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Lightsheet Fluorescence Microscopy of Branching Human Fetal Kidney

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In the fifth week of human development, the ureteric bud forms as a diverticulum off of the intermediate mesoderm-derived mesonephric duct. The ureteric bud epithelium is induced by the adjacent metanephric mesenchyme to invade and undergo repeated branching events, forming the collecting system of the metanephric kidney [1, 2]. Transcription factors expressed in the mesenchyme, including the *Hox11* paralogous group members *Hoxa11*, *Hoxc11* and *Hoxd11*, have been extensively implicated in promotion and maintenance of GDNF/RET signaling furthering ureteric bud branching [2–5].

The depicted human fetal kidney was collected with IRB-approval following elective dilation and curettage. Fetal age was estimated to be 11.0 weeks by heel-toe length. It was fixed in 4% paraformaldehyde, embedded in acrylamide hydrogel matrix and rendered transparent with the PAssive Clarity Technique (PACT) [6]. The specimen was immersed in primary antibodies against E-cadherin and HoxA11 and flurophore-conjugated secondary antibodies. The specimen was embedded in low-melting point agarose, equilibrated to $n=1.46$ in refractory index matching solution and imaged on a Lightsheet Z.1 microscope (Carl Zeiss AG, Oberkochen, Germany). 3D models were constructed using Imaris (Bitplane AG, Zurich, Switzerland).

The imaged lobulated fetal kidney demonstrates extensive branching morphology of E-cadherin immunolabeled ureteric bud epithelium (Fig. 1) and adjacent regions of HoxA11-expressing metanephric mesenchyme (Fig. 2). Kidneys of these age should continue to branch until about week 15 of gestation as the mature renal collecting system continues to develop [2].

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Figure 1. Lighsheet Fluorescence Microscopy (LSFM) image of a lobulated 11-week human fetal kidney stained with anti-E-Cadherin demonstrating branching morphogenesis of the ureteric bud epithelium.

Figure 2. Lightsheet Fluorescence Microscopy (LSFM) image of the same lobulated 11-week human fetal kidney stained with anti-E-Cadherin (red) and anti-HoxA11 (green) demonstrating sites of interaction of ureteric bud epithelium with overlying HoxA11-expressing metanephric mesenchyme.