Single Cell 'Omics Technologies for Contraceptive Discovery

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Around half of all pregnancies occur unintentionally. While female hormonal pills and barrier-based contraceptive options for men are currently available, there is a critical need to identify new, effective, and safe non-hormonal contraceptive alternatives. Meiosis is a specialized reductive division during gametogenesis that results in the production of haploid gametes. Recent work from our lab has shown that meiosis represents a suitable time point to target for non-hormonal contraception. As meiosis is critical for proper gamete formation in both sexes, we hypothesize that this stage is a promising timepoint to target for novel male and female non-hormonal contraceptives.

In males, spermatogonia enter meiosis at around the time of puberty and this continues in waves throughout life. A complex gene regulation network exists in cells undergoing meiotic entry, providing us with many possible gene targets. By contrast, females undergo meiotic entry and progression through prophase I *in utero*, but oocytes arrest in an extended diplotene phase of meiosis I (known as "dictyate arrest"). Importantly, throughout dictyate arrest, which can last years to decades in women, the oocyte is transcriptionally inert. At puberty, meiosis is resumed in a cohort of oocytes in response to hormonal stimuli that result in a cascade of protein signaling pathways between the somatic cells and the oocytes, leading to the completion of meiosis I and subsequent ovulation.

Given this considerable sexual dimorphism in meiosis, there is a need for differing technologies to study these processes and to identify potential contraceptive targets at single-cell resolution. As we are interested in targeting meiotic entry in males, we have employed cutting-edge spatial transcriptomics platforms that provide information at a single-cell resolution. In this study, we optimized Slide-seq and Stereo-seq for Spatial Total RNA Sequencing, which provides us with the entire repertoire of RNA species (coding and non-coding RNAs) that are involved in meiotic regulation. By contrast, since meiotic entry is inaccessible in female meiosis, we are interested in maintaining dictyate arrest as a non-hormonal contraceptive strategy. Owing to the low transcriptional abundance during this phase, we instead focused on identifying proteins present during dictyate arrest at single-cell resolution, reasoning that only a few oocytes at each cycle can respond to stimuli that lead to ovulation. Thus, we want to understand the proteins involved in meiotic resumption following dictyate arrest. We hope targeting these proteins achieves a contraceptive effect by maintaining oocytes in arrest. As oocytes undergo meiotic resumption in cyclical cohorts, the number of cells undergoing these complex mechanisms is very few. In addition, it is unknown what contributes to the selection of certain oocytes into resuming meiosis during these cycles. Current proteomics techniques require significantly large input material to obtain sufficient peptide information, which has hitherto prohibited the study of these cells for their differences. To overcome these limitations, we are developing a novel proteomics technology that enables the identification of thousands of peptides with as few as ten oocytes. This technology will allow us to identify novel protein targets for the use of non-hormonal contraception, far outcompeting existing methods in the field. This study outlines our efforts to

develop state-of-the-art single-cell techniques to further our understanding of possible targets in meiosis in both males and females, which will advance options for non-hormonal contraceptives.