RESULTS

In this study, 211 proteins were differentially regulated in the spermatozoa of men with NSGCT compared to NC.

1. The IPA analysis showed that spermatozal proteins implicated in canonical pathways such as RNA binding, EIF2 signaling, mitochondrial dysfunction, mitochondrial-L-carnitine shuttle pathway, oxidative phosphorylation were downregulated significantly (P<0.01) whereas the proteins playing a role in LXR/RXR activation were significantly upregulated (P<0.01) in NSGCT compared with NC (Figure 1).

2. The reproductive system development and function was differentially regulated in the spermatozoa of men with NSGCT compared to NC.

3. Most of the DEPs in diseases and disorders, molecular and cellular functions and physiological system development and function were downregulated in the spermatozoa of men with NSGCT.

4. Proteins responsible for proper mitochondrial function in the spermatozoa were downregulated (P<0.01) leading to mitochondrial dysfunction in men with NSGCT. On the other hand, proteins implicated in LXR/RXR and FXR/THR activation respectively were significantly upregulated (P<0.01) in the spermatozoa of men with NSGCT when compared with NC.

5. Downregulation of proteins involved in biological pathways such as spermatogenesis, gonadogenesis, and fertility potential could cause a reduction in the fertility potential (Figure 3). The downregulation key proteins shown in the molecular networks (Z Score ≥ 3) lead to abnormal spermatozoa, sperm disorder and abnormal morphology of male germ cells in men with NSGCT (Figure 4).

6. DEPs associated with spermatozoan, ATP production, acrosome reaction and fertilization were downregulated in NSGCT group (Table 1).

7. The comparison of DEPs, upregulated and downregulated proteins in the spermatozoa of men with NSGCT showed that stream regulators were activated (Z Score ≥ 2.0), whereas molecular networks were inhibited (Z Score ≤ 2.0) in the spermatozoa of men with NSGCT compared to NC (Figure 5).

8. Molecular network and core analyses in IPA showed the activation of RICTOR that could lead to the downregulation of proteins involved in EIF2 signaling, mitochondrial dysfunction, oxidative phosphorylation and protein ubiquitination in the spermatozoa of men with NSGCT.

CONCLUSIONS

1. Proteins required for molecular and cellular functions such as energy production, cell-to-cell signaling and interaction, reproductive system development and function, cell assembly and organization, protein degradation, and protein synthesis were significantly impacted in the spermatozoa of men with NSGCT.

2. Key proteins involved in acrosome reaction, binding of sperm to the zona pellucida, fertilization, spermatogenesis, sperm function, sperm motility etc., were downregulated in the spermatozoa of men with NSGCT.

3. The molecular mechanism(s) of reduced fertility in men with NSGCT might be ascribed to the stimulation of up-stream regulators such as RICTOR, leading to the downregulation of proteins involved in EIF2 signaling, mitochondrial dysfunction, oxidative phosphorylation and protein ubiquitination in the spermatozoa of men with NSGCT.

4. Our findings may help explain the impaired semen quality and fertility observed in many patients with NSGCT even before initiating cancer treatment.