**Supplementary Table 1: Comparative seminal plasma proteomics studies in male infertility**

| *Authors* | *Year* | *Study design* | *MS strategy* | *Identified and differentially expressed proteins* | *Main molecular functions and biological processes* |
|---|---|---|---|---|---|
| Starita-Geribaldi *et al.* | 2001 | 5 fertile men<br>4 vasectomized men<br>4 azoospermic men (SCOS) | Individual samples<br>2DE followed by MALDI-TOF/MS | 757 spots were identified in fertile men<br>Vasectomy: 25 spots were absent and 9 were decreased in one man compared to pre-vasectomy and in all men compared to fertile men<br>Azoospermia: 8 spots were absent in all patients, compared to fertile men<br>One spot was present in all fertile men and absent in all infertile men | - |
| Starita-Geribaldi *et al.* | 2003 | 5 fertile men<br>4 vasectomized men<br>4 azoospermic men (SCOS) | Individual samples<br>2DE with different pH followed by MALDI-TOF/MS | 937 spots were identified in fertile men<br>5 proteins were underexpressed in vasectomized and azoospermic men | - |
| Yamakawa *et al.* | 2007 | 10 fertile men<br>7 men with NOA<br>3 men with obstructive azoospermia | Individual samples<br>2DE followed by LC-MS/MS | 63 spots were identified in all fertile men<br>73% of proteins varied more than 50% between different fertile men<br>NPC2 was absent in obstructive azoospermia, but not in NOA<br>4 proteins were absent in more than 3 patients with NOA | - |
| Batruch *et al.* | 2011 | 5 fertile men<br>5 vasectomized men | Pooled proteins<br>Strong-cation exchange LC-MS/MS | In fertile men, 2022 proteins were identified, of which 32 proteins were exclusive (testicular and epididymal proteins)<br>In vasectomy, 2096 proteins were identified, of which 49 proteins were underexpressed (proteins expressed in the testis and epididymis, but also in other regions of the male reproductive tract), 3 proteins were exclusive and 25 proteins were overexpressed<br>A total of 2360 proteins were identified | Protein binding<br>Catalytic activity<br>Metal ion binding<br>Nucleotide binding |
| Batruch *et al.* | 2012 | 5 men with NOA compared to 5 fertile men and 5 vasectomized men (previous study) | Pooled proteins<br>Strong-cation exchange LC-MS/MS | NOA: 2048 proteins were identified. Comparing to control, 18 proteins were exclusive or overexpressed, and 34 proteins were underexpressed or absent. Comparing to vasectomy, 59 proteins were exclusive or overexpressed, and 16 proteins were underexpressed<br>Total of 2500 different proteins in all groups | NOA<br>Protein binding<br>Catalytic activity<br>Metabolism<br>Regulation<br>Response to stimulus<br>Cell organization and biogenesis<br>Transport<br>Development<br>Reproduction |
| Drabovich *et al.* | 2011 | 12 fertile men<br>10 men with NOA<br>8 vasectomized men | 79 underexpressed or uniquely expressed proteins in postvasectomy men (previous study), pooled and individually analyzed by SRM | A total of 2100 proteins were identified, of which some proteins were associated to a differential diagnosis between<br>Control versus vasectomy (16 proteins)<br>Control versus NOA (3 proteins)<br>Vasectomy versus NOA (11 proteins) | - |
| Drabovich *et al.* | 2013 | 119 fertile, NOA and vasectomized men | 18 proteins suggested as biomarkers of azoospermia in a previous study, analyzed by multiplex SRM | TEX101 expression was higher in fertile men<br>ECM1 expression was higher in fertile men and NOA, but was highly decreased in vasectomy | - |
| Freour *et al.* | 2013 | 20 men with NOA presenting sperm in TESE (NOA+)<br>20 men with NOA without sperm in TESE (NOA–) | 6 samples analyzed by isotope-coded protein label nanoLC-MS/MS<br>40 samples utilized for validation by ELISA | 68 proteins were differentially expressed, of which 3 proteins were suggested as potential spermatogenesis biomarkers in the seminal plasma | - |
| Rolland *et al.* | 2013 | Normal men<br>Infertile men (nonliquefied semen) | Validation of previous findings by western blot of seminal plasma and immunohistochemistry of testicular extracts | LDHC, PGK2 and TKTL1 were expressed both in germ cells and in normal seminal plasma, but not (or less) observed in the seminal plasma from NOA, obstructive azoospermia and postvasectomy groups | - |

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|---|---|---|---|---|---|
| Wang *et al.* | 2009 | 20 fertile men<br>38 asthenozoospermic men | Individual samples<br>1DE followed by LC-MS/MS<br>Validation with western blot in pooled samples | 741 proteins were identified, of which 45 proteins were overexpressed and 56 proteins were underexpressed in the asthenozoospermia group<br>PARK7 protein was validated and presented 49.1% lower expression in asthenozoospermia | Asthenozoospermia<br>Catalytic activity (mostly hydrolase activity)<br>Enzyme regulator activity |
| Davalieva *et al.* | 2012 | 4 men with NOA<br>4 fertile men<br>4 oligozoospermic men<br>4 asthenozoospermic men | Pooled proteins<br>2D DIGE followed by MALDI-TOF/MS | 1291 spots were identified.<br>Comparison of the NOA groups and the other groups demonstrated some differentially expressed proteins<br>Compared to fertile: 5 proteins<br>Compared to oligozoospermia: 4 proteins<br>Compared to asthenozoospermia: 4 proteins | - |
| Herwig *et al.* | 2013 | 11 fertile men<br>11 iOAT men | Measurement of levels of carbonyl proteins<br>Pooled proteins and one single individual from each group (lowest and highest carbonyl protein levels)<br>Reversed-phase LC-MS/MS | 2489 proteins were identified, of which 505 proteins were exclusive, 24 were overexpressed proteins (>1.5-fold) and 744 were absent in iOAT<br>27 proteins were common to all iOAT | Overexpressed proteins<br>Response to stress<br>System development<br>Anatomical structure development |
| Sharma *et al.* | 2013 | 21 healthy men of unproven fertility and 43 men presenting for infertility evaluation, divided into<br>Normozoospermia (NN, *n*=26)<br>Normal sperm concentration and teratozoospermia (NA, *n*=23)<br>Oligozoospermia and normal sperm morphology (ON, *n*=6)<br>Oligoteratozoospermia (OA, *n*=10) | Pooled proteins<br>LC-MS/MS | 35 proteins were identified.<br>Comparison of the study groups and NN group demonstrated<br>3 underexpressed proteins in NA<br>1 underexpressed and 2 overexpressed proteins in ON<br>1 underexpressed and 2 overexpressed proteins in OA<br>2 proteins differentially expressed in all the study groups<br>11 proteins were observed in all the groups | Regulation<br>Response to stress<br>Cellular process<br>Development<br>Reproduction |
| Intasqui *et al.* | 2013 | 89 normozoospermic men grouped as<br>Low sperm DNA fragmentation (bottom 25%, *n*=18)<br>High sperm DNA fragmentation (top 25%, *n*=18) | Pooled proteins<br>LC-MS/MS | 72 proteins were identified, of which 9 proteins were absent or underexpressed and<br>21 proteins were exclusive or overexpressed in the high sperm DNA fragmentation group | Low sperm DNA fragmentation<br>Phospholipase activity<br>Lipoproteins remodeling and level regulation<br>High sperm DNA fragmentation<br>Acute-phase response<br>Fatty acid binding<br>Endoribonuclease activity |
| Sharma *et al.* | 2013 | 20 healthy, normozoospermic men with unproven fertility and 32 infertile men, grouped as<br>ROS positive<br>ROS negative | Pooled proteins<br>LC-MS/MS | 14 proteins were identified, of which 7 were exclusive or overexpressed in each group | ROS positive<br>Cell morphogenesis, differentiation, motility and cycle<br>Aging<br>ROS negative<br>Antioxidant activity<br>DNA binding<br>Enzyme regulation<br>Catalytic activity<br>Homeostasis |
| Intasqui *et al.* | 2015 | 156 normozoospermic men, grouped as<br>Low lipid peroxidation levels (bottom 15%, *n*=23)<br>High lipid peroxidation levels (top 15%, *n*=23) | Pooled proteins<br>LC-MS/MS | 629 proteins were identified, of which 23 were absent or underexpressed and 71 were exclusive or overexpressed in the High lipid peroxidation levels group<br>MUC5B was proposed as biomarker of semen oxidative stress | Cellular response to heat stress<br>Cellular response to superoxide anion<br>Cellular response to transition metal ions<br>Chemokine production<br>Homeostasis<br>Unsaturated fatty acid biosynthesis |

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| da Silva *et al.* | 2013 | 12 men with SCI, which retrieved semen by PVS, *n*=6 or EEJ, *n*=6<br>10 healthy, normozoospermic men | Pooled, unfractionated samples<br>Individual samples separated by 2DE<br>LC-MS/MS | In unfractionated samples, 637 proteins were identified. Of these, 88 proteins were exclusive from the PVS group and 66 from the EEJ group<br>2DE: 18 proteins of interest | All groups<br>Smooth muscle function<br>Cytoskeletal binding<br>Homeostasis<br>Oxidation of iron<br>Response to calcium<br>Control<br>Cell adhesion<br>Locomotion<br>Prostate gland growth<br>All SCI groups<br>Iron metabolism<br>Humoral immune response<br>PVS<br>Hydrogen peroxide response<br>Hypoxia<br>Inflammatory response |
| Milardi *et al.* | 2014 | 10 men with proven fertility<br>20 men with hypogonadism secondary to neurosurgery<br>Of these, 10 men were also evaluated after one week of a 6-month treatment with testosterone enanthate | Individual samples<br>LC-MS/MS | 61 proteins were identified in the fertile group. Of these, 33 proteins were absent in all hypogonadic men<br>14 of the absent proteins were identified in posttherapy patients (testosterone-dependent proteins) | Absent proteins<br>Catalytic activity<br>Binding activity<br>Hydrolase was the most impaired enzyme by T deficiency |
| Cadavid *et al.* | 2014 | 9 infertile men<br>7 proven fertile men | Individual samples<br>SELDI-TOF/MS followed by protein chip arrays | 10 proteins were overexpressed in infertile men, but were not identified by MS/MS | - |

MS: mass spectrometry; SCOS: sertoli cell-only syndrome; TESE: testicular sperm extraction; NOA: Nonobstructive azoospermia; iOAT: idiopathic oligoasthenoteratozoospermia; OA: oligoasthenozoospermia; LC-MS: liquid chromatography-mass spectrometry; ROS: reactive oxygen species; SCI: spinal cord injury; PVS: penile vibratory stimulation; EEJ: electroejaculation; 2DE: two-dimensional electrophoresis; MALDI-TOF/MS: matrix-assisted laser desorption ionization time-of-flight mass spectrometry; SRM: selected reaction monitoring; 1DE: one-dimensional electrophoresis; DIGE: difference gel electrophoresis; SELDI-TOF/MS: surface-enhanced laser desorption ionization time-of-flight mass spectrometry; ECM1: extracellular matrix protein 1; TEX101: testis-expressed sequence 101 protein; LDHC: L-lactate dehydrogenase C chain; PGK2: phosphoglycerate kinase 2; TKTL1: transketolase-like protein 1; PARK7: protein deglycase DJ-1; NPC2: epididymal secretory protein E1.