Dissecting the spatiotemporal development of the human reproductive ducts through the lens of single-cell and spatial genomics

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The Müllerian and Wolffian ducts - the precursors of the female and male reproductive tracts, respectively - develop in both male and female embryos. Over the course of gestation, the sexually matched ducts continue to differentiate, while the sexually unmatched ducts regress. The development, differentiation, and regression of the Müllerian and Wolffian ducts are regulated by a complex interplay of paracrine interactions and hormonal signalling, which is essential for the proper development of the reproductive tracts. Defects in these processes can lead to congenital anomalies and infertility. However, our understanding of the Müllerian and Wolffian ducts in humans has been hindered by the lack of reliable in vitro models and the significant differences between humans and mice.

Using a combination of single-cell (scRNA-seq, scATAC-seq) and spatial (spatial transcriptomics, *In Situ* Sequencing) genomics techniques, we characterised for the first time the temporal and spatial distribution of cells of the human reproductive tracts during prenatal development (from 6 to 21 weeks post conception). Our analysis revealed transient upregulation of neuronal/axonal growth genes in the Müllerian epithelium during migration as well as an increase in the expression of WNT inhibition and autophagy markers during regression in male embryos. We then linked histological features with gene expression changes along the cranio-caudal axis of the differentiating Müllerian and Wolffian ducts to pinpoint transcription factors beyond the *HOX* code likely responsible for mesenchymal patterning (of both the Müllerian and Wolffian ducts). Moreover, we shed light onto the spatially variable mesenchymal ligands that likely signal to the adjacent epithelium how to differentiate accordingly. Finally, we compared our developmental dataset with publicly available post-pubertal datasets of female and male reproductive tissues to evaluate how much of the spatial organisation and cell type diversity observed in adulthood is already established in utero.

Overall, our study elucidates the still poorly-understood mechanisms that underlie the healthy emergence, differentiation and regression of the human reproductive ducts, while representing a significant step towards better addressing developmental anomalies leading to infertility and other reproductive disorders later on in life.