Reduced semen quality in patients with testicular cancer seminoma is associated with alterations in the expression of sperm proteins

Tânia R Dias¹,²,³, Ashok Agarwal¹, Peter N Pushparaj⁴, Gulfam Ahmad⁵, Rakesh Sharma¹

Testicular cancer seminoma is one of the most common types of cancer among men of reproductive age. Patients with this condition usually present reduced semen quality, even before initiating cancer therapy. However, the underlying mechanisms by which testicular cancer seminoma affects male fertility are largely unknown. The aim of this study was to investigate alterations in the sperm proteome of men with seminoma undergoing sperm banking before starting cancer therapy, in comparison to healthy proven fertile men (control group). A routine semen analysis was conducted before cryopreservation of the samples (n = 15 per group). Men with seminoma showed a decrease in sperm motility (P = 0.019), total motile count (P = 0.001), concentration (P = 0.003), and total sperm count (P = 0.001). Quantitative proteomic analysis identified 393 differentially expressed proteins between the study groups. Ten proteins involved in spermatogenesis, sperm function, binding of sperm to the oocyte, and fertilization were selected for validation by western blot. We confirmed the underexpression of heat shock-related 70 kDa protein 2 (P = 0.041), ubiquinol-cytochrome C reductase core protein 2 (P = 0.026), and testis-specific sodium/potassium-transporting ATPase subunit alpha-4 (P = 0.016), as well as the overexpression of angiotensin I converting enzyme (P = 0.005) in the seminoma group. The altered expression levels of these proteins are associated with spermatogenesis dysfunction, reduced sperm kinematics and motility, failure in capacitation and fertilization. The findings of this study may explain the decrease in the fertilizing ability of men with seminoma before starting cancer therapy.

Keywords: male fertility; proteomics; seminoma; sperm proteins; sperm quality; testicular cancer

INTRODUCTION

Germ cell tumors (GCTs) represent the most common type of testicular cancer, accounting for about 90%–95% of all cases. The principal types of GCTs are nonseminomas and seminomas; the latter usually grows and spreads more slowly. In the last decades, there is a growing trend in the proportion of seminomas.¹ The survival rate of men with seminoma is very high (over 95%); thus, it is generally not seen as a threat to public health. However, its impact on male fertility represents a major concern for reproductive medicine as it frequently affects men in reproductive age (20–44 years).²

Men with seminoma present impaired fertilizing ability, even before diagnosis.³ Testicular cancer seminoma affects the hypothalamic-pituitary-gonadal (HPG) axis and consequently disturbs spermatogenesis.⁴ These deleterious effects are dependent on the stage and type of seminoma, resulting in poor semen quality or even azoospermia.³ The treatment for this type of cancer, usually performed by surgery, chemotherapy, or radiotherapy, further affects semen quality³ and hormonal function,⁴ thus highly impairing male fertility. In fact, after cancer therapy, patients may become temporarily or permanently infertile.³ For that reason, it is strongly recommended that men diagnosed with seminoma undergo sperm banking to increase the probability to father a child in the future.⁴ The chances to establish a pregnancy by natural conception are 30% lower after the cancer therapy and the recovery of fertilizing ability usually takes several years.⁵ Therefore, in many surviving patients with seminoma, assisted reproductive technology (ART) with cryopreserved samples is the only option for having children.⁶ Still, sperm banking is not possible for many patients due to the high cost or lack of facilities, urgency to initiate the treatment, impaired spermatogenesis, and/or poor semen quality at the time of specimen collection.⁷

Proteomics studies have been recently used as a valuable tool to explore how certain health conditions affect male reproductive potential, especially by evaluating spermatozoa and seminal plasma proteome.⁸,⁹ Although spermatozoa are transcriptionally and translationally silent after being produced in the testis, the acquisition of sperm function occurs during maturation in the epididymis and transit through the female reproductive tract.¹⁰ Therefore, the sperm proteome is highly susceptible to alterations according to the health status of

¹American Center for Reproductive Medicine, Cleveland Clinic, Cleveland, OH 44195, USA; ²Department of Health Sciences, Faculty of Health Sciences, University of Beira Interior, Covilhã 6201-001, Portugal; ³Department of Microscopy and Unit for Multidisciplinary Research in Biomedicine, Institute of Biomedical Sciences Abel Salazar, University of Porto, Porto 4050-313, Portugal; ⁴Center of Excellence in Genomic Medicine Research, Faculty of Applied Medical Sciences, Jeddah 21589, Saudi Arabia; ⁵Division of Pathology, School of Medical Sciences, Sydney University, Lidcombe NSW 2141, Australia.

Correspondence: Dr. A Agarwal (agarwaa@ccf.org)
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the individual, and this impacts the quality of sperm parameters. The deleterious effects of seminoma treatment represent a challenge to understand the mechanisms behind the impairment of male fertility caused by the disease. In this study, we used semen samples from men with testicular cancer seminoma that were cryopreserved before starting cancer therapy, to investigate alterations in the sperm proteome in comparison with healthy proven fertile men.

PARTICIPANTS AND METHODS

Semen analysis and cryopreservation

This study was conducted after approval by the Institutional Review Board (IRB) of Cleveland Clinic, Cleveland, OH, USA. Semen samples were obtained from healthy volunteers with proven fertility (control, \( n = 15 \)) and patients with seminoma (\( n = 15 \)). All the participants signed informed written consent to allow the use of their samples in this study. The inclusion criteria were as follows: (1) control group, healthy fertile men who had fathered a child in the last 2 years; (2) seminoma group, patients diagnosed with seminoma and undergoing sperm banking before starting cancer therapy. Following 2–3 days of abstinence, semen samples were collected at the Andrology Center, Cleveland Clinic. Samples were liquefied for 20–30 min in an incubator (Panasonic, Newark, NJ, USA) at 37°C, and a routine semen analysis was conducted according to the World Health Organization (WHO) 2010 guidelines.\(^{15} \) Semen volume, sperm motility, and sperm concentration were recorded. Total sperm count and total motile count were also calculated and the results were expressed as mean ± standard error of the mean (s.e.m.). Whole ejaculate samples were immediately cryopreserved in TEST-yolk buffer (TYB; Irvine Scientific, Santa Ana, CA, USA) in a ratio of 1:1 as previously described\(^{16} \) and finally labeled and stored in liquid nitrogen at −196°C.

Protein extraction and estimation

Samples were thawed on ice and centrifuged at 4000g for 10 min (Eppendorf, Hauppauge, NY, USA). To remove the freezing medium (TYB) as much as possible, the sperm pellet was washed four times in phosphate-buffered saline (PBS; Sigma-Aldrich, St. Louis, MO, USA) and centrifuged at 4000g for 10 min at 4°C. Total sperm protein was extracted overnight at 4°C with radioimmunoprecipitation assay (RIPA) buffer (Sigma-Aldrich). Subsequently, samples were centrifuged at 10000g for 30 min at 4°C, to recover the protein fraction (supernatant). Pierce BCA Protein Assay kit (Thermo Fisher Scientific, Waltham, MA, USA) was used to estimate the protein concentration, according to the manufacturer's instructions.

Quantitative proteomic analysis

Three samples from the control or seminoma group were randomly selected for the proteomic analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Samples were pooled (\( n = 3 \)) using the same amount of protein from each sample. Each pool was then evaluated as an individual sample in the proteomic analysis. The system used was a Finnigan LTQ-Orbitrap Elite hybrid mass spectrometer (Thermo Fisher Scientific) using the previously described conditions and software.\(^{17} \) Scaffold (version 4.0.6.1, Proteome Software Inc., Portland, OR, USA) was used for the identification of differentially expressed proteins (DEPs) between the control and seminoma groups. The spectral counts were used to determine the abundance of each protein (very low, low, medium, or high). The identified DEPs were categorized as underexpressed, overexpressed, or unique to one of the groups, based on the normalized spectral abundance factor (NSAF) ratio according to previously reported criteria.\(^{17} \)

Bioinformatic analysis

Bioinformatic analysis of DEPs identified by LC-MS/MS was carried out using the Ingenuity Pathway Analysis software (IPA; Qiagen, Hilden, Germany). IPA was used to evaluate the canonical pathways, top diseases and bio-functions, and upstream regulators related to the identified DEPs. Proteins were selected for validation by western blot considering the following criteria: (1) proteins involved in reproductive system development and function; (2) proteins involved in the top canonical pathways; (3) proteins with a higher difference of abundance between the experimental groups; and (4) proteins with a well-described function in the literature. Only proteins that met all the above-mentioned criteria were subjected to western blot.

Western blot

Western blot was performed using individual samples from the control and seminoma groups (\( n = 6 \) per group). A total of 25 µg protein per sample was mixed with 4 × Laemmli sample buffer (Bio-Rad, Hercules, CA, USA) in a ratio of 1:3 and made up to 25 µl with PBS. Samples were boiled at 95°C for 10 min and immediately loaded into a 4%–15% (w/v) polyacrylamide gel (Bio-Rad). Electrophoresis was performed with constant voltage (90 V) for 2 h. Precision Plus Protein™ Dual Xtra Standard (Thermo Fisher Scientific) was used as the molecular weight marker. The resolved proteins were transferred (20 V for 30 min) to methanol-activated polyvinylidene difluoride (PVDF) membranes (GE Healthcare, Marlborough, MA, USA) and blocked for 90 min at room temperature, with a 5% (w/v) nonfat milk (Bio-Rad) solution prepared in tris-buffered saline with tween-20 (TBST; Sigma-Aldrich). Membranes were incubated overnight (4°C) with specific primary antibodies followed by the respective secondary antibodies at room temperature, for 90 min (Supplementary Table 1). Membranes were incubated with enhanced chemiluminescence (ECL) reagent (GE Healthcare) for 5 min, and the chemiluminescence signals were read in the ChemiDoc™ MP Imaging System (Bio-Rad). Densities from each band were quantified with Image Lab™ Software (version 6.0.1, Bio-Rad) and divided by the corresponding total protein lane density. Total protein density was obtained by incubation of the membranes with total colloidal gold protein stain (BioRad). The results were expressed as fold variation relative to the control group.

Statistical analyses

After testing normal distribution by the Kolmogorov–Smirnov test, semen parameters and western blot results were analyzed by Mann–Whitney U test for independent samples, using the MedCalc Software (version 17.8; MedCalc Software, Ostend, Belgium). All data are presented as mean ± s.e.m., and differences with \( P < 0.05 \) were considered statistically significant.

RESULTS

Semen quality in patients with testicular cancer seminoma

The average volume of the ejaculates was very similar between the control and seminoma groups (Table 1). However, there was a decrease in sperm parameters of fertile men (control) and patients with testicular cancer seminoma

Table 1: Semen parameters of fertile men (control) and patients with testicular cancer seminoma

| Parameter                          | Control  | Seminoma | \( P \) |
|-----------------------------------|----------|----------|--------|
| Semen volume (ml)                 | 3.53±0.35| 3.33±0.42| 0.541  |
| Sperm motility (%)                | 67±3     | 54±5     | 0.019  |
| Sperm concentration (10⁶ ml⁻¹)    | 95.49±7.79| 46.72±12.19| 0.003 |
| Total sperm count (10⁶)           | 316.92±45.41| 136.11±41.55| 0.001 |
| Total motile count (10⁶)          | 211.88±30.09| 75.63±22.44| 0.001 |

Results are presented as mean±s.e.m. (\( n=15 \) per group). Statistical significance was considered for \( P < 0.05 \). s.e.m.: standard error of the mean.
motility ($P = 0.019$), sperm concentration ($P = 0.003$), total sperm count ($P = 0.001$), and total motile count ($P = 0.001$) in patients with seminoma relative to control (Table 1). Nevertheless, all the samples were considered normozoospermic according to the WHO 2010 criteria.\(^\text{15}\)

**Differentially expressed proteins**

Proteomic analysis identified 1149 proteins in the control group and 911 in the seminoma group. After comparative analysis between the experimental groups, a total of 1192 proteins were quantified and 393 were found to be differentially expressed (Supplementary Table 2). More than half (52.7%) of the DEPs were underexpressed, while 20.1% were overexpressed in spermatozoa of patients with seminoma. Furthermore, 4.1% of the DEPs were unique to the seminoma group and 23.1% unique to the control group (Figure 1).

**Selection of proteins for validation**

According to the IPA analysis, among the top diseases and biofunctions related to “physiological system development and function,” the category with the highest $P$ value was “reproductive system development and function.” Within this category, we selected seven proteins involved in specific reproductive processes (Table 2): angiotensin-converting enzyme (ACE), acrosin precursor (ACR), T-complex protein 1 subunit gamma (CCT3), sperm surface protein Sp17 (SPA17), sodium/potassium-transporting ATPase subunit alpha-4 (ATP1A4), heat shock-related 70 kDa protein 2 (HSPA2), and proteasome activator complex subunit 4 (PSME4). Some of these proteins were also involved in the top canonical pathways identified in this dataset. While HSPA2 participates in the “protein ubiquitination pathway” and “unfolded protein response,” ACE is related to “phagosome maturation.” Other top five canonical pathways included “mitochondrial dysfunction” and “oxidative phosphorylation.” Among the proteins involved in those pathways were NADH-ubiquinone oxidoreductase 75 kDa subunit (NDUFS1), cytochrome b-c1 complex subunit 2 (UQCRC2), and ATP synthase subunit alpha (ATP5A), which are subunits of the mitochondrial complexes I, III, and V, respectively. These three proteins were also selected for analysis by western blot. The abundance and expression pattern of the ten selected proteins obtained by the proteomic analysis is presented in Table 3.

**Prediction of the upstream regulators**

The IPA analysis predicted the activation or inhibition of several proteins, which could be responsible for the altered expression in the sperm proteome of men with seminoma. The rapamycin-insensitive companion of mammalian target of rapamycin (RICTOR) was predicted to be activated, thus leading to the underexpression of NDUFS1, UQCRC2, ATP5A1, and PSME4. Moreover, it was predicted that the underexpression of ATP5A1 and ATP1A4 may involve the activation of the amyloid-beta A4 protein (APP). On the other hand, the inhibition of the heat shock factor protein 2 (HSF2) was predicted to regulate the underexpression of CCT3, as well as six other chaperonins of the T-complex protein-1 (TCP-1) family (CCT2, CCT4, CCT5, CCT6A, CCT7, and CCT8).

**Western blot analysis**

All proteins selected for western blot analysis were identified. There was an increase in the protein expression of ACE ($P = 0.005$) and ACR ($P = 0.009$) in the seminoma group ($2.61 \pm 0.38$ and $2.02 \pm 0.26$-fold variation to control, respectively) in comparison with the

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**Table 2: Specific functions of the differentially expressed proteins related to reproductive system development and function identified by the bioinformatic analysis when comparing the sperm proteome of patients with testicular cancer seminoma with fertile men**

| Process | Protein | $P$ |
|---------|---------|-----|
| Binding of sperm | ACE, ACR, CCT3, SPA17 | $<0.0001$ |
| Fertilization | ACE, ACR, ATP1A4, SPA17 | $<0.0001$ |
| Cell movement of sperm | ATP1A4 | $<0.0001$ |
| Spermatogenesis | ACE, ATP1A4, HSPA2, PSME4, SPA17 | $0.0036$ |
| Function of sperm | ATP1A4 | $0.0028$ |
| Acrosome reaction | ACR | $0.0037$ |
| Fertility | ACE, ACR, PSME4 | $0.0067$ |
| Morphology of male germ cells | ACR, PSME4 | $0.0089$ |
| Morphology of sperm | ACR | $0.0120$ |
| Hyperactivation of sperm | ATP1A4 | $0.0133$ |

ACE: angiotensin-converting enzyme; ACR: acrosin precursor; ATP1A4: sodium/potassium-transporting ATPase subunit alpha-4; CCT3: T-complex protein 1 subunit gamma; HSPA2: heat shock-related 70 kDa protein 2; PSME4: proteasome activator complex subunit 4; SPA17: sperm surface protein Sp17

**Table 3: Proteomic data of the differentially expressed proteins identified in the spermatozoa samples of fertile men (control) and men with testicular cancer seminoma before cancer therapy, which were selected for validation by western blot**

| Protein | Abundance | NSAF ratio | Expression profile | $P$ |
|---------|-----------|------------|--------------------|-----|
| ACE | High | 1.62 | Overexpressed in seminoma | 0.0131 |
| ACR | Medium | 0.34 | Underexpressed in seminoma | 0.001 |
| ATP1A4 | Medium | 0.07 | Underexpressed in seminoma | 0.0001 |
| ATP5A1 | High | 0.18 | Underexpressed in seminoma | $<0.0001$ |
| CCT3 | High | 0.09 | Underexpressed in seminoma | $<0.0001$ |
| HSPA2 | High | 0.53 | Underexpressed in seminoma | $<0.0001$ |
| NDUFS1 | Medium | 0.42 | Underexpressed in seminoma | 0.0307 |
| PSME4 | Medium | 0.13 | Underexpressed in seminoma | 0.0006 |
| SPA17 | Medium | 0.02 | Underexpressed in seminoma | 0.0001 |
| UQCRC2 | High | 0.23 | Underexpressed in seminoma | 0.0001 |

ACE: angiotensin-converting enzyme; ACR: acrosin; ATP1A4: sodium/potassium-transporting ATPase subunit alpha-4; ATP5A1: ATP synthase subunit alpha, CCT3: T-complex protein 1 subunit gamma; HSPA2: heat shock-related 70 kDa protein 2; NDUFS1: NADH-ubiquinone oxidoreductase 75 kDa subunit; NSAF: normalized spectral abundance factor; PSME4: proteasome activator complex subunit 4; SPA17: sperm surface protein Sp17; UQCRC2: cytochrome b-c1 complex subunit 2
control (1.00 ± 0.25 and 1.00 ± 0.19, respectively) (Figure 2). On the other hand, there was a decrease in the protein levels of ATP1A4 (P = 0.016) and HSPA2 (P = 0.041) in men with seminoma (0.53 ± 0.03 and 0.32 ± 0.11-fold variation to control, respectively) when compared with the control group (1.00 ± 0.25 and 1.00 ± 0.22, respectively) (Figure 2). The protein levels of CCT3, SPA17, and PSME4 were similar between the study groups. There was also a decrease (P = 0.026) in the protein expression levels of UQCRC2 (0.54 ± 0.14-fold variation to control) in the seminoma group relative to the control (1.00 ± 0.14) (Figure 3). No differences were found in the protein levels of NDUFS1 or ATP5A1 between the experimental groups.

**DISCUSSION**

The present study is the first attempt to identify alterations in spermatozoa proteome of patients with seminoma before initiating cancer therapy, using fertile donors as control group. Our goal was to evaluate the expression levels of proteins involved in reproductive function from spermatogenesis to sperm function and fertilization. This may provide new insights on the underlying mechanisms responsible for the reduced sperm quality in men with seminoma.

Spermatogenesis consists of a complex process of spermatozoa production that involves several steps of germ cell differentiation. The bioinformatic analysis identified an underexpression of PSME4 in spermatozoa of patients with seminoma. PSME4 plays a role in the morphology of male germ cells; it is particularly important for histone replacement during chromatin remodeling and DNA double-strand break repair. It has been reported that mice lacking this protein present impaired spermatogenesis and reduced fertility. Thus, the downregulation of this protein may contribute to reduced fertility in men with seminoma. Although we were not able to confirm the underexpression of PSME4 by western blot in our dataset, we observed the underexpression of the molecular chaperone HSPA2 by both proteomics and western blot analysis. Molecular chaperones are essential for normal sperm production and functional transformation. HSPA2 acts as a protein quality control system as it ensures the correct folding/refolding of proteins and activates the degradation of misfolded proteins. It has been described that HSPA2 participates in the stability of the microtubules during the meiotic process of germ cell differentiation. In fact, animal studies show that knockout mice for Hspa2 exhibit an enormous number of apoptotic germ cells, resulting in infertility. Men with abnormal spermatogenesis frequently present a reduced hspa2 mRNA expression. Thus, the downregulation of HSPA2 protein in men with seminoma may contribute to the decreased production of normal spermatozoa during spermatogenesis, which is in accordance with the observed reduction in sperm concentration and total sperm count in seminoma group.

The protein ATP1A4 was identified as downregulated in seminoma group by the proteomic analysis, and this result was confirmed by the western blot technique. IPA analysis revealed that ATP1A4 participates in several reproductive processes, including spermatogenesis, function of sperm, cell movement of sperm, hyperactivation, and fertilization. ATP1A4 is the catalytic subunit of the Na+/K+ ATPase membrane protein, which controls the exchange of sodium and potassium ions across the plasma membrane in an ATP-dependent reaction. The regulation of ions in spermatozoa is essential for the acquisition of motility and fertilizing ability. ATP1A4 plays a key role in maintaining human sperm motility. It has been shown that male mice lacking this subunit are completely sterile and their spermatozoa present not only reduced motility but also impaired hyperactivation and inability to fertilize in vitro. These studies highlight the importance of ATP1A4 for male fertility, and the underexpression of this protein in spermatozoa of men with seminoma may explain the decrease in sperm motility and total motile count relative to proven fertile men (control group). The downregulation of ATP1A4 was related to the activation of APP. In fact, this protein has been identified in human spermatozoa and suggested to play an important role in sperm function, especially in signaling events involved in sperm motility.

Another important process crucial for sperm function is mitochondrial function. It is required for energy production necessary for spermatogenesis, sperm movement and production of reactive oxygen species (ROS) in physiological amounts to trigger capacitation and regulate hyperactivation and acrosome reaction. Mitochondrial function relies on the expression of the mitochondrial complexes I–IV for oxidative phosphorylation (OXPHOS) and complex V for ATP production. Our proteomic data showed a downregulation of NDUFS1, UQCRC2, and ATP5A1 in the seminoma group, which are subunits of complex I, III, and V, respectively. The downregulation of these three proteins was predicted to be induced by the activation of RICTOR, which plays a key role in spermatogenesis and sperm maturation signaling.

**Figure 2:** Graphical representation of the expression levels of proteins involved in reproductive functions (ACE, ACR, ATP1A4, CCT3, HSPA2, PSME4, and SPA17) in spermatozoa samples obtained from fertile men (control) and men with testicular cancer seminoma. Results are presented as fold variation to control and expressed as mean ± standard error of the mean (n = 15 per group). Statistical significance is indicated as: “*P < 0.05,” “**P < 0.01,” seminoma versus control. Representative blots for each protein are also presented. ACE: angiotensin-converting enzyme; ACR: acrosin precursor; ATP1A4: sodium/potassium-transporting ATPase subunit alpha; CCT3: t-complex protein 1 subunit gamma; HSPA2: heat shock-related 70 kDa protein 2; PSME4: proteasome activator complex subunit 4; SPA17: sperm surface protein Sp17.

**Figure 3:** Graphical representation of the protein expression levels of mitochondrial complex subunits NDUFS1, UQCRC2, and ATP5A1 in spermatozoa samples obtained from fertile men (control) and men with testicular cancer seminoma. Results are presented as fold variation to control and expressed as mean ± standard error of the mean (n = 15 per group). Statistical significance is indicated as: “*P < 0.05,” seminoma versus control. Representative blots for each protein are also presented. NDUFS1: NADH-ubiquinone oxidoreductase 75 kDa subunit; UQCRC2: cytochrome b-c1 complex subunit 2; ATP5A: ATP synthase subunit alpha.
pathways. The mitochondrial subunits are essential for the proper assembly of the complexes; thus, alterations in their protein expression in spermatozoa are indicative of mitochondrial dysfunction, as reported by the IPA canonical pathways. Although the western blot analysis demonstrated a tendency of reduced expression of the three mitochondrial subunits, only the UQCRCC2 was decreased in patients with seminoma. Downregulation of UQCRCC2 was associated with reduced sperm kinematics, ATP production, and capacitation, which ultimately compromises sperm binding and fertilization. In fact, an underexpression of UQCRCC2 was observed in infertile men with varicocele.

The acquisition of sperm fertilizing ability involves a timed triggering of events in the female reproductive system, culminating in sperm–oocyte binding. SPA17 and CCT3 are two sperm proteins involved in this function, which were identified as downregulated in the seminoma group by the proteomic analysis. SPA17 is a mannose-binding protein that binds to zona pellucida carbohydrates during fertilization. It also plays an important role in germ cell differentiation during spermatogenesis, as its expression increases from early to late stages. CCT3 is one of the subunits of the TCP-1 complex. Although we selected to evaluate the expression levels of this subunit, six other subunits of this complex (CCT2, CCT4, CCT5, CCT6A, CCT7, and CCT8) were also downregulated in men with seminoma. These subunits mediate capacitation-dependent binding of spermatozoa to the zona pellucida. Thus, the downregulation of this system may compromise sperm fertilization. The downregulation of TCP-1 complex subunits was predicted to be due to HS2 inhibition. In fact, disruption of hsf2 in mice affected testicular size and spermatogenic defects. When active, HS2 is likely to induce the upregulation of HSPA2. Thus, the predicted inhibition of HS2 in men with seminoma is in accordance with the downregulation of HSPA2. Although the underexpression of SPA17 and CCT3 was not confirmed by the western blot, the downregulation of HSPA2 in men with seminoma may contribute to the loss of sperm function. In fact, this protein is known to regulate the formation of zona pellucida-binding sites in spermatozoa during spermatogenesis. In addition, it regulates fertilization by mediating the function of sperm surface receptors, such as sperm adhesion molecule 1 (SPAM1) and arylsulfatase A (ARSA), during sperm-egg recognition. Previous proteomic studies have shown low expression levels of HSPA2 in men with asthenozoospermia and primary or secondary infertility. Another study also reported a downregulation of HSPA2, ATP1A4, and SPA17 in infertile varicocele patients. Our results suggest that the altered expression levels of these proteins in men with seminoma may contribute to the impairment of male fertility.

The proteomic analysis also identified ACE as overexpressed in the seminoma group, and this result was confirmed by western blot. This protein is a zinc metalloendopeptidase responsible for the conversion of angiotensin I to angiotensin II. The role of ACE in male reproductive function is not completely understood. Studies with ACE-deficient mice reported that these animals produce a normal number of spermatozoa and present normal motility and morphology. However, the spermatozoa were unable to bind and fertilize the egg. A negative correlation between sperm-bound ACE activity and sperm motility has also been observed. The testis-specific isoform of this protein (tACE) is believed to be released from functional spermatozoa during capacitation and acrosome reaction to increase the fertilizing ability. In fact, a lower tACE activity was detected in spermatozoa from normozoospermic men relative to those with oligoasthenozoospermia. Thus, the overexpression of this protein in spermatozoa from men with seminoma may be responsible for the decrease in sperm motility observed in this group, and possibly explains why some men with seminoma are not able to have children even before the treatment.

Finally, the protein ACR, in its precursor form (proacrosin), was identified as underexpressed in the seminoma group by the proteomic analysis. This protein is activated and converted to its active form during acrosome reaction, playing a role in sperm–oocyte binding. In contrast, using western blot, we found a high overexpression of this protein in men with seminoma. Although we cannot clearly infer about the molecular mechanisms, any of the scenarios (underexpression/overexpression) could lead to a defective acrosome reaction and impaired fertilization. The difference on these results may be due to the sensitivity of each technique and to the sample size. Further studies to assess acrosin activity in men with seminoma are needed to clarify the impact of this condition in acrosome reaction.

Overall, our study points toward important alterations in sperm proteins with a key role in male fertility in men with seminoma. As of today, no specific sperm markers have been identified for the clinical diagnosis and monitoring of testicular cancer seminoma development. The expression levels of HSPA2, ATP1A4, UQCRCC2, and ACE can be helpful sperm biomarkers when evaluating the fertility status of a man, which may allow the early diagnosis of seminomas in a noninvasive approach. Although there is still a lot to explore in the pathophysiology of male subfertility/infertility in men with seminoma, our results represent a step forward in understanding the molecular mechanisms behind the reduced sperm quality in these patients. Future advances in mass spectrometry and bioinformatics will improve our understanding on human sperm function in healthy and disease conditions.

**AUTHOR CONTRIBUTIONS**

AA and RS were responsible for the conception and design of the study. TRD was responsible for the acquisition and interpretation of data, as well as writing the first draft. GA helped in samples processing and PNP performed the bioinformatic analysis. All authors read and approved the final manuscript.

**COMPETING INTERESTS**

All authors declared no competing interests.

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Supplementary Information is linked to the online version of the paper on the Asian Journal of Andrology website.

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### Supplementary Table 1: List of the primary and secondary antibodies used in this study

| Antibody | Source | KDa | Dilution | Vendor | Catalog Number |
|----------|--------|-----|----------|--------|----------------|
| ACE      | Rabbit | 200 | 1:1000   | Abcam  | ab85955        |
| ACR      | Rabbit | 46  | 1:1000   | Abcam  | ab203289       |
| ATP1A4   | Rabbit | 100 | 1:10000  | Abcam  | ab76020        |
| ATP5A    | Mouse  | 54  | 1:1000   | Abcam  | ab110411       |
| CCT3     | Rabbit | 61  | 1:2000   | Abcam  | ab225878       |
| HSPA2    | Mouse  | 70  | 1:500    | Abcam  | ab89130        |
| NDUF51   | Rabbit | 79  | 1:10000  | Abcam  | ab157221       |
| PSME4    | Rabbit | 211 | 1:500    | Abcam  | ab181203       |
| SPA17    | Rabbit | 17  | 1:1000   | Abcam  | ab172626       |
| UQRC2    | Mouse  | 48  | 1:1000   | Abcam  | ab110411       |
| Mouse    | Rabbit | -   | 1:10000  | Abcam  | ab6728         |
| Rabbit*  | Goat   | -   | 1:10000  | Abcam  | ab97051        |

*Secondary antibody. ACE: angiotensin-converting enzyme; ACR: acrosin precursor; CCT3: T-complex protein 1 subunit gamma; SPA17: sperm surface protein Sp17; ATP1A4: sodium/potassium-transporting ATPase subunit alpha-4; HSPA2: heat shock-related 70 kDa protein 2; PSME4: proteasome activator complex subunit 4; NDUF51: NADH-ubiquinone oxidoreductase 75 kDa subunit; UQRC2: cytochrome b-c1 complex subunit 2; ATP5A: ATP synthase subunit alpha

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### Supplementary Table 2: List of the differentially expressed proteins identified by the bioinformatic analysis when comparing the sperm proteome of fertile men (control) and patients with testicular cancer seminoma

| Protein                                                                 | Accession | Average SC | Abundance | NSAF ratio | t-test | Expression       |
|------------------------------------------------------------------------|-----------|------------|-----------|------------|--------|-----------------|
| 1. Transmembrane and coiled-coil domain-containing protein 2            | 56847610  | 23.3       | 0         | M          | ni     | 0.00            | Unique to Control |
| 2. Isocitrate dehydrogenase (NAD) subunit alpha, mitochondrial precursor| 5031777   | 48.0       | 0         | M          | ni     | 0.00            | Unique to Control |
| 3. Succinyl-CoA ligase (ADP-forming) subunit beta, mitochondrial precursor| 11321583  | 25.7       | 0         | M          | ni     | 0.00            | Unique to Control |
| 4. Short-chain specific acyl-CoA dehydrogenase, mitochondrial precursor | 4557233   | 50.0       | 0         | M          | ni     | 0.00            | Unique to Control |
| 5. Probable serine carboxypeptidase CPVL isoform X1                     | 530384848 | 27.0       | 0         | M          | ni     | 0.00            | Unique to Control |
| 6. ATP synthase subunit O, mitochondrial precursor                      | 4502303   | 33.7       | 0         | M          | ni     | 0.00            | Unique to Control |
| 7. Doublecortin domain-containing protein 2C                            | 566006166 | 21.7       | 0         | M          | ni     | 0.00            | Unique to Control |
| 8. Bifunctional glutamate/proline-tRNA ligase                          | 62241042  | 21.0       | 0         | M          | ni     | 0.00            | Unique to Control |
| 9. Exportin-7                                                           | 154448982 | 27.3       | 0         | M          | ni     | 0.00            | Unique to Control |
| 10. Uncharacterized protein KIAA1683 isoform X1                          | 530415216 | 23.3       | 0         | M          | ni     | 0.00            | Unique to Control |
| 11. Leucine-rich repeat-containing protein 37A3 isoform X14             | 578840218 | 12.3       | 0         | L          | ni     | 0.00            | Unique to Control |
| 12. Heme oxygenase 2 isoform a                                          | 555943918 | 11.3       | 0         | L          | ni     | 0.00            | Unique to Control |
| 13. Actin-related protein T3                                            | 221139714 | 17.7       | 0         | L          | ni     | 0.00            | Unique to Control |
| 14. Ubiquitin carboxy-terminal hydrolase 7 isoform 1                    | 150378533 | 18.3       | 0         | L          | ni     | 0.00            | Unique to Control |
| 15. Tetratricopeptide repeat protein 25                                  | 13899233  | 12.7       | 0         | L          | ni     | 0.00            | Unique to Control |
| 16. Actin-like protein 7A                                               | 5729720   | 16.7       | 0         | L          | ni     | 0.00            | Unique to Control |
| 17. Dynne intermediate chain 2, axonemal isoform X4                     | 530412670 | 15.7       | 0         | L          | ni     | 0.00            | Unique to Control |
| 18. Four and a half LIM domains protein 1 isoform 5                      | 228480205 | 18.7       | 0         | L          | ni     | 0.00            | Unique to Control |
| 19. Putative lipoyltransferase 2, mitochondrial precursor               | 221554520 | 9.0        | 0         | L          | ni     | 0.00            | Unique to Control |
| Protein                                             | Accession | Average SC | Abundance | NSAF ratio | t-test | Expression          |
|-----------------------------------------------------|-----------|------------|-----------|------------|--------|---------------------|
| 20 Tubulin polymerization-promoting protein family member 2 | 226491350 | 16.3       | 0         | L           | 0.00   | 0.00012 Unique to Control |
| 21 Isocitrate dehydrogenase (NAD) subunit beta, mitochondrial isomorf a precursor | 2817821 | 19.3       | 0         | L           | 0.00   | 0.00019 Unique to Control |
| 22 Protein DPCD                                       | 39930355  | 18.3       | 0         | L           | 0.00   | 0.00028 Unique to Control |
| 23 Long-chain-fatty-acid-CoA ligase 3                | 42794754  | 9.7        | 0         | L           | 0.00   | 0.00031 Unique to Control |
| 24 Sodium/potassium-transporting ATPase subunit alpha-3 isofrm 1 | 22748667 | 17.3       | 0         | L           | 0.00   | 0.00047 Unique to Control |
| 25 26S proteasome non-ATPase regulatory subunit 4    | 5292161   | 9.0        | 0         | L           | 0.00   | 0.00047 Unique to Control |
| 26 ATP synthase subunit g, mitochondrial             | 51479156  | 9.0        | 0         | L           | 0.00   | 0.00049 Unique to Control |
| 27 Acyl-CoA dehydrogenase family member 9, mitochondrial | 21361497 | 18.0       | 0         | L           | 0.00   | 0.00061 Unique to Control |
| 28 Transcription factor A, mitochondrial isofrm 1 precursor | 4507401 | 15.0       | 0         | L           | 0.00   | 0.00065 Unique to Control |
| 29 Elongation factor Tu, mitochondrial precursor     | 34147630  | 10.7       | 0         | L           | 0.00   | 0.00066 Unique to Control |
| 30 Eukaryotic translation elongation factor 1 epsilon-1 isofrm 2 | 208879470 | 8.0        | 0         | L           | 0.00   | 0.00068 Unique to Control |
| 31 Voltage-dependent calcium channel subunit alpha-2/delta-2 isofrm X1 | 530373385 | 8.0        | 0         | L           | 0.00   | 0.00075 Unique to Control |
| 32 Armadillo repeat-containing protein 12 isofrm X1 | 530381603 | 12.0       | 0         | L           | 0.00   | 0.00079 Unique to Control |
| 33 Deoxyuridine 5'-triphosphate nucleotidohydrolase, mitochondrial isofrm 3 | 70906444 | 13.0       | 0         | L           | 0.00   | 0.00081 Unique to Control |
| 34 Probable inactive serine protease 37 isofrm 1 precursor | 28539164 | 9.0        | 0         | L           | 0.00   | 0.00087 Unique to Control |
| 35 26S proteasome non-ATPase regulatory subunit 14    | 5031981   | 9.0        | 0         | L           | 0.00   | 0.00088 Unique to Control |
| 36 Mitochondria-eating protein isofrm X4             | 530376736 | 16.7       | 0         | L           | 0.00   | 0.00097 Unique to Control |
| 37 Mitochondrial fission 1 protein                   | 151108473 | 8.7        | 0         | L           | 0.00   | 0.00110 Unique to Control |
| 38 Alpha-soluble NSF attachment protein              | 47933379  | 8.0        | 0         | L           | 0.00   | 0.00137 Unique to Control |
| 39 Maleylacetoacetate isomerase isofrm 1             | 22202624  | 9.0        | 0         | L           | 0.00   | 0.00179 Unique to Control |
| 40 40S ribosomal protein S15                         | 4506687   | 10.0       | 0         | L           | 0.00   | 0.00184 Unique to Control |
| 41 Aladin isofrm 2                                   | 291045307 | 8.3        | 0         | L           | 0.00   | 0.00186 Unique to Control |
| 42 Ubiquitin carboxyl-terminal hydrolase isoyme L1   | 21361091  | 14.3       | 0         | L           | 0.00   | 0.00195 Unique to Control |
| 43 Stomatin-like protein 2, mitochondrial isofrm a    | 7305503   | 12.3       | 0         | L           | 0.00   | 0.00200 Unique to Control |
| 44 Protein FAM209B isofrm X2                         | 578835992 | 8.0        | 0         | L           | 0.00   | 0.00205 Unique to Control |
| 45 Putative protein FAM71E2                          | 223972704 | 12.3       | 0         | L           | 0.00   | 0.00229 Unique to Control |
| 46 Acyl-protein thioesterase 1 isofrm 1              | 5453722   | 11.7       | 0         | L           | 0.00   | 0.00240 Unique to Control |
| 47 Histone H1t                                       | 20544168  | 8.0        | 0         | L           | 0.00   | 0.00244 Unique to Control |
| 48 Armadillo repeat-containing protein 4 isofrm X3   | 578818430 | 18.7       | 0         | L           | 0.00   | 0.00324 Unique to Control |
| 49 Dnaj homolog subfamily B member 1 isofrm X1       | 578833210 | 13.3       | 0         | L           | 0.00   | 0.00375 Unique to Control |
| 50 Calcium-binding mitochondrial carrier protein Aralar2 isofrm 1 | 237649019 | 14.0       | 0         | L           | 0.00   | 0.00450 Unique to Control |
| Protein Accession | Average SC | Abundance Control | Abundance Seminoma | NSAF ratio Seminoma/Control | t-test P | Expression |
|-------------------|------------|-------------------|-------------------|----------------------------|----------|------------|
| 51 ACSBG2 isoform a | 574584557  | 17.7              | 0                 | L ni                       | 0.00     | 0.00479    | Unique to Control |
| 52 Methionine-tRNA ligase, cytoplasmic | 14043022  | 10.0              | 0                 | L ni                       | 0.00     | 0.00512    | Unique to Control |
| 53 60S acidic ribosomal protein P0 | 4506667   | 13.3              | 0                 | L ni                       | 0.00     | 0.00722    | Unique to Control |
| 54 Cytoplasmic dynein 1 heavy chain 1 | 33350932  | 11.3              | 0                 | L ni                       | 0.00     | 0.00728    | Unique to Control |
| 55 ADP-ribosylation factor 6 | 4502211   | 9.0               | 0                 | L ni                       | 0.00     | 0.00763    | Unique to Control |
| 56 Glycine-tRNA ligase precursor | 116805340 | 15.0              | 0                 | L ni                       | 0.00     | 0.00770    | Unique to Control |
| 57 BAG family molecular chaperone regulator 5 isoform b | 6631077   | 9.3               | 0                 | L ni                       | 0.00     | 0.00818    | Unique to Control |
| 58 60S ribosomal protein L7a | 4506661   | 5.3               | 0                 | VL ni                      | 0.00     | 0.00000    | Unique to Control |
| 59 Isobutyl-CoA dehydrogenase, mitochondrial | 7656849   | 7.0               | 0                 | VL ni                      | 0.00     | 0.00000    | Unique to Control |
| 60 cAMP-dependent protein kinase catalytic subunit gamma | 15619015  | 7.0               | 0                 | VL ni                      | 0.00     | 0.00000    | Unique to Control |
| 61 Vitamin K epoxide reductase complex subunit 1-like protein 1 isoform 1 | 46309463  | 3.7               | 0                 | VL ni                      | 0.00     | 0.00000    | Unique to Control |
| 62 Translocation protein SEC63 homolog | 6005872   | 2.0               | 0                 | VL ni                      | 0.00     | 0.00001    | Unique to Control |
| 63 UDP-N-acetylhexosamine pyrophosphorylase | 156627575 | 3.0               | 0                 | VL ni                      | 0.00     | 0.00001    | Unique to Control |
| 64 Guanine nucleotide-binding protein subunit beta-2-like 1 | 5174447   | 2.0               | 0                 | VL ni                      | 0.00     | 0.00003    | Unique to Control |
| 65 Dynein intermediate chain 1, axonemal isoform 2 | 526479830 | 7.0               | 0                 | VL ni                      | 0.00     | 0.00003    | Unique to Control |
| 66 Fibronectin type III domain-containing protein 8 | 8922138   | 2.0               | 0                 | VL ni                      | 0.00     | 0.00009    | Unique to Control |
| 67 40S ribosomal protein S26-like | 530438702 | 3.0               | 0                 | VL ni                      | 0.00     | 0.00009    | Unique to Control |
| 68 Mitochondrial import receptor subunit TOM22 homolog | 9910382   | 6.0               | 0                 | VL ni                      | 0.00     | 0.00009    | Unique to Control |
| 69 Cation channel sperm-associated protein subunit beta precursor | 51339295  | 2.0               | 0                 | VL ni                      | 0.00     | 0.00009    | Unique to Control |
| 70 Maestro heat-like repeat-containing protein family member 7 | 223278410 | 3.3               | 0                 | VL ni                      | 0.00     | 0.00010    | Unique to Control |
| 71 ADP-ribosylation factor-like protein 2 isoform 1 | 148612885 | 2.7               | 0                 | VL ni                      | 0.00     | 0.00015    | Unique to Control |
| 72 protein NDRG1 isoform 1 | 207028748 | 4.0               | 0                 | VL ni                      | 0.00     | 0.00016    | Unique to Control |
| 73 Speriolin isoform 1 | 197276668 | 6.3               | 0                 | VL ni                      | 0.00     | 0.00017    | Unique to Control |
| 74 Radial spoke head protein 6 homolog A | 13540559  | 3.3               | 0                 | VL ni                      | 0.00     | 0.00018    | Unique to Control |
| 75 DCN1-like protein 1 | 36030883  | 4.7               | 0                 | VL ni                      | 0.00     | 0.00025    | Unique to Control |
| 76 dnaJ homolog subfamily C member 3 precursor | 5453980   | 3.7               | 0                 | VL ni                      | 0.00     | 0.00025    | Unique to Control |
| 77 Sialic acid synthase | 12056473  | 3.0               | 0                 | VL ni                      | 0.00     | 0.00028    | Unique to Control |
| 78 Glutamine-tRNA ligase isoform b | 441478305 | 3.7               | 0                 | VL ni                      | 0.00     | 0.00028    | Unique to Control |
| 79 Mimitin, mitochondrial | 29789409  | 4.3               | 0                 | VL ni                      | 0.00     | 0.00031    | Unique to Control |
| 80 60S ribosomal protein L22 proprotein | 4506613   | 5.0               | 0                 | VL ni                      | 0.00     | 0.00032    | Unique to Control |
| 81 EF-hand calcium-binding domain-containing protein 14 | 7662160   | 6.7               | 0                 | VL ni                      | 0.00     | 0.00033    | Unique to Control |
| Protein                                                                 | Accession | Average SC | Abundance | NSAF ratio | t-test | Expression  |
|------------------------------------------------------------------------|-----------|------------|-----------|------------|--------|------------|
| Iron-sulfur cluster assembly enzyme ISCU, mitochondrial isoform X1     | 530400013 | 4.7        | 0         | VL ni      | 0.00   | Unique to Control |
| Growth hormone-inducible transmembrane protein                         | 118200356 | 4.7        | 0         | VL ni      | 0.00   | Unique to Control |
| S-phase kinase-associated protein 1 isoform b                          | 25777713  | 4.0        | 0         | VL ni      | 0.00   | Unique to Control |
| Calcium-binding mitochondrial carrier protein Aralar1                   | 21361103  | 3.3        | 0         | VL ni      | 0.00   | Unique to Control |
| Diphosphomevalonate decarboxylase                                      | 4505289   | 2.3        | 0         | VL ni      | 0.00   | Unique to Control |
| V-type proton ATPase subunit E 2 isoform X1                             | 530368260 | 4.0        | 0         | VL ni      | 0.00   | Unique to Control |
| Nucleosome assembly protein 1-like 1                                    | 21327708  | 4.3        | 0         | VL ni      | 0.00   | Unique to Control |
| 26S proteasome regulatory subunit 4                                     | 24430151  | 6.0        | 0         | VL ni      | 0.00   | Unique to Control |
| Mitochondrial ornithine transporter 1                                   | 7657585   | 5.3        | 0         | VL ni      | 0.00   | Unique to Control |
| 60S ribosomal protein L5                                               | 14591909  | 3.7        | 0         | VL ni      | 0.00   | Unique to Control |
| Dynein heavy chain 17, axonemal                                        | 256542310 | 88.0       | 1.0       | H VL       | 0.01   | UE in Seminoma |
| L-amino-acid oxidase isoform 2 precursor                               | 384381475 | 76.0       | 0.3       | M VL       | 0.01   | UE in Seminoma |
| Sperm-associated antigen 6 isoform X1                                   | 530392552 | 58.0       | 0.3       | M VL       | 0.01   | UE in Seminoma |
| Nuclear pore complex protein Nup93 isoform X1                          | 530424559 | 37.7       | 0.3       | M VL       | 0.01   | UE in Seminoma |
| Valine-RNA ligase                                                      | 5454158   | 87.7       | 1.7       | H VL       | 0.01   | UE in Seminoma |
| Sperm surface protein Sp17                                              | 8394343   | 31.3       | 0.3       | M VL       | 0.02   | UE in Seminoma |
| Exportin-2 isoform 1                                                   | 29029559  | 16.7       | 0.3       | L VL       | 0.02   | UE in Seminoma |
| 26S proteasome non-ATPase regulatory subunit 13 isoform 1              | 157502193 | 19.7       | 0.3       | L VL       | 0.02   | UE in Seminoma |
| Cathepsin F precursor                                                  | 6042196   | 21.0       | 0.3       | M VL       | 0.03   | UE in Seminoma |
| 26S proteasome non-ATPase regulatory subunit 7                         | 25777615  | 13.7       | 0.3       | L VL       | 0.03   | UE in Seminoma |
| Uncharacterized protein C7orf61                                        | 51972226  | 14.3       | 0.3       | L VL       | 0.03   | UE in Seminoma |
| Vacuolar protein sorting-associated protein 13A isoform C              | 66346672  | 19.7       | 0.3       | L VL       | 0.03   | UE in Seminoma |
| Mitochondrial pyruvate carrier 1-like protein                          | 306922396 | 18.0       | 0.3       | L VL       | 0.03   | UE in Seminoma |
| Plasma membrane calcium-transporting ATPase 4 isoform 4b               | 48255957  | 52.3       | 2.7       | M VL       | 0.03   | UE in Seminoma |
| Presequence protease, mitochondrial isoform 2 precursor                | 41352061  | 50.3       | 1.0       | M VL       | 0.03   | UE in Seminoma |
| Exportin-1 isoform X1                                                  | 530368070 | 8.3        | 0.3       | L VL       | 0.03   | UE in Seminoma |
| Ras-related protein Rab-11B                                            | 190358517 | 15.7       | 0.3       | L VL       | 0.03   | UE in Seminoma |
| Phosphatidylethanolamine-binding protein 4 precursor                   | 116812622 | 15.0       | 0.3       | L VL       | 0.04   | UE in Seminoma |
| Protein FAM71A                                                          | 282721094 | 12.3       | 0.3       | L VL       | 0.04   | UE in Seminoma |
| Puromycin-sensitive aminopeptidase                                     | 158937236 | 45.0       | 1.3       | M VL       | 0.04   | UE in Seminoma |
| Epimerase family protein SDR39U1 isoform 1                              | 116812630 | 13.3       | 0.3       | L VL       | 0.04   | UE in Seminoma |
| Protein Description | Accession | Average SC Control | Abundance Control | NSAF ratio | t-test P | Expression |
|---------------------|-----------|---------------------|-----------------|------------|---------|------------|
| V-type proton ATPase catalytic subunit A | 19913424 | 15.7 | 0.3 | L | VL | 0.04 | 0.00313 | UE in Seminoma |
| Cullin-associated NEDD8-dissociated protein 1 | 21361794 | 143.3 | 7.0 | H | VL | 0.05 | 0.00000 | UE in Seminoma |
| Low molecular weight phosphorylase protein phosphatase isoform c | 4757714 | 8.7 | 0.3 | L | VL | 0.05 | 0.00005 | UE in Seminoma |
| Dynamin heavy chain B, axonemal isoform X1 | 578811443 | 132.3 | 6.3 | H | VL | 0.05 | 0.00003 | UE in Seminoma |
| Heat shock protein 75, mitochondrial isoform 1 precursor | 15572983 | 8.3 | 0.3 | L | VL | 0.05 | 0.00081 | UE in Seminoma |
| Cullin-3 isoform 3 | 380714665 | 58.3 | 2.3 | M | VL | 0.05 | 0.00012 | UE in Seminoma |
| Lysozyme alpha-glucosidase isoform X1 | 530411863 | 5.0 | 0.3 | VL | VL | 0.05 | 0.00074 | UE in Seminoma |
| Isoleucine-tRNA ligase, mitochondrial precursor | 46852147 | 40.7 | 1.7 | M | VL | 0.05 | 0.00001 | UE in Seminoma |
| Protein FAM71B | 222418633 | 46.7 | 1.3 | M | VL | 0.05 | 0.00050 | UE in Seminoma |
| Actin-related protein T2 | 29893808 | 45.7 | 1.7 | M | VL | 0.06 | 0.00004 | UE in Seminoma |
| Thioredoxin domain-containing protein 3 | 148839372 | 18.3 | 1.0 | L | VL | 0.06 | 0.00016 | UE in Seminoma |
| Carnitine O-palmitoyltransferase 1, muscle isoform a | 4758050 | 11.3 | 1.0 | L | VL | 0.06 | 0.00107 | UE in Seminoma |
| Phosphoglycolate phosphatase | 108796653 | 15.3 | 0.3 | L | VL | 0.06 | 0.00014 | UE in Seminoma |
| Ecto-ADP-ribose transferase 3 isoform X8 | 530377706 | 38.3 | 1.7 | M | VL | 0.06 | 0.00004 | UE in Seminoma |
| EF-hand calcium-binding domain-containing protein 1 isoform a | 13375787 | 11.3 | 0.3 | L | VL | 0.07 | 0.00341 | UE in Seminoma |
| Izumo sperm-egg fusion protein 2 isoform X1 | 578833932 | 9.0 | 0.3 | L | VL | 0.07 | 0.00273 | UE in Seminoma |
| Sodium/potassium-transporting ATPase subunit alpha-4 isoform 1 | 153946397 | 59.7 | 5.3 | M | VL | 0.07 | 0.00006 | UE in Seminoma |
| Enolase-2, mitochondrial isoform 2 | 260274832 | 25.3 | 1.0 | M | VL | 0.07 | 0.00081 | UE in Seminoma |
| Casein kinase II subunit beta isoform 1 | 23503295 | 9.3 | 0.3 | L | VL | 0.07 | 0.00181 | UE in Seminoma |
| Sodium/potassium-transporting ATPase subunit alpha-4 isoform 1 | 153946397 | 59.7 | 5.3 | M | VL | 0.07 | 0.00006 | UE in Seminoma |
| Casein kinase II subunit beta isoform 1 | 23503295 | 9.3 | 0.3 | L | VL | 0.07 | 0.00181 | UE in Seminoma |
| Small membrane A-kinase anchor protein | 110349742 | 9.3 | 0.3 | L | VL | 0.07 | 0.00193 | UE in Seminoma |
| 60S ribosomal protein L12 | 4506597 | 14.3 | 0.7 | L | VL | 0.07 | 0.00044 | UE in Seminoma |
| Leucine-rich repeat-containing protein 37A3 precursor | 75677612 | 20.3 | 1.3 | M | VL | 0.07 | 0.00021 | UE in Seminoma |
| NADH dehydrogenase (ubiquinone) iron-sulfur protein B, mitochondrial isoform X1 | 530396818 | 8.0 | 0.3 | L | VL | 0.07 | 0.00305 | UE in Seminoma |
| Heat shock 70 protein 4L | 31541941 | 93.3 | 2.3 | H | VL | 0.08 | 0.00012 | UE in Seminoma |
| Sperm equatorial segment protein 1 precursor | 21717832 | 100.7 | 5.0 | H | VL | 0.08 | 0.00000 | UE in Seminoma |
| Pyruvate dehydrogenase E1 component subunit beta, mitochondrial isoform 1 precursor | 156564403 | 67.3 | 3.3 | M | VL | 0.08 | 0.00002 | UE in Seminoma |
| Choline transporter-like protein 5 isoform B | 194239633 | 8.0 | 1.3 | L | VL | 0.08 | 0.00340 | UE in Seminoma |
| 6-Phosphofructokinase type C isoform 1 | 11321601 | 131.3 | 10.7 | H | L | 0.09 | 0.00000 | UE in Seminoma |
| 26S proteasome non-ATPase regulatory subunit 12 isoform 1 | 4506221 | 10.7 | 0.7 | L | VL | 0.09 | 0.00427 | UE in Seminoma |
| rUVB-like 2 | 5730023 | 137.3 | 7.7 | H | VL | 0.09 | 0.00007 | UE in Seminoma |
| T-complex protein 1 subunit gamma isoform a | 63162572 | 128.7 | 7.7 | H | VL | 0.09 | 0.00000 | UE in Seminoma |
### Supplementary Table 2: Contd...

| Protein                                                                 | Accession     | Average SC            | Abundance | NSAF ratio | t-test | Expression         |
|------------------------------------------------------------------------|---------------|-----------------------|-----------|------------|--------|--------------------|
| ATP synthase subunit beta, mitochondrial precursor                      | 32189394      | 354.3                 | 21.7      | H          | M      | 0.09               |
| Phosphatidylglycerophosphatase and protein-tyrosine phosphatase 1 isoform 1 | 148224884     | 11.7                  | 0.7       | L          | VL     | 0.09               |
| 26S proteasome non-ATPase regulatory subunit 3                         | 25777612      | 35.3                  | 3.0       | M          | VL     | 0.09               |
| Importin-5 isoform X2                                                  | 530423350     | 24.7                  | 1.7       | M          | VL     | 0.10               |
| Mitochondrial dicarboxylate carrier isoform 2                          | 20149598      | 56.0                  | 3.7       | M          | VL     | 0.10               |
| TMEM189-UBE2V1 fusion protein                                          | 40806190      | 8.3                   | 0.7       | L          | VL     | 0.10               |
| Dynein heavy chain 7, axonemal                                         | 151301127     | 18.0                  | 1.0       | L          | VL     | 0.11               |
| Lysozyme-like protein 1                                                | 73390143      | 9.7                   | 0.7       | L          | VL     | 0.11               |
| Importin subunit alpha-1                                               | 4504897       | 54.7                  | 3.3       | M          | VL     | 0.11               |
| Nuclear pore complex protein Nup155 isoform 1                          | 24430149      | 86.0                  | 8.3       | H          | L      | 0.12               |
| Mitochondrial 2-oxoglutarate/malate carrier protein isoform 1           | 21361114      | 39.0                  | 2.7       | M          | VL     | 0.12               |
| Hyaluronidase PH-20 isoform 2                                           | 23510418      | 35.3                  | 2.3       | M          | VL     | 0.12               |
| 40S ribosomal protein S16                                               | 4506691       | 10.7                  | 0.7       | L          | VL     | 0.12               |
| 26S proteasome non-ATPase regulatory subunit 11                         | 28872725      | 13.0                  | 1.0       | L          | VL     | 0.12               |
| 26S proteasome non-ATPase regulatory subunit 6 isoform 2                | 7661914       | 18.7                  | 1.7       | L          | VL     | 0.13               |
| T-complex protein 1 subunit zeta-2 isoform X1                           | 578830267     | 36.7                  | 3.3       | M          | VL     | 0.13               |
| Bifunctional ATP-dependent dihydroxyacetone kinase/FAD-AMP lyase (cyclizing) isoform X1 | 530396576 | 29.0                  | 3.0       | M          | VL     | 0.13               |
| Ropporin-1B                                                            | 59891409      | 92.7                  | 7.3       | H          | VL     | 0.13               |
| Dynactin subunit 2 isoform 3                                            | 387527974     | 15.7                  | 1.7       | L          | VL     | 0.13               |
| ras-related protein Rab-14                                             | 19923483      | 19.0                  | 1.3       | L          | VL     | 0.13               |
| Proteasome activator complex subunit 4                                 | 163644283     | 52.7                  | 5.7       | M          | VL     | 0.13               |
| T-complex protein 1 subunit alpha isoform a                            | 57863257      | 132.3                 | 13.0      | H          | L      | 0.13               |
| Pyruvate dehydrogenase E1 component subunit alpha, testis-specific form, mitochondrial precursor | 4885543 | 50.0                  | 3.7       | M          | VL     | 0.13               |
| Dynactin light chain roadblock-type 2                                   | 18702323      | 8.7                   | 0.7       | L          | VL     | 0.13               |
| Nuclear transport factor 2                                             | 5031985       | 9.7                   | 0.7       | L          | VL     | 0.13               |
| Metalloeductase STEAP4 isoform 1                                       | 100815815     | 13.3                  | 1.7       | L          | VL     | 0.13               |
| Prenylated Rab acceptor protein 1                                      | 222144309     | 8.3                   | 1.0       | L          | VL     | 0.13               |
| Heat shock protein 105 isoform 1                                       | 42544159      | 8.7                   | 0.7       | L          | VL     | 0.14               |
| ATP synthase subunit gamma, mitochondrial isoform L (liver) precursor   | 50345988      | 48.7                  | 3.3       | M          | VL     | 0.14               |
| 3-Hydroxyisobutyryl-CoA hydrolase, mitochondrial isoform 1 precursor   | 37594471      | 11.7                  | 1.0       | L          | VL     | 0.14               |
| Transmembrane protein 89 precursor                                     | 56847630      | 12.7                  | 1.0       | L          | VL     | 0.14               |
| Protein                                                                 | Accession | Average SC Control | Abundance Seminoma Control | Abundance Seminoma | NSAF ratio Seminoma/Control | t-test P | Expression |
|-----------------------------------------------------------------------|-----------|--------------------|-----------------------------|-------------------|-----------------------------|---------|------------|
| T-complex protein 1 subunit beta isoform 1                            | 5453603   | 120.7              | 12.0                        | H                 | L                           | 0.14    | 0.00005    |
| T-complex protein 1 subunit zeta isoform a                            | 4502643   | 71.7               | 7.3                         | M                 | VL                          | 0.15    | 0.00013    |
| Inactive serine protease 54 precursor                                 | 122937420 | 19.0               | 1.7                         | L                 | VL                          | 0.15    | 0.00060    |
| Nucleoporin p54 isoform 1                                              | 26051237  | 21.7               | 2.3                         | M                 | VL                          | 0.16    | 0.01278    |
| T-complex protein 1 subunit theta isoform 1                           | 48762932  | 77.7               | 8.3                         | M                 | L                           | 0.16    | 0.00032    |
| Sperm protein associated with the nucleus on the X chromosome B/F     | 190570192 | 22.0               | 2.7                         | M                 | VL                          | 0.16    | 0.00428    |
| Histone H2A-Bbd type 2/3                                              | 63029935  | 21.7               | 2.0                         | M                 | VL                          | 0.16    | 0.00688    |
| Transcription elongation factor B polypeptide 2 isoform a              | 6005890   | 11.0               | 1.3                         | L                 | VL                          | 0.16    | 0.00017    |
| Protein MENT isoform X1                                               | 579801150 | 97.7               | 11.0                        | H                 | L                           | 0.17    | 0.00005    |
| ATP synthase subunit d, mitochondrial isoform a                       | 5453559   | 33.0               | 3.0                         | M                 | VL                          | 0.17    | 0.00091    |
| Ropporin-1A isoform X1                                                | 530374814 | 55.7               | 5.7                         | M                 | VL                          | 0.17    | 0.00001    |
| ATP synthase F (0) complex subunit B1, mitochondrial precursor        | 21361565  | 35.3               | 3.7                         | M                 | VL                          | 0.17    | 0.00076    |
| NADH dehydrogenase (ubiquinone) flavoprotein 1, mitochondrial isoform 1 precursor | 20149568 | 14.0               | 1.7                         | L                 | VL                          | 0.17    | 0.00400    |
| Apolipoprotein 0 isoform X1                                           | 578837961 | 40.3               | 4.3                         | M                 | VL                          | 0.17    | 0.00011    |
| 26S proteasome non-ATPase regulatory subunit 1 isoform 1               | 25777600  | 49.0               | 8.3                         | M                 | L                           | 0.17    | 0.00016    |
| Elongation factor 1-delta isoform 1                                   | 304555881 | 32.3               | 3.7                         | M                 | VL                          | 0.18    | 0.00006    |
| 26S proteasome non-ATPase regulatory subunit 8                        | 156631005 | 25.7               | 2.3                         | M                 | VL                          | 0.18    | 0.00001    |
| ATP synthase subunit alpha, mitochondrial isoform a precursor         | 50345984  | 265.3              | 33.0                        | H                 | M                           | 0.18    | 0.00001    |
| Heat shock 70 protein 1-like isoform X1                               | 530381921 | 207.0              | 24.3                        | H                 | M                           | 0.19    | 0.00038    |
| Nitriilase homolog 1 isoform 3                                         | 297632348 | 18.7               | 2.0                         | L                 | VL                          | 0.19    | 0.00095    |
| T-complex protein 1 subunit eta isoform a                             | 5453607   | 129.7              | 16.0                        | H                 | L                           | 0.19    | 0.00010    |
| Calcium-binding tyrosine phosphorylation-regulated protein isoform a  | 24797108  | 63.3               | 9.0                         | M                 | L                           | 0.20    | 0.00012    |
| Tricarboxylate transport protein, mitochondrial isoform b             | 374717343 | 15.3               | 1.7                         | L                 | VL                          | 0.20    | 0.00049    |
| T-complex protein 1 subunit epsilon                                   | 24307939  | 78.7               | 11.3                        | M                 | L                           | 0.20    | 0.00001    |
| Tissue alpha-L-fucosidase precursor                                   | 119360348 | 19.0               | 1.7                         | L                 | VL                          | 0.20    | 0.00008    |
| GTP-binding nuclear protein Ran                                       | 5453555   | 22.0               | 2.7                         | M                 | VL                          | 0.20    | 0.00007    |
| Dipeptidyl peptidase 2 isoform X1                                     | 530426726 | 17.7               | 1.7                         | L                 | VL                          | 0.20    | 0.00216    |
| 3(2'),5'-Bisphosphate nucleotidase 1 isoform X3                       | 530365931 | 19.3               | 2.3                         | L                 | VL                          | 0.20    | 0.00871    |
| Lysine-tRNA ligase isoform 1                                           | 194272210 | 30.0               | 3.0                         | M                 | VL                          | 0.20    | 0.00462    |
| Mitochondrial thiamine pyrophosphate carrier isoform X1               | 530412630 | 16.3               | 2.0                         | L                 | VL                          | 0.21    | 0.00139    |
| Vesicle-fusing ATPase isoform X1                                       | 578831007 | 16.7               | 2.3                         | L                 | VL                          | 0.21    | 0.00045    |

**Contd...**
| Protein                                                                                                                                                                                                                                                                                                                                 | Accession   | Average SC Control | Abundance Control | Abundance Seminoma | NSAF ratio Seminoma/Control | t-test P | Expression |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|--------------------|-------------------|-------------------|----------------------------|-------------|------------|
| 206 FUN14 domain-containing protein 2                                                                                                                                                                                                                                                                                                | 24371248    | 60.3               | 8.0               | M                 | L             | 0.22       | 0.00023    | UE in Seminoma |
| 207 Mitochondrial pyruvate carrier 2                                                                                                                                                                                                                                                                                                 | 219521872   | 25.7               | 5.0               | M                 | VL            | 0.22       | 0.00370    | UE in Seminoma |
| 208 Dynein light chain 1, axonemal isoform 1                                                                                                                                                                                                                                                                                    | 164607156   | 14.3               | 2.0               | L                 | VL            | 0.23       | 0.00000    | UE in Seminoma |
| 209 Cytochrome b-c1 complex subunit 2, mitochondrial precursor                                                                                                                                                                                                                                                                   | 50592988    | 111.0              | 14.0              | H                 | L             | 0.23       | 0.00006    | UE in Seminoma |
| 210 ADP/ATP translocase 4                                                                                                                                                                                                                                                                                                           | 13775208    | 140.3              | 25.3              | H                 | M             | 0.23       | 0.00180    | UE in Seminoma |
| 211 26S protease regulatory subunit 7 isoform 1                                                                                                                                                                                                                      | 4506209     | 9.7                | 1.7               | L                 | VL            | 0.23       | 0.00149    | UE in Seminoma |
| 212 Uncharacterized protein C9orf9                                                                                                                                                                                                                                                                                                | 33285006    | 45.0               | 6.7               | M                 | VL            | 0.23       | 0.00005    | UE in Seminoma |
| 213 ADP/ATP translocase 2                                                                                                                                                                                                                                                                                                          | 156071459   | 35.7               | 8.3               | M                 | L             | 0.24       | 0.00081    | UE in Seminoma |
| 214 Synaptojanin-2-binding protein                                                                                                                                                                                                                                                                                              | 157388993   | 28.3               | 5.0               | M                 | VL            | 0.24       | 0.00057    | UE in Seminoma |
| 215 Heat shock 70 protein 1A/1B                                                                                                                                                                                                                                                                                                  | 167466173   | 54.7               | 8.0               | M                 | L             | 0.24       | 0.00086    | UE in Seminoma |
| 216 26S protease regulatory subunit 6B isoform 1                                                                                                                                                                                                                      | 5729991     | 16.0               | 2.3               | L                 | VL            | 0.24       | 0.00222    | UE in Seminoma |
| 217 Mannose-6-phosphate isomerase isoform 1                                                                                                                                                                                                                           | 4505235     | 9.0                | 1.3               | L                 | VL            | 0.25       | 0.00220    | UE in Seminoma |
| 218 Nuclear pore membrane glycoprotein 210 precursor                                                                                                                                                                                                                  | 27477134    | 23.3               | 3.3               | M                 | VL            | 0.25       | 0.00026    | UE in Seminoma |
| 219 Arylsulfatase A isoform a precursor                                                                                                                                                                                                                               | 313569791   | 28.0               | 3.3               | M                 | VL            | 0.25       | 0.00335    | UE in Seminoma |
| 220 Leucine-rich repeat-containing protein 37A isoform X5                                                                                                                                                                                                         | 530413292   | 165.7              | 27.7              | H                 | M             | 0.26       | 0.00002    | UE in Seminoma |
| 221 Solute carrier family 2, facilitated glucose transporter member 5 isoform X2                                                                                                                                                                                      | 578799621   | 31.0               | 7.7               | M                 | VL            | 0.26       | 0.00472    | UE in Seminoma |
| 222 Protein-glutamine gamma-glutamyltransferase 4                                                                                                                                                                                                                 | 156627577   | 232.3              | 44.0              | H                 | M             | 0.26       | 0.00006    | UE in Seminoma |
| 223 Protein FAM162A                                                                                                                                                                                                                                                   | 49355721    | 9.0                | 1.3               | L                 | VL            | 0.26       | 0.00288    | UE in Seminoma |
| 224 26S protease regulatory subunit 6A                                                                                                                                                                                                                               | 21361144    | 22.3               | 4.0               | M                 | VL            | 0.27       | 0.00780    | UE in Seminoma |
| 225 Myosin regulatory light chain 12B                                                                                                                                                                                                                                | 15809016    | 11.7               | 2.3               | L                 | VL            | 0.27       | 0.00058    | UE in Seminoma |
| 226 Hexokinase-1 isoform X2                                                                                                                                                                                                                                           | 530393498   | 345.3              | 64.0              | H                 | M             | 0.27       | 0.00001    | UE in Seminoma |
| 227 NADH dehydrogenase (ubiquinone) iron-sulfur protein 7, mitochondrial                                                                                                                                                                                              | 187281616   | 9.7                | 2.0               | L                 | VL            | 0.27       | 0.00101    | UE in Seminoma |
| 228 Cytochrome b-c1 complex subunit Rieske, mitochondrial                                                                                                                                                                                                         | 163644321   | 27.0               | 4.7               | M                 | VL            | 0.27       | 0.00004    | UE in Seminoma |
| 229 Leucine-rich repeat-containing protein 37B isoform X5                                                                                                                                                                                                           | 53829385    | 176.3              | 40.3              | H                 | M             | 0.27       | 0.00007    | UE in Seminoma |
| 230 ES1 protein homolog, mitochondrial-like isoform X1                                                                                                                                                                                                               | 578797780   | 35.0               | 5.7               | M                 | VL            | 0.27       | 0.00001    | UE in Seminoma |
| 231 Lysosomal Pro-X carboxypeptidase isoform 1 preproprotein                                                                                                                                                                                                       | 4826940     | 13.3               | 1.7               | L                 | VL            | 0.27       | 0.00077    | UE in Seminoma |
| 232 Transmembrane protein 190 precursor                                                                                                                                                                                                                               | 21040263    | 33.3               | 5.7               | M                 | VL            | 0.28       | 0.00004    | UE in Seminoma |
| 233 ATP-glucose-1-phosphate uridylyltransferase isoform a                                                                                                                                                                                                        | 48255966    | 20.3               | 5.0               | M                 | VL            | 0.28       | 0.00603    | UE in Seminoma |
| 234 26S protease regulatory subunit 10B                                                                                                                                                                                                                               | 195539395   | 21.3               | 4.3               | M                 | VL            | 0.29       | 0.00659    | UE in Seminoma |
| 235 Dynactin subunit 1 isoform 4                                                                                                                                                                                                                                      | 205277396   | 21.7               | 3.7               | M                 | VL            | 0.29       | 0.00497    | UE in Seminoma |
| 236 26S protease regulatory subunit 8 isoform 1                                                                                                                                                                                                                      | 24497435    | 17.0               | 3.7               | L                 | VL            | 0.29       | 0.00479    | UE in Seminoma |
| Protein                                                                 | Accession       | Average SC Abundance | NSAF ratio | t-test | Expression |
|------------------------------------------------------------------------|-----------------|-----------------------|------------|--------|------------|
| Ethanolamine-phosphate cytidylyltransferase isoform 6                  | 532524977       | 16.0 3.0 L VL         | 0.29       | 0.00018 | UE in Seminoma |
| 60 heat shock protein, mitochondrial isoform X1                        | 530370277       | 125.7 16.3 H L        | 0.30       | 0.00029 | UE in Seminoma |
| Beta-galactosidase-1-like protein isoform X1                           | 530370954       | 35.3 4.7 M VL         | 0.30       | 0.00099 | UE in Seminoma |
| Adenylate kinase isoenzyme 1 isoform X1                               | 530390694       | 45.3 7.7 M VL         | 0.30       | 0.00114 | UE in Seminoma |
| Dynein light chain Tctex-type 1                                        | 5730085         | 10.0 1.7 L VL         | 0.30       | 0.00600 | UE in Seminoma |
| Chitinase domain-containing protein 1 isoform X2                       | 530395670       | 17.3 3.3 L VL         | 0.31       | 0.00482 | UE in Seminoma |
| A-kinase anchor protein 4 isoform 1                                    | 21493037        | 156.3 28.0 H M        | 0.31       | 0.00000 | UE in Seminoma |
| Hypoxia up-regulated protein 1 isoform X2                             | 530397761       | 177.0 37.0 H M        | 0.31       | 0.00002 | UE in Seminoma |
| Diablo homolog, mitochondrial isoform 1 precursor                      | 9845297         | 27.3 6.7 M VL         | 0.32       | 0.00223 | UE in Seminoma |
| Zona pellucida-binding protein 2 isoform 2 precursor                  | 40556389        | 45.7 9.0 M L          | 0.32       | 0.00009 | UE in Seminoma |
| Ubiquitin-like modifier-activating enzyme 1 isoform X1                | 530421539       | 75.0 11.3 M L         | 0.32       | 0.00035 | UE in Seminoma |
| 40S ribosomal protein S15a                                            | 14165469        | 12.7 2.3 L VL         | 0.33       | 0.00231 | UE in Seminoma |
| Prohibitin isoform 1                                                  | 527498279       | 22.7 4.7 M VL         | 0.33       | 0.00192 | UE in Seminoma |
| Long-chain-fatty-acid-CoA ligase 6 isoform e                            | 327412327       | 39.0 6.0 M VL         | 0.33       | 0.00042 | UE in Seminoma |
| Importin subunit beta-1 isoform 1                                      | 19923142        | 54.7 11.7 M L         | 0.34       | 0.00045 | UE in Seminoma |
| Long-chain-fatty-acid-CoA ligase 1 isoform X3                          | 530377352       | 170.7 45.7 H M        | 0.34       | 0.00024 | UE in Seminoma |
| AP-1 complex subunit beta-1 isoform b                                 | 260436860       | 12.0 2.3 L VL         | 0.34       | 0.00257 | UE in Seminoma |
| Acrosin precursor                                                     | 148613878       | 255.7 65.3 H M        | 0.34       | 0.00011 | UE in Seminoma |
| Glutathione S-transferase omega-2 isoform 2                            | 300360567       | 10.3 2.0 L VL         | 0.34       | 0.00400 | UE in Seminoma |
| Carboxypeptidase D isoform 1 precursor                                 | 22202611        | 36.3 7.0 M VL         | 0.34       | 0.02105 | UE in Seminoma |
| Phosphate carrier protein, mitochondrial isoform b precursor           | 4505775         | 38.3 14.0 M L         | 0.35       | 0.00176 | UE in Seminoma |
| Heat shock 70 protein 4                                                | 38327039        | 44.7 7.7 M VL         | 0.35       | 0.00303 | UE in Seminoma |
| Fatty acid-binding protein, epidermal                                  | 4557581         | 30.7 6.0 M VL         | 0.35       | 0.00328 | UE in Seminoma |
| Ras-related protein Rab-2A isoform a                                  | 4506365         | 156.7 37.3 H M        | 0.36       | 0.00002 | UE in Seminoma |
| 3-hydroxyacyl-CoA dehydrogenase type-2 isoform 1                       | 4758504         | 43.0 9.3 M L          | 0.36       | 0.00138 | UE in Seminoma |
| T-complex protein 1 subunit delta isoform a                            | 38455427        | 108.3 25.7 H M        | 0.36       | 0.00075 | UE in Seminoma |
| cAMP-dependent protein kinase type II-alpha regulatory subunit isoform X1 | 530372834       | 109.7 29.0 H M        | 0.36       | 0.00000 | UE in Seminoma |
| Glutamine synthetase isoform X1                                        | 578800828       | 23.3 5.3 M VL         | 0.36       | 0.01626 | UE in Seminoma |
| Calmodulin isoform X1                                                 | 578826144       | 75.0 20.7 M M         | 0.37       | 0.00004 | UE in Seminoma |
| Elongation factor 1-beta                                              | 4503477         | 10.0 2.3 L VL         | 0.37       | 0.00022 | UE in Seminoma |
| ruvB-like 1                                                           | 4506753         | 99.7 20.3 H M         | 0.37       | 0.00684 | UE in Seminoma |

Contd...
| Protein | Accession | Average SC Control | Abundance Control | NSA F ratio | t-test | Expression |
|---------|-----------|---------------------|------------------|-------------|--------|------------|
| 268 Elongation factor 1-alpha 1 | 4503471 | 155.0 | 51.3 | H | M | 0.38 | 0.00001 | UE in Seminoma |
| 269 hsc70-interacting protein isoform 1 | 19923193 | 30.3 | 7.3 | M | VL | 0.38 | 0.01230 | UE in Seminoma |
| 270 Transmembrane protein 126A isoform 1 | 14150017 | 22.0 | 6.0 | M | VL | 0.38 | 0.00020 | UE in Seminoma |
| 271 26S proteasome non-ATPase regulatory subunit 2 isoform 1 | 25777602 | 56.0 | 9.0 | M | L | 0.39 | 0.00049 | UE in Seminoma |
| 272 Arachidonate 15-lipoxygenase B isoform d | 85067501 | 23.7 | 4.3 | M | VL | 0.40 | 0.00236 | UE in Seminoma |
| 273 NADH dehydrogenase (ubiquinone) 1 alpha subcomplex subunit 9, mitochondrial precursor | 6681764 | 9.7 | 2.3 | L | VL | 0.40 | 0.00078 | UE in Seminoma |
| 274 electron transfer flavoprotein subunit beta isoform 1 | 4503609 | 22.0 | 5.0 | M | VL | 0.41 | 0.00000 | UE in Seminoma |
| 275 Tubulin alpha-3CD chain | 156564363 | 242.3 | 60.7 | H | M | 0.41 | 0.00001 | UE in Seminoma |
| 276 Fumarylacetoacetate hydrolase domain-containing protein 2B | 40786394 | 31.0 | 7.7 | M | VL | 0.41 | 0.00018 | UE in Seminoma |
| 277 Peroxiredoxin-5, mitochondrial isoform a precursor | 6912238 | 78.0 | 20.7 | M | M | 0.41 | 0.00039 | UE in Seminoma |
| 278 NADH-ubiquinone oxidoreductase 75 subunit, mitochondrial isoform 4 | 316983156 | 21.0 | 4.0 | M | VL | 0.42 | 0.03074 | UE in Seminoma |
| 279 Probable C-mannosyltransferase DPY19L2 isoform X1 | 578823598 | 101.0 | 26.0 | H | M | 0.42 | 0.00052 | UE in Seminoma |
| 280 L-lactate dehydrogenase A-like 6B | 15082234 | 145.0 | 42.7 | H | M | 0.42 | 0.00021 | UE in Seminoma |
| 281 Acrosin-binding protein precursor | 17999524 | 288.3 | 74.7 | H | M | 0.43 | 0.00003 | UE in Seminoma |
| 282 Tubulin beta-4B chain | 5174735 | 287.3 | 81.3 | H | L | 0.43 | 0.00004 | UE in Seminoma |
| 283 Electron transfer flavoprotein subunit alpha, mitochondrial isoform a | 4503607 | 46.0 | 12.0 | M | L | 0.44 | 0.00065 | UE in Seminoma |
| 284 Serpin B6 isoform d | 425876768 | 79.0 | 33.3 | M | M | 0.46 | 0.00013 | UE in Seminoma |
| 285 Lysozyme-like protein 4 isoform X2 | 578805633 | 29.3 | 8.0 | M | L | 0.46 | 0.00114 | UE in Seminoma |
| 286 cAMP-dependent protein kinase type I-alpha regulatory subunit isoform a | 4503607 | 46.0 | 12.0 | M | L | 0.47 | 0.00245 | UE in Seminoma |
| 287 Vesicle-associated membrane protein-associated protein A isoform 2 | 94721252 | 38.7 | 12.0 | M | L | 0.47 | 0.00220 | UE in Seminoma |
| 288 Acrosomal protein SP-10 isoform a precursor | 4501879 | 64.3 | 20.0 | M | M | 0.48 | 0.00064 | UE in Seminoma |
| 289 Carnitine O-acetyltransferase isoform 2 | 383209673 | 41.0 | 11.7 | M | L | 0.48 | 0.00294 | UE in Seminoma |
| 290 Endoplasmin precursor | 4507677 | 543.7 | 146.0 | H | H | 0.49 | 0.00066 | UE in Seminoma |
| 291 Izumo sperm-egg fusion protein 4 isoform 1 precursor | 89903025 | 119.3 | 39.0 | H | M | 0.53 | 0.00058 | UE in Seminoma |
| 292 Heat shock-related 70 protein 2 | 13676857 | 442.0 | 126.0 | H | H | 0.53 | 0.00000 | UE in Seminoma |
| 293 Elongation factor 2 | 4503483 | 84.3 | 30.0 | H | M | 0.56 | 0.00021 | UE in Seminoma |
| 294 Clathrin heavy chain 1 isoform X2 | 530411491 | 122.7 | 51.3 | H | M | 0.58 | 0.00014 | UE in Seminoma |
| 295 Sperm acrosome membrane-associated protein 1 precursor | 13569934 | 176.0 | 83.7 | H | H | 0.60 | 0.00073 | UE in Seminoma |
| 296 Zona pellucida-binding protein 1 isoform 1 precursor | 229577313 | 278.7 | 110.3 | H | H | 0.61 | 0.00002 | UE in Seminoma |
| 297 Phosphoglycerate kinase 2 | 3154397 | 204.3 | 79.0 | H | M | 0.65 | 0.00034 | UE in Seminoma |
| 298 2,4-Dienoyl-CoA reductase, mitochondrial precursor | 4503301 | 180.7 | 70.0 | H | M | 0.65 | 0.00211 | UE in Seminoma |

Contd...
| Protein Accession | Protein Name | Average SC Abundance | NSAF ratio | t-test P-value | Expression |
|-------------------|--------------|----------------------|------------|---------------|------------|
| 299 530107092     | Aminopeptidase N isoform X1 | 218.0 186.3 | H H 1.54 | 0.00040 | OE in Seminoma |
| 300 4757900       | Calreticulin precursor | 125.0 106.7 | H H 1.55 | 0.00511 | OE in Seminoma |
| 301 18765694      | Dipeptidyl peptidase 4 | 138.0 122.0 | H H 1.61 | 0.00164 | OE in Seminoma |
| 302 530402335     | Plastin-2 isoform X2 | 116.7 100.0 | H H 1.62 | 0.00036 | OE in Seminoma |
| 303 4503273       | Angiotensin-converting enzyme isoform 1 precursor | 141.3 124.7 | H H 1.62 | 0.01310 | OE in Seminoma |
| 304 6005942       | Transitional endoplasmic reticulum ATPase | 145.0 97.0 | H H 1.75 | 0.00238 | OE in Seminoma |
| 305 116256327     | Nepriysin | 85.3 59.3 | H H 2.01 | 0.00052 | OE in Seminoma |
| 306 24308201      | Adipocyte plasma membrane-associated protein | 54.0 66.7 | M M 2.01 | 0.00011 | OE in Seminoma |
| 307 62388777      | Carboxypeptidase Z isoform 1 precursor | 29.3 28.0 | M M 2.06 | 0.00040 | OE in Seminoma |
| 308 4502107       | Annexin A5 | 41.0 48.0 | M M 2.08 | 0.00049 | OE in Seminoma |
| 309 50845386      | Annexin A2 isoform 2 | 46.7 57.7 | M M 2.09 | 0.00126 | OE in Seminoma |
| 310 112380628     | Lysozyme-associated membrane glycoprotein 1 precursor | 18.0 20.0 | L M 2.14 | 0.00275 | OE in Seminoma |
| 311 194018472     | Plasma serine protease inhibitor preproprotein | 40.7 45.7 | M M 2.15 | 0.00208 | OE in Seminoma |
| 312 530403978     | Dehydrogenase/reductase SDR family member 7 isoform X1 | 22.3 35.7 | M M 2.46 | 0.00033 | OE in Seminoma |
| 313 25121982      | Cysteine-rich secretory protein 1 isoform 1 precursor | 24.0 35.7 | M M 2.53 | 0.00587 | OE in Seminoma |
| 314 4502105       | Annexin A4 | 22.3 31.3 | M M 2.56 | 0.01438 | OE in Seminoma |
| 315 66933005      | Calnexin precursor | 34.3 51.3 | M M 2.56 | 0.00094 | OE in Seminoma |
| 316 578815184     | Clusterin isoform X1 | 116.7 175.3 | H H 2.71 | 0.00034 | OE in Seminoma |
| 317 4505757       | Gastricsin isoform 1 preproprotein | 18.0 29.7 | L M 2.76 | 0.00296 | OE in Seminoma |
| 318 4507509       | Metalloproteinase inhibitor 1 precursor | 9.3 14.7 | L L 2.80 | 0.00553 | OE in Seminoma |
| 319 54607120      | Lactotransferrin isoform 1 precursor | 702.3 996.7 | H H 2.87 | 0.00000 | OE in Seminoma |
| 320 4506773       | Protein S100-A9 | 23.7 45.0 | M M 3.35 | 0.00011 | OE in Seminoma |
| 321 7657116       | Glyceraldehyde-3-phosphate dehydrogenase, testis-specific | 48.7 106.7 | M H 3.41 | 0.00000 | OE in Seminoma |
| 322 20070125      | Protein disulfide-isomerase precursor | 85.0 185.0 | H H 3.53 | 0.00012 | OE in Seminoma |
| 323 4504301       | Histone H4 | 13.7 27.7 | L M 3.56 | 0.00040 | OE in Seminoma |
| 324 578814724     | Malatase-glucoamylase, intestinal isoform X1 | 16.7 33.0 | L M 3.58 | 0.00342 | OE in Seminoma |
| 325 12025678      | Alpha-actinin-4 | 44.0 48.7 | M M 3.62 | 0.00001 | OE in Seminoma |
| 326 4557894       | Lysozyme C precursor | 7.7 16.3 | VL L 3.76 | 0.00087 | OE in Seminoma |
| 327 50659080      | Alpha-1-antichymotrypsin precursor | 18.7 33.7 | L M 3.82 | 0.00044 | OE in Seminoma |
| 328 21614544      | Protein S100-A8 | 15.0 33.3 | L M 3.98 | 0.00483 | OE in Seminoma |
| 329 32483377      | Thioredoxin-dependent peroxide reductase, mitochondrial isoform b | 3.3 8.3 | VL L 4.38 | 0.00328 | OE in Seminoma |
## Supplementary Table 2: Contd...

| Protein                                      | Accession   | Average SC Abundance | NSAF ratio | t-test | Expression |
|----------------------------------------------|-------------|----------------------|------------|--------|------------|
| Control          | Seminoma     | Control          | Seminoma     |        |            |
| 330  | Semenogelin-2 precursor | 4506885 | 261.3 | 682.7 | H | H | 4.41 | 0.00044 | OE in Seminoma |
| 331  | Prosaposin isoform a preproprotein            | 11386147 | 20.0  | 48.0  | M | M | 4.73 | 0.00704 | OE in Seminoma |
| 332  | Olfactomedin-4 precursor                      | 32313593 | 15.3  | 35.7  | L | M | 4.90 | 0.00039 | OE in Seminoma |
| 333  | Lactadherin isoform a preproprotein           | 167830475 | 8.3   | 25.0  | L | M | 4.91 | 0.00144 | OE in Seminoma |
| 334  | Mucin-5B precursor                             | 301172750 | 22.0  | 65.0  | M | M | 4.97 | 0.00001 | OE in Seminoma |
| 335  | Prolactin-inducible protein precursor         | 4505821 | 238.3 | 849.0 | H | H | 4.99 | 0.00045 | OE in Seminoma |
| 336  | Alpha-1-antitrypsin precursor                 | 189163528 | 13.7  | 34.7  | L | M | 5.01 | 0.00000 | OE in Seminoma |
| 337  | Histone H3.3                                  | 4885385 | 5.7   | 18.7  | VL | L | 5.70 | 0.00001 | OE in Seminoma |
| 338  | Annexin A11 isoform X1                        | 530393508 | 4.0   | 16.0  | VL | L | 6.08 | 0.00010 | OE in Seminoma |
| 339  | Ectonucleotide pyrophosphatase/phosphodiesterase family member 3 | 111160296 | 7.0   | 24.3  | VL | M | 6.24 | 0.00214 | OE in Seminoma |
| 340  | Cathepsin D preproprotein                     | 4503143 | 3.3   | 11.7  | VL | L | 6.46 | 0.00352 | OE in Seminoma |
| 341  | BPI fold-containing family B member 1 precursor | 40807482 | 4.7   | 15.3  | VL | L | 6.64 | 0.00033 | OE in Seminoma |
| 342  | Fibronectin isoform 1 preproprotein           | 47132557 | 112.7 | 505.0 | H | H | 7.15 | 0.00001 | OE in Seminoma |
| 343  | Nucleobindin-2 isoform X1                     | 578820554 | 13.0  | 51.3  | L | M | 7.44 | 0.00026 | OE in Seminoma |
| 344  | Semenogelin-1 preproprotein                   | 4506883 | 94.0  | 422.3 | H | H | 7.78 | 0.00187 | OE in Seminoma |
| 345  | Cytoskeleton-associated protein 4              | 19920317 | 1.3   | 8.0   | VL | L | 8.24 | 0.00084 | OE in Seminoma |
| 346  | Ribonuclease pancreatic precursor             | 38201684 | 0.7   | 4.7   | VL | VL | 8.79 | 0.00074 | OE in Seminoma |
| 347  | Transketolase isoform 1                       | 205277463 | 3.7   | 15.3  | VL | L | 8.84 | 0.00097 | OE in Seminoma |
| 348  | Neutrophil defensin 1 precursor               | 124248516 | 8.7   | 36.0  | L | M | 9.32 | 0.00010 | OE in Seminoma |
| 349  | Neutrophil gelatinase-associated lipocalin precursor | 38455402 | 9.3 | 58.7  | L | M | 9.97 | 0.00000 | OE in Seminoma |
| 350  | Myeloperoxidase precursor                     | 4557759 | 69.3  | 368.7 | M | H | 10.30 | 0.00000 | OE in Seminoma |
| 351  | Myeloblastin precursor                        | 71361688 | 6.0   | 34.0  | VL | M | 10.33 | 0.00005 | OE in Seminoma |
| 352  | Catalase                                     | 4557014 | 1.7   | 8.3   | VL | L | 10.41 | 0.00009 | OE in Seminoma |
| 353  | Azurcidin preproprotein                       | 11342670 | 15.7  | 95.0  | L | H | 11.52 | 0.00000 | OE in Seminoma |
| 354  | Carcinoembryonic antigen-related cell adhesion molecule 1 isoform 1 precursor | 19923195 | 2.0  | 14.0  | VL | L | 12.42 | 0.00321 | OE in Seminoma |
| 355  | Erythrocyte band 7 integral membrane protein isoform a | 38016911 | 9.3  | 58.7  | L | M | 13.46 | 0.00055 | OE in Seminoma |
| 356  | Apolipoprotein B-100 precursor                | 105990532 | 1.7  | 12.0  | VL | L | 13.65 | 0.00031 | OE in Seminoma |
| 357  | Cysteine-rich secretory protein 3 isoform 1 precursor | 300244560 | 0.7  | 5.3   | VL | VL | 14.12 | 0.00098 | OE in Seminoma |
| 358  | Mucin-6 isoform X1                            | 578840955 | 3.7   | 29.3  | VL | M | 14.30 | 0.00214 | OE in Seminoma |
| 359  | ERO1-like protein alpha precursor             | 7657069 | 0.7   | 8.7   | VL | L | 15.45 | 0.00087 | OE in Seminoma |
| 360  | Annexin A3                                   | 4826643 | 4.0   | 45.7  | VL | M | 17.62 | 0.00001 | OE in Seminoma |
| Protein                                                                 | Accession   | Average SC | Abundance | NSAF ratio | t-test  | Expression                |
|------------------------------------------------------------------------|-------------|------------|-----------|------------|---------|---------------------------|
| 361 Neutrophil elastase preproprotein                                  | 4503549     | 2.3        | 23.0      | VL M       | 17.84   | 0.00006 OE in Seminoma   |
| 362 Phospholipase B-like 1 precursor                                   | 110227598   | 1.7        | 22.7      | VL M       | 22.14   | 0.00036 OE in Seminoma   |
| 363 Laminin subunit alpha-5 precursor                                  | 21264602    | 4.3        | 39.0      | VL M       | 22.30   | 0.00613 OE in Seminoma   |
| 364 Moesin isoform X1                                                  | 530421753   | 0.3        | 4.0       | VL VL      | 25.18   | 0.00003 OE in Seminoma   |
| 365 Eosinophil cationic protein precursor                              | 45243507    | 1.0        | 20.3      | VL M       | 27.03   | 0.00057 OE in Seminoma   |
| 366 Carcinomembryonic antigen-related cell adhesion molecule 6 precursor| 40255013    | 2.0        | 24.3      | VL M       | 27.08   | 0.00170 OE in Seminoma   |
| 367 Syntenin-1 isoform X1                                              | 530388518    | 0.3        | 5.7       | VL VL      | 30.17   | 0.00085 OE in Seminoma   |
| 368 CD63 antigen isoform A                                             | 383872447    | 1.0        | 14.7      | VL L       | 31.84   | 0.00518 OE in Seminoma   |
| 369 Collagen alpha-1 (XVIII) chain isoform 1 precursor                 | 110611235   | 1.0        | 27.0      | VL M       | 33.62   | 0.00154 OE in Seminoma   |
| 370 Laminin subunit gamma-1 precursor                                  | 145309326   | 1.0        | 22.0      | VL M       | 40.70   | 0.00062 OE in Seminoma   |
| 371 Integrin alpha-M isoform 1 precursor                               | 224831239   | 5.3        | 176.0     | VL H       | 50.91   | 0.00002 OE in Seminoma   |
| 372 Laminin subunit beta-2 isoform X1                                  | 530372442   | 1.3        | 45.0      | VL M       | 61.08   | 0.00000 OE in Seminoma   |
| 373 Alpha-1-acid glycoprotein 1 precursor                              | 167857790   | 0.7        | 20.7      | VL M       | 64.68   | 0.00005 OE in Seminoma   |
| 374 Integrin beta-2 precursor                                          | 188595677   | 2.3        | 124.0     | VL H       | 71.24   | 0.00000 OE in Seminoma   |
| 375 Carcinoembryonic antigen-related cell adhesion molecule 8 precursor| 21314600    | 0.3        | 18.0      | VL L       | 103.73  | 0.00001 OE in Seminoma   |
| 376 Cytochrome b-245 heavy chain                                       | 6996021     | 0.3        | 15.0      | VL L       | 112.04  | 0.00002 OE in Seminoma   |
| 377 Bactericidal permeability-increasing protein precursor             | 157276999   | 0.3        | 49.0      | VL M       | 300.57  | 0.00002 OE in Seminoma   |
| 378 Matrix metalloproteinase-9 preproprotein                           | 74272287    | 0.0        | 90.0      | ni H       | Seminoma only 0.00000 Unique to Seminoma |
| 379 Leukocyte elastase inhibitor                                       | 13489087    | 0.0        | 23.0      | ni M       | Seminoma only 0.00006 Unique to Seminoma |
| 380 Arachidonate 5-lipoxygenase isoform 2                              | 371877525   | 0.0        | 12.7      | ni L       | Seminoma only 0.00000 Unique to Seminoma |
| 381 Prostate and testis expressed protein 4 precursor                  | 221554530   | 0.0        | 8.0       | ni L       | Seminoma only 0.00002 Unique to Seminoma |
| 382 Chitinase-3-like protein 1 precursor                                | 144226251   | 0.0        | 10.3      | ni L       | Seminoma only 0.00003 Unique to Seminoma |
| 383 ADP-ribosyl cyclase 2 precursor                                     | 168229159   | 0.0        | 13.7      | ni L       | Seminoma only 0.00009 Unique to Seminoma |
| 384 Peptidoglycan recognition protein 1 precursor                      | 4827036     | 0.0        | 10.7      | ni L       | Seminoma only 0.00059 Unique to Seminoma |
| 385 Neutrophil collagenase preproprotein                               | 4505221     | 0.0        | 16.3      | ni L       | Seminoma only 0.00120 Unique to Seminoma |
| 386 Haptoglobin isoform 2 preproprotein                                | 186910296   | 0.0        | 11.3      | ni L       | Seminoma only 0.00207 Unique to Seminoma |
| 387 Resistin precursor                                                 | 301129180   | 0.0        | 6.0       | ni VL      | Seminoma only 0.00001 Unique to Seminoma |
| 388 Matriilin-2 isoform a precursor                                    | 62548860    | 0.0        | 3.0       | ni VL      | Seminoma only 0.00002 Unique to Seminoma |
| 389 Immunoglobulin alpha Fc receptor isoform a precursor               | 4503673     | 0.0        | 3.7       | ni VL      | Seminoma only 0.00018 Unique to Seminoma |
| 390 Vascular non-inflammatory molecule 2 isoform X1                    | 578813045   | 0.0        | 6.0       | ni VL      | Seminoma only 0.00025 Unique to Seminoma |
| 391 Integrin beta-2 isoform X1                                         | 578836536   | 0.0        | 6.3       | ni VL      | Seminoma only 0.00029 Unique to Seminoma |

Contd...
### Supplementary Table 2: Contd...

| Protein                        | Accession | Average SC | Abundance | NSAF ratio | t-test | Expression |
|--------------------------------|-----------|------------|-----------|------------|--------|------------|
|                                |           | Control    | Seminoma  | Control    | Seminoma/C |           |            |
|                                |           | Control    | Seminoma  |            |         |            |            |
| 392 Flotillin-2 isoform X1     | 530410971 | 0.0        | 6.3       | ni         | VL      | 0.00055    | Unique to  |
|                                |           |            |           |            |         |            |  Seminoma  |
| 393 Cathepsin G preproprotein | 4503149   | 0.0        | 3.3       | ni         | VL      | 0.00065    | Unique to  |
|                                |           |            |           |            |         |            |  Seminoma  |

H: high; L: low; M: medium; ni: not identified; NSAF: normalized spectral abundance factor; OE: overexpressed; SC: spectral counts; UE: underexpressed; VL: very low