

D. Histology of human fetal bladders: We wanted to harvest other urogenital organs to confirm that we could produce high quality tissue samples from other developing genitourinary organs. We focused on the bladder as the urothelium of the bladder is notoriously difficult to fix and stain with high quality due to damage that occurs directly after harvesting. In our first set of samples, we saw a similar urothelial damage phenotype due to post harvesting damage. We consulted (b)(6) (GUDMAP) and (b)(6) (GUDMAP) who advised that immediately post-harvesting the bladders should be bisected and immediately immersed in fixative. This approach has been very successful; please see representative images from a 21-week human fetal bladder, with a continuous urothelium and well-preserved lamina propria (**Figure 4A**) and muscle (**Figure 4B**). These images were evaluated by the Bladder evaluation team including (b)(6) as well as GUDMAP members (b)(6) (**letter of support**) and (b)(6) (**letter of support**).

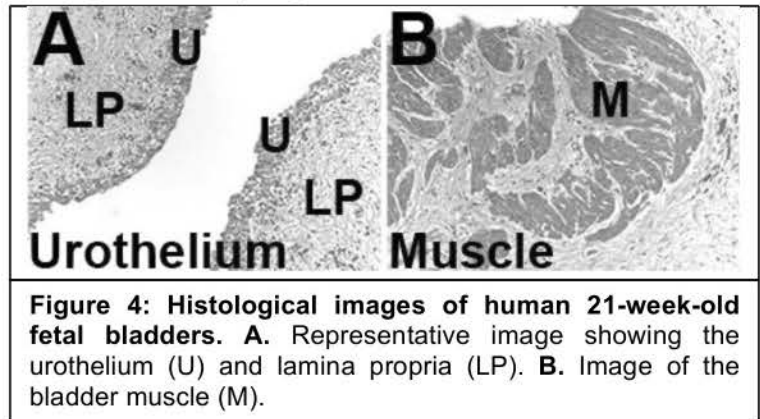


Figure 4: Histological images of human 21-week-old fetal bladders. A. Representative image showing the urothelium (U) and lamina propria (LP). **B.** Image of the bladder muscle (M).

e. Immunostaining of developing bladder urothelium and muscle: As with the kidney tissue we wanted to confirm that the bladder tissue had preserved antigens. We stained for urothelial (Uroplakin 3a and Cadherin 20) and muscle markers (α SMA) [38]. We observed well-preserved urothelium (**Figure 5A-B**), lamina propria (data not shown) and muscle layers (**Figure 5C**). The bladder evaluation team confirmed these findings.

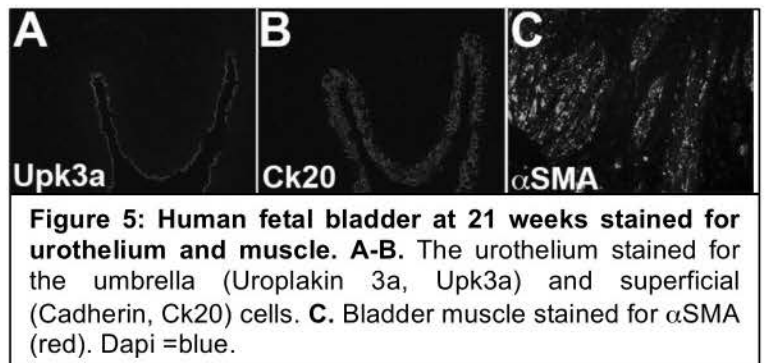


Figure 5: Human fetal bladder at 21 weeks stained for urothelium and muscle. A-B. The urothelium stained for the umbrella (Uroplakin 3a, Upk3a) and superficial (Cadherin, Ck20) cells. **C.** Bladder muscle stained for α SMA (red). Dapi =blue.

3. Experimental Approach:

A. Assessing genitourinary histology: When the genitourinary tissues are received, we will process a third of the tissue into paraffin for histological examination. Sections will be cut at $4\mu\text{m}$ and stained with hematoxylin and eosin to evaluate basic morphology. Our pathologist and anatomist will evaluate tissue quality. High-resolution images will then be taken and these images will be uploaded and be available for GUDMAP projects and the scientific community. Our target goal is to have available a minimum of 5 cases (tissues and if possible other biologics) per week of gestational age for ages 6-42 weeks.

B. Evaluation of tissue specific antibodies: A subsequent third of the tissue will be embedded into TissueTek optimum cutting temperature (OCT) for immunofluorescence (IF). Based on the needs of GUDMAP projects, we will section at $6\mu\text{m}$ and perform IF to confirm the presence of antigens. These stained slides will be reviewed to assess for antigen preservation. Similarly, these high-resolution images will be uploaded and made available to the successful Atlas projects and the scientific community.

C. *In situ* hybridization to assess mRNA integrity: The remaining third of the tissue will be embedded into TissueTek optimum cutting temperature (OCT) for *in situ* hybridization (ISH). Based on the needs of GUDMAP projects, we will section at $6\mu\text{m}$ and perform ISH to confirm preservation of mRNA. These stained slides will be reviewed to assess mRNA degradation. Similarly, these high-resolution images will be uploaded and made available to the successful Atlas projects and the scientific community.

4. Anticipated Results, Pitfalls and Alternatives: We anticipate being able to provide high quality tissue to the successful GUDMAP projects by a combination of the HUB branch of the HSTB and the IIAM (for the later gestation time points). As an alternative for these later gestational time points, we are in the process of updating our IRB protocol at the University of Pittsburgh to include consenting for tissues from neonates of later gestational ages that have undergone sudden death. This would likely produce improved specimen quality and turnaround time since we can immediately harvest the tissue and provide these to the GUDMAP Atlas projects. Furthermore, if in the unlikely event that we are unable to acquire enough tissue from our in house tissue hub and collection site, we will utilize other tissue banks that are known to supply embryonic