP groups. Scale bar = 200 μ m.

4 Follicle segmentation



Category	Oocyte diameter (μ m)	AMH coverage	Antrum percentage	Max granulosa cell layer thickness (μ m)
Primordial	10-20			
Primordial	20-30	$\leq 1\%$ (in computed follicle ring)		
Primary	20-30	$> 10\%$ (in computed follicle ring)		
Primary	30-40	+		
Secondary	40-65	+	$\leq 1\%$	
Antral	40-65	+	$> 1\%$	
Antral	65+	$> 0\%$, or granulosa thickness $> 80\mu$ m		$\leq 80\mu$ m, or AMH $> 0\%$
Pre-ovulatory	65+	0%		$> 80\mu$ m

Figure 4. Follicle segmentation and criteria Top panel - Raw image (left) with follicle segmentation (right), follicles colour-coded based on follicle stage. Scale bar = 300 μ m. Bottom panel - Table showing criteria for how follicles were grouped, based on a combination of oocyte diameter, AMH coverage, antrum percentage and max granulosa cell layer thickness.

5 Quantitative endpoints

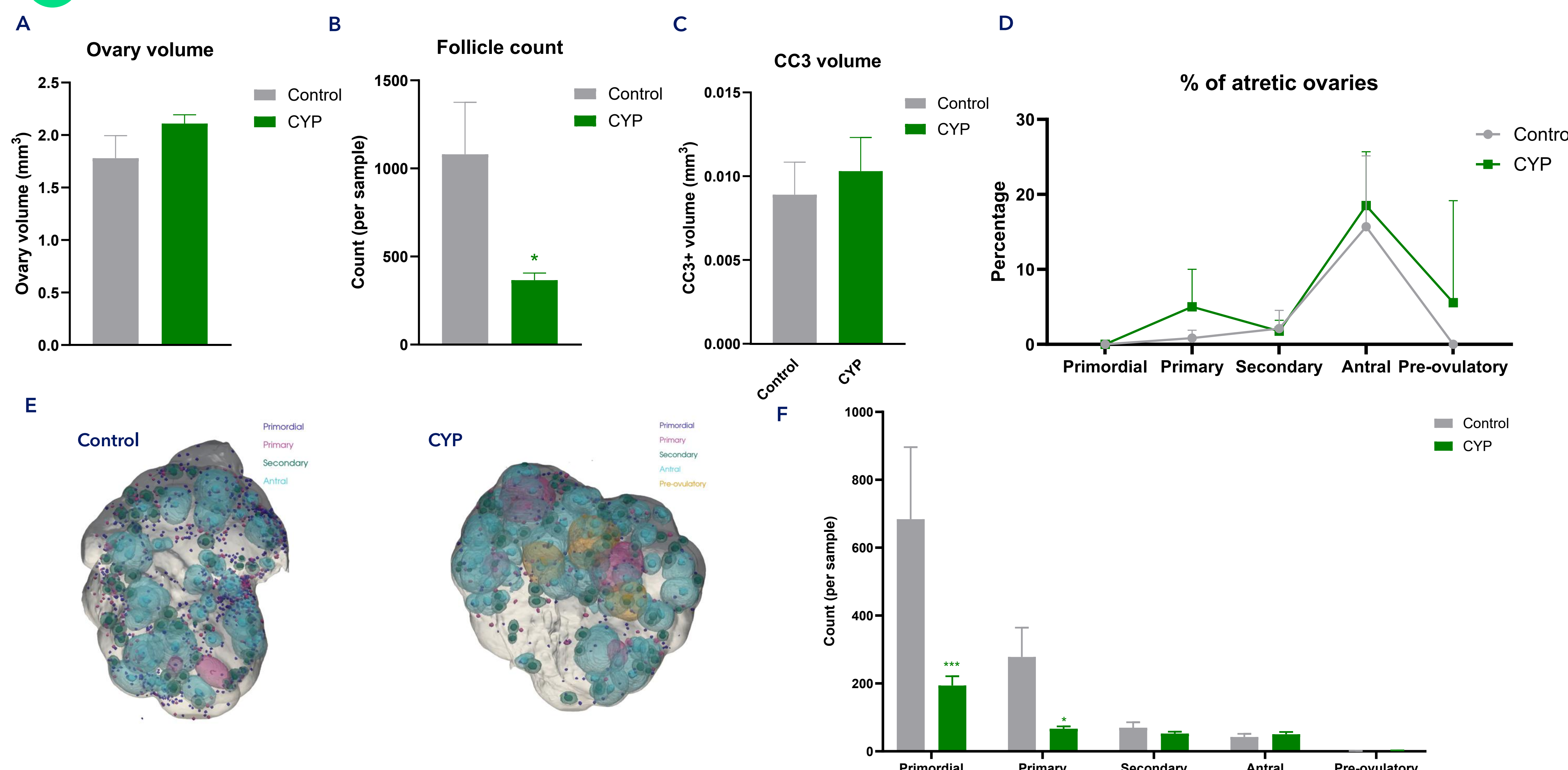


Figure 5. Quantification of ovary endpoints and follicle categorization (A) Ovary volume (B) Follicle count (C) CC3 volume (D) % of atretic ovaries (CC3 coverage $> 5\%$) (E) Follicle segmentation of representative whole mouse ovaries from control and CYP group. n.b. the evidently lower reserves of primary and primordial follicles in the CYP group (F) Follicle categorization. Mean $\pm$ SEM, n = 6 per group. For A-C, Dunnett's test on one-factor linear model. For D and F, two-factor linear model. * $p < 0.05$, *** $p < 0.001$ compared to Control.