Second ejaculation produces good quality sperm and decreases the rate of unexpected ICSI cycle

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Abstract

Background: Second ejaculation can influence sperm quality which may define the first line treatment. The purpose of this study was to evaluate the effectiveness of a second ejaculation in decreasing the intracytoplasmic sperm injection (ICSI) rate in unexpected ICSI cycle.

Methods: A retrospective study of unexpected ICSI cycle and conventional in vitro fertilization IVF was conducted. The unexpected ICSI group was subdivided into the second ejaculation IVF and ICSI groups based on second ejaculation semen parameters after swim-up. To predict the influence of sperm protein between two ejaculation semen, the PXD010695 dataset and differentially expressed proteins (DEPs) were downloaded and subjected to bioinformatics analysis.

Results: Semen volume and sperm DNA fragmentation (SDF) were lower, whereas sperm concentration, total sperm count, progressive motility and total progressive motility sperm count after swim-up were higher during the second ejaculation. A lower rate of fertilization, a higher rate of good-quality embryos on day 3 and good-quality blastocyst were found in the second ejaculation IVF group. There was no significant difference among the groups in rate of rescue ICSI, oocytes utilization rate, clinical pregnancy, implantation, miscarriage, and live birth. Bioinformatics analysis indicated that the second ejaculation with downregulated proteins is related to a reduction in motility and forms a protein module with glutathione metabolism.

Conclusion: For unexpected ICSI group, second ejaculation IVF produced good quality sperm and could be an economical and secure alternative to decrease the rate of unexpected ICSI.

Background

The semen quality can be affected considerably by abstinence times and will define the first line treatment options for infertility couples undergoing assisted reproductive technologies. Unhealthy lifestyles, psychological factors, and other factors may lead to poor semen quality on the day of ovum pick-up in patients preparing for IVF, following with increasing unexpected ICSI. Studies have shown that the duration of abstinence affects both conventional sperm parameters and sperm DNA fragmentation (SDF) [1–3]). Semen volume and sperm concentration rise with the prolongation of abstinence time, but this abstinence will have a negative effect on motility, viability and morphology [4]. Similarly, when a second ejaculate was collected within 1 to 3h of the first ejaculate, the second ejaculation of men showed a significant enhancement in terms of total motility sperm count [4, 5] and a significant lower SDF [6]. Compromised spermatozoa quality varies with different sexual abstinence periods and may influence SDF as well as pregnancy outcomes [2, 7]. However, despite ICSI being widely used, recent studies found that moderate oligoasthenospermia did not influence the overall clinical outcomes through different assisted reproductive treatments [8, 9]. Furthermore, ICSI may require increased laboratory experience, time, and is associated with increased effort for embryologists and economic burden for patients than
conventional in vitro fertilization (IVF) [10]. Therefore, it is important to secure availability of high-quality spermatozoa with second ejaculation if the first line IVF treatment is to be effective.

Since, human sperm proteins play a major role in sperm motility, fertilization and embryogenesis [11, 12]. As a result, a recent sperm proteome study proposed that the differential abundance of sperm proteins after a decreased male ejaculatory abstinence period might play a significant role in sperm quality and reproductive potential [13]. However, their research ignores the difference between up-regulated proteins and down-regulated proteins as well as molecule compounds, which are crucial to understanding the molecular mechanism of second ejaculation. In the present study, we downloaded the PXD010695 and obtained differentially expressed proteins (DEPs) from the archive Database. Then gene ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of both up-regulated proteins and down-regulated proteins were performed. Finally, to determine the modules through protein–protein interaction (PPI) network and subsequently analyze their functions.

Given the findings of recent studies on the positive impact of shorter abstinence times on pregnancy outcomes, we aimed to verify whether second ejaculation could be a good solution to obtain more progressive motility spermatozoa for successful IVF with unexpected ICSI couples and how the molecular mechanism of second ejaculation proteins influence the reproductive potential. For this purpose, we compared sperm quality and DEPs between first and second ejaculation, as well as the subsequent pregnancy outcomes among second ejaculation IVF, ICSI and traditional IVF.

Methods

Study design and participants

This retrospective study of 255 infertile couples undergoing IVF or ICSI cycles was conducted at our hospital between January 2016 and November 2021. All male partners were diagnosed when sperm normal in at least two samples and prepared for IVF. All patients provided a semen sample after 2–7 days of abstinence. The unexpected ICSI patients were checked for the first ejaculation on the day of ovum pick-up after swim-up under the conventional ICSI criteria (progressive motility < 2 ×10^6/mL), while the men were rejected to undergo ICSI and provided a second semen sample followed by the first sample after only 1–3 h.

Retrospectively, three groups were analyzed. The patients with second semen parameters after swim-up beyond the conventional ICSI criteria outlined below were included in the second ejaculation IVF group (N = 55), whereas those with semen quality after swim-up within the conventional ICSI criteria were included in the ICSI group (N = 59). Patients with traditional IVF on the same ovum pick-up day were assessed as the conventional IVF group (N = 141).

Semen handling and analysis
Semen samples were obtained in a private room by masturbation into a sterile wide-mouthed plastic container. After liquefaction at 37°C for 30 minutes, the samples were assessed under an inverted microscope at 200× magnification. Semen analyses were performed according to the fifth edition of the World Health Organization guidelines and included sperm concentration and motility. A Sperm Class Analyzer CASA system (Microptic S.L., Barcelona, Spain) was used to assess sperm concentration and motility. Motile sperm fractions were isolated after density gradients and the “swim-up” process. Morphology was assessed by smearing 10 µL of the semen samples and subsequently washed and fixed using MGG Quick Stain (Bio-optica, Milan, Italy). The SDF was assessed by the sperm chromatin dispersion test using the Halosperm kit (Halotech DNA S.L., Madrid, Spain).

**Ovarian stimulation protocols**

When more than three follicles were larger than 18 mm in diameter, a dose of 5000–10,000 IU human chorionic gonadotropin was injected intramuscularly. After 36–40 hours oocytes were retrieved under ultrasonographic guidance. According to clinical indications, the patient underwent conventional IVF or ICSI 3–5 hours after oocyte retrieval. When the amount of second polar bodies released from mature oocytes was less than 30%, culture was continued for more than 2 hours and then the second PB exposure was checked again. When < 50% of the mature oocytes were exposed to the second PB, rescue ICSI was carried out immediately for the oocytes with only one PB. The operation of rescue ICSI was like that of conventional ICSI.

**Embryo assessment and embryo transfer protocols**

All oocytes were cultured in a culture medium with 6% CO₂, 5% O₂, and 89% N₂ at 37°C. The conventional morphology, cell count, cleavage patterns, and degree of fragmentation of the embryos were determined. To minimize operator-dependent variations, especially in blastocyst annotation, two embryologists were specifically trained and annotation was performed according to published guidelines. Good-quality embryos were defined as having 8–10 cells, no embryo fragmentation, and cells that were very even, regular, and similarly sized.

Fresh embryo transfer was performed on days 3 or 5, based on the number and quality of embryos available, according to the clinical protocol. Intramuscular progesterone (50 mg/day) was started on the day after oocyte retrieval for those undergoing a fresh transfer and continued until 10 weeks of gestation if conception occurred.

**Confirmation of pregnancy**

Biochemical pregnancy was verified if β-hCG was positive in the blood 14 days after embryo transfer. After 35 days, the gestational sac was detected as a clinical pregnancy by vaginal ultrasound. The implantation rate was calculated as the percentage of embryonic sacs surviving in the uterus as implanted embryos. We calculated the ongoing pregnancy as an intrauterine pregnancy lasting more than 12 weeks. Miscarriage was defined as fetal loss prior to 12 weeks of gestation, despite the presence of a gestational sac in the first trimester ultrasound.
Functional Prediction of sperm Proteins Using Public Databases

To predict the functional involvement of sperm proteins in ejaculates, data were retrieved from the PXD010695 (https://www.ebi.ac.uk/pride/archive/) to compare the difference between 1–3 hours of short-term abstinence and 3–7 days of long-term abstinence. Only 322 DEPs with 224 upregulated and 98 downregulated proteins were included [13]. The DAVID 6.8 database (https://david.ncifcrf.gov/) was used to perform GO functional and KEGG pathway analysis of the integrated DEPs. GO functional analysis of integrated DEPs involves three parts: biological process (BP), cell component, and molecular function. $P<0.05$ indicated statistical significance. Using DAVID software, functional and pathway enrichment analyses were conducted separately for the upregulated and downregulated proteins. $P<0.05$ indicated a statistically significant difference for the screening of significant GO terms and KEGG pathways.

A PPI network of integrated DEPs was constructed using STRING (http://string-db.org) with default medium confidence (0.4). The PPI networks were visualized using Cytoscape software (version 3.7.1). Finally, MCODE was used to determine the most significant module of the PPI network. The module was selected according to the following criteria: MCODE score $>5$, degree cutoff $=2$, node score cutoff $=0.2$, max depth $=100$, and k-score $=2$. Then, DAVID was used to analyze KEGG and GO of the two most significant modules to gain a deeper biological understanding.

Statistical analyses

IBM SPSS Statistics (version 19.0; IBM Corp., Armonk, NY, USA) was used to analyze the data. The results are expressed as mean ± standard deviation or proportions (%). Student's t-test was used to compare the clinical baseline data of continuous variables between the two groups, and the Mann–Whitney U test was used to compare continuous variables with a skewed distribution. The Chi-square test was used to compare categorical data between groups. All statistical tests were two-sided, and values were considered significant at $P<0.05$.

Results

The baseline characteristics of the three groups are shown in Table 1.
The sperm parameters of the ejaculate after 3–7 days of abstinence and the second ejaculate after 1–3 hours were compared using non-parametric Mann–Whitney U test. There were significant differences in semen volume, concentration, total sperm count, progressive motility, SDF and total progressive motility sperm count after swim-up between the two ejaculates, but no significant differences were found in total progressive motile sperm count, sperm morphology, concentration and progressive motility after swim-up (Table 2).
Table 2
Comparison of sperm parameters between the two ejaculates of unexpected ICSI couples

<table>
<thead>
<tr>
<th>Parameters</th>
<th>1st</th>
<th>2nd</th>
<th>Z</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (milliliter)</td>
<td>2.00 (1.50,3.00)</td>
<td>1.0 (0.50,1.00)</td>
<td>-6.314</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Concentration (million/mL)</td>
<td>15.95 (9.60,24.75)</td>
<td>21.70 (10.33,40.25)</td>
<td>-2.211</td>
<td>0.027</td>
</tr>
<tr>
<td>Total sperm count (million)</td>
<td>32.35 (15.15,59.60)</td>
<td>23.35 (11.85,42.18)</td>
<td>-2.224</td>
<td>0.026</td>
</tr>
<tr>
<td>Progressive motility (%)</td>
<td>12.20 (4.75,24.60)</td>
<td>20.00 (10.43,31.30)</td>
<td>-2.871</td>
<td>0.004</td>
</tr>
<tr>
<td>Total progressive motile sperm count (million)</td>
<td>22.20 (9.45,47.15)</td>
<td>19.45 (9.64,36.45)</td>
<td>-1.099</td>
<td>0.272</td>
</tr>
<tr>
<td>Morphology (%)</td>
<td>1.0 (1.0,2.0)</td>
<td>1.0 (1.0,3.0)</td>
<td>-0.867</td>
<td>0.386</td>
</tr>
<tr>
<td>SDF</td>
<td>40.0 (26.0,59.0)</td>
<td>31.0 (17.0,43.0)</td>
<td>-1.985</td>
<td>0.047</td>
</tr>
<tr>
<td>Concentration after swim-up (million/mL)</td>
<td>2.10 (1.15,3.40)</td>
<td>3.10 (1.30,5.90)</td>
<td>-1.959</td>
<td>0.050</td>
</tr>
<tr>
<td>Progressive motility after swim-up (%)</td>
<td>69.10 (58.30,78.70)</td>
<td>73.10 (59.00,86.80)</td>
<td>-1.720</td>
<td>0.085</td>
</tr>
<tr>
<td>Total progressive motile sperm count after swim-up (million)</td>
<td>0.67 (0.31,1.08)</td>
<td>1.04 (0.38,2.38)</td>
<td>-2.438</td>
<td>0.015</td>
</tr>
</tbody>
</table>

There was a lower fertilization rate per oocyte retrieved in the second ejaculation IVF group compared with both the ICSI and conventional IVF groups (56.48% vs. ICSI 67.12%, and IVF 63.07%; all P<0.05).

There was no significant difference in the rescue ICSI rate between the second ejaculation IVF group (5.17%) and conventional IVF group (5.37%). The good-quality embryo rate on day 3 was significantly higher in the second ejaculation IVF group (55.60%) than in the ICSI (42.47%, P<0.001) and conventional IVF (45.96%, P=0.005) groups. There was a higher good-quality blastocyst rate in the second ejaculation IVF group compared with the ICSI group (33.58% vs. 22.64%; all P<0.05). No significant differences in terms of oocytes utilization rate, clinical pregnancy, implantation, miscarriage and live birth rates were observed between second ejaculation IVF group and ICSI, conventional IVF groups (Table 3).
Table 3
Embryological and clinical outcomes

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unexpected ICSI</th>
<th>Conventional ICSI</th>
<th>P1</th>
<th>P2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Second ejaculation IVF</td>
<td>ICSI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fertilization rate per oocyte retrieved</td>
<td>279/494 (56.48)</td>
<td>449/669 (67.12)</td>
<td>859/1362 (63.07)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Rescue ICSI rate</td>
<td>3/58 (5.17)</td>
<td>8/149 (5.37)</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Good-quality embryo rate on day 3</td>
<td>154/277 (55.60)</td>
<td>189/445 (42.47)</td>
<td>392/853 (46.96)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Good-quality blastocyst rate</td>
<td>45/133 (33.58)</td>
<td>48/212 (22.64)</td>
<td>125/414 (30.19)</td>
<td>0.025</td>
</tr>
<tr>
<td>Oocytes utilization rate</td>
<td>178/494 (36.03)</td>
<td>242/669 (36.17)</td>
<td>558/1362 (40.97)</td>
<td>0.961</td>
</tr>
<tr>
<td>Fresh transfer</td>
<td>32/65 (49.23)</td>
<td>36/69 (52.17)</td>
<td>87/143 (60.84)</td>
<td>0.733</td>
</tr>
<tr>
<td>Clinical pregnancy rate</td>
<td>29/65 (44.62)</td>
<td>36/69 (52.17)</td>
<td>60/143 (41.96)</td>
<td>0.382</td>
</tr>
<tr>
<td>Implantation rate</td>
<td>37/118 (31.36)</td>
<td>45/128 (35.16)</td>
<td>74/275 (26.91)</td>
<td>0.528</td>
</tr>
<tr>
<td>Miscarriage rate</td>
<td>4/65 (6.15)</td>
<td>9/69 (13.04)</td>
<td>8/143 (5.59)</td>
<td>0.178</td>
</tr>
<tr>
<td>Live birth rate</td>
<td>25/65 (38.46)</td>
<td>27/69 (39.13)</td>
<td>52/143 (36.36)</td>
<td>0.937</td>
</tr>
</tbody>
</table>

P1: second ejaculation IVF vs. ICSI; P2: second ejaculation IVF vs. conventional IVF

We reanalyzed a publicly available dataset PXD010695, comparing ejaculates from short (1–3 h) to long (3–7 days) periods of abstinence, and identified the top five GO terms and KEGG pathways of upregulated and downregulated DEPs are shown in Fig. 1. Upregulated proteins were mainly enriched in the BP of actin filament bundle assembly, actin filament organization, actin cytoskeleton organization, cell-cell adhesion, and muscle contraction. Downregulated proteins were mainly enriched in the BP of flagellated sperm motility, binding of sperm to the zona pellucida, spermatogenesis, protein import into the mitochondrial inner membrane, and defense response to bacteria.

Based on the information in the STRING database, we used the lug-in MCODE in Cytoscape to identify 319 nodes and 945 edges in the PPI network. Figure 2a presents the visualization of the network generated using the Cytoscape software; Fig. 2b and 2c represent two important modules obtained from the PPI network. The results of subsequent GO function annotation and KEGG pathway analyses for the modules are summarized in Supplementary Tables S1 and S2. However, the results showed that there were no specific GO enrichment and KEGG pathways in module 1, while module 2 most significantly took
part in BP, as shown in Table 4. KEGG pathway analysis showed that it was mainly distributed in glutathione metabolism.

### Table 4

<table>
<thead>
<tr>
<th>Description</th>
<th>$P$ Value</th>
<th>Count</th>
<th>Proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>GO:0098869 cellular oxidant detoxification (BP)</td>
<td>1.63×10$^{-8}$</td>
<td>5</td>
<td>GLRX, GSTP1, GSTO1, GSR, TXN</td>
</tr>
<tr>
<td>GO:0045454 cell redox homeostasis (BP)</td>
<td>5.76×10$^{-7}$</td>
<td>4</td>
<td>PRDX1, PRDX2, GSR, TXN</td>
</tr>
<tr>
<td>GO:0006749 glutathione metabolic process (BP)</td>
<td>7.57×10$^{-7}$</td>
<td>4</td>
<td>GSTP1, GSTO1, GLO1, GSR</td>
</tr>
<tr>
<td>GO:0042744 hydrogen peroxide catabolic process (BP)</td>
<td>5.68×10$^{-5}$</td>
<td>3</td>
<td>PRDX1, PRDX2, CAT</td>
</tr>
<tr>
<td>GO:0043066 negative regulation of apoptotic process (BP)</td>
<td>1.024×10$^{-2}$</td>
<td>4</td>
<td>PRDX2, GSTP1, CAT, GLO1</td>
</tr>
<tr>
<td>hsa00480: Glutathione metabolism</td>
<td>7.07×10$^{-4}$</td>
<td>3</td>
<td>GSTP1, GSTO1, GSR</td>
</tr>
</tbody>
</table>

### Discussion

Most studies have demonstrated an improvement in sperm quality following second ejaculation [1, 2, 4, 6, 7]. However, only a few studies have focused on whether a second ejaculation can offer enough spermatozoa with progressive motility for IVF [13]. Therefore, we comprehensively compared and analyzed the sperm quality and bioinformatics analysis of DEPs between two ejaculates as well as the pregnancy outcomes of second ejaculation IVF, ICSI, and conventional IVF. We showed that the second ejaculation could produce good quality sperm and lower the rate of application of unexpected ICSI without affecting pregnancy outcomes.

In the present study, we performed semen analysis between the first and second ejaculations. There was higher concentration, progressive motility, total progressive motility sperm count after swim-up and lower SDF in the second ejaculation. Similar to our study, studies have indicated that an increase in motility and decrease in SDF are associated with second ejaculation [6, 7]. It is not clear why the second ejaculation with a short abstinence period was associated with higher-quality sperm. Human spermatozoa are produced in the seminiferous tubules and are stored in the epididymis. During epididymal transit, a series of elaborate interactions between spermatozoa and epididymal secretions occurs [14], which may influence flagellar beating and sperm motility [15]. This is consistent with our bioinformatics analysis, which showed that downregulated proteins were mainly enriched in flagellated sperm motility, sperm binding to the zona pellucida, and spermatogenesis. However, further bioinformatics analysis showed that the significant module of the PPI network was mainly enriched in cellular oxidant detoxification, cell redox homeostasis, glutathione metabolic processes, hydrogen peroxide catabolic processes, and
negative regulation of apoptotic processes, which are involved in oxidative stress and infertility [16–21]. These may be explained that although the downregulation protein from second ejaculation with short storage time in the epididymis will reduce the sperm motility, glutathione metabolism may play a major role in this process to improve the overall sperm quality of the second ejaculation. Another reasonable explanation might be that second ejaculation is associated with shorter storage time in the epididymis and lower oxidative stress. Shorten exposure to reactive oxygen species arising from dead spermatozoa and leukocytes in the epididymis [22, 23] will also be the reason for the association between increases in sperm quality and a decrease in SDF with second ejaculation.

We detected a lower fertilization rate in the second ejaculation IVF groups. However, our study found a similar rate of rescue ICSI between couples with second ejaculation IVF and those with conventional IVF. Though with the variety of semen quality and fertilization modes, fertilization ability will differ. Previous research has demonstrated that sperm from the proximal cauda shows higher fertilizing potential than those from the distal cauda in animals [24]. Second ejaculation could decrease the storage and transfer time of spermatozoa in the epididymis, indirectly influencing a series of physiological and biochemical changes, which may consequently decrease fertilizing capacity. Furthermore, the downregulated DEPs were enriched in flagellated sperm motility and binding of sperm to the zona pellucida, which may also decrease the fertilizing capacity.

In this study, there was an increase in the rate of good-quality embryos on day 3 and good-quality blastocyst in the second ejaculation IVF group. The significant increase in good embryos obtained in the second ejaculation IVF group highlighted by our study could be explained by lower exposure to higher oxidative stress in the epididymis than elsewhere in the reproductive tract. Many studies have shown that oxidative stress results in increased SDF and poor embryonic development [18, 25]; therefore, shorter storage time in the epididymis might lead to lower SDF and higher good-quality embryo rates. As confirmed in our study, the significant module of the PPI network was mainly enriched in cellular oxidant detoxification, cell redox homeostasis, glutathione metabolism, hydrogen peroxide catabolism and the negative regulation of apoptosis, which also involved glutathione metabolism related to sperm DNA damage, apoptosis and embryogenesis [16, 26–28]. Furthermore, only good-quality sperm can fertilize oocytes with good quality using natural processes.

Nevertheless, no significant differences in oocytes utilization rate and the pregnancy outcomes were observed. Although the fertilization rate of second ejaculation IVF groups was decreased, the overall egg utilization rate remained unchanged may due to the increasing of high-quality embryo rate on day 3 and high-quality blastocyst rate, so as the pregnancy outcomes. Similarly, owing to higher fertilization rate but higher immaturity eggs not used for ICSI compared to IVF, the percentage of embryos formed or stored from 2PN were equal [29]. Originally, ICSI was applied to overcome the risk of low or total failed fertilization and increase the number of embryos [30]. However, no difference in the rescue ICSI rate between the second ejaculation IVF group and the conventional IVF group, nor did the oocyte utilization rate between the second ejaculation IVF group and the ICSI group was found in our study. Considering the
high cost and additional required laboratory experience, a second ejaculation IVF could be used to decrease the application of unexpected ICSI in selected male infertile couples.

Conclusions

This study showed that a second ejaculation could be a simple, low-cost, and effective way to improve sperm quality and decrease the rate of unexpected ICSI. The biological pathways and molecule compound identified by bioinformatics analysis could infer the potential mechanism of second ejaculation in IVF. However, further studies are needed to validate these findings.

Abbreviations

ICSI intracytoplasmic sperm injection
DEPs differentially expressed proteins
SDF sperm DNA fragmentation
IVF in vitro fertilization
GO gene ontology
KEGG Kyoto Encyclopedia of Genes and Genomes
PPI protein–protein interaction
BP biological process

Declarations

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AUTHOR CONTRIBUTIONS

Conceived and designed the study: XHZ, SKW, DWL and LTX. Performed the experiments: XHZ, YYH, XBM, ZDL, PPW and LSC. Analyzed the data: XHZ. Wrote the manuscript: XHZ. All authors read and approved the final manuscript.

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Data Availability Statement

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethical considerations

All procedures were performed in accordance with the ethical standards of the ethics committee of our hospital and with the 1964 Declaration of Helsinki and its later amendments.

Competing interests

The authors declare no competing interests.

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Figures
The GO analysis and KEGG pathway analysis of upregulated and downregulated differentially expressed proteins (a) Biological process; (b) Cellular component localization; (c) Molecular function; (d) KEGG pathway
Figure 2

Protein–protein interaction (PPI) network analysis (a) Using the STRING online database, 322 differentially expressed proteins (224 upregulated [blue] and 98 downregulated gene [red]) were filtered into the PPI network. The top two modules from the PPI network. (b) Module 1 and (c) module 2

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTableS1.docx
- SupplementaryTableS2.docx