Single cell RNA sequencing shed light on the cellular and molecular alterations behind distinct types of cryptozoospermia.

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Incomplete understanding of the cellular and molecular alterations underlying male infertility leads to a high degree of diagnostic uncertainty, in fact over 70% of infertile men do not receive a causal diagnosis. The complexity of their reproductive disorder is often reduced to abnormal findings in their semen and hormone analyses. Consequently, patients with distinct etiologies are grouped together based on a similar descriptive diagnosis. An example for this, are men diagnosed with cryptozoospermia (Crypto), in which sperm is only found after centrifugation of the ejaculate (HP:0030974), who can though be highly heterogeneous in terms of testicular tissue composition. Here, we aimed at improving the classification of cryptozoospermic patients based on their cellular composition and evaluating potential distinct cellular and molecular alterations among different patient categories. To unravel the testicular tissue composition and transcriptional profile, we performed single cell RNA sequencing in testis samples from 13 infertile patients (obstructive azoospermia (OA), n=4, Crypto, n=9), who had undergone a testicular biopsy at the Centre of Reproductive Medicine and Andrology. We assigned the identity of over 65,000 cells, which - based on their transcriptional profile - included all the different germ cell subtypes (spermatogonia, spermatocytes, and spermatids), as well as testicular somatic cells (Sertoli, Leydig, peritubular, endothelial, perivascular, and immune cells). We performed principal component analysis followed by hierarchical clustering on the principal components using the proportion of the different cell types as input. Interestingly, the clustering resulted in four groups. In group 1 (Control), which included 3 of the OA patients, the spermatids were the most abundant cell type. The Crypto patients were subdivided equally into three groups (n=3 each), which presented immune cells (immune-rich), spermatogonia (SPG-rich) and spermatocytes (SPC-rich) as the most abundant cell type, respectively. One of the patients diagnosed with obstructive azoospermia clustered together with the SPC-rich group. Using a combination of differential gene expression and gene ontology analysis, we evaluated common and distinct molecular changes among the different groups. Interestingly, we could not find any differentially expressed genes between the spermatids of the Control group and those of the three-cryptozoospermic groups, suggesting no transcriptional alterations in this cell type. The immune-rich group showed unique changes in the somatic compartment. Sertoli cells showed signs of reversion to a more immature state, as indicated by increased expression of the Inhibin

Subunit Alpha (INHA) and genes involved in oxidative phosphorylation. Leydig cells showed higher expression of genes involved in the “steroid biosynthetic process”; however, the serum testosterone level in these patients did not differ from the controls. The SPG-rich group showed the highest number of differentially expressed genes in the germ cell stages up to diplotene spermatocytes. Gene ontology analyses revealed that the genes were involved in cell cycle as well as DNA repair. Finally, the SPC-rich groups showed the highest number of differentially expressed genes in the early spermatocytes, which were enriched in terms associated with meiotic cell cycle and chromosome segregation. In summary, our study revealed three subgroups of cryptozoospermic patients based on the testicular tissue composition and identified common and specific cellular and molecular alterations among them. Our approach can pave the way to more precise diagnoses for these infertile patients.