Evaluation of Total Antioxidant Capacity in Serum and Follicular Fluid of Women Undergoing ICSI and its Association with Implantation Failure

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Abstract

Purpose: Total antioxidant capacity (TAC) in women serum and follicular fluid (FF) which surrounding oocytes may be related to the implantation failure. Therefore, we herein examined the relationship between total antioxidant capacity status in serum and FF and its association with implantation failure.

Method: One hundred and seventeen of non-reproductive women who underwent intra-cytoplasmic sperm injection (ICSI) included in this study and conducted between March 2018 and April 2019 in Kamal AL-Samarrai Hospital, center of fertility and IVF. Serum and follicular fluid were collected from non-reproductive women aged ranged 20-45 years and BMI (ranged 21.9-36.3kg/m^2), TAC were measured using sandwich ELISA in serum and follicular fluid specimen of 21 women of successful implantation compared to 96 experienced implantation failures.

Results: TAC was increased in serum of implantation failure compared to successful but not significant differences between two study groups. Whereas TAC levels were highly significant (P=0.002) in FF of women who had successful implantation (1.08 ± 0.64mmol/L) whereas in failure were lower (0.55 ± 0.42 mmol/L). In addition to that, when evaluating the frequency of TAC category in FF revealed highly significant differences (P=0.003) between two groups, the majority of failure groups (84.4%) had low TAC compared with (40%) in successful groups, while a significant increase of sufficient TAC in successful than failure groups (50% versus 6.1% respectively), whereas the borderline TAC were (9.1% versus 10%) in failure and successful groups.

Conclusions: TAC in FF may be potential markers for implantation successful in ICSI cycle.

Keywords: Follicular fluid, antioxidant, implantation failure, intra-cytoplasmic sperm injection.

Introduction

In fact, despite advances in assisted reproduction treatment, poor oocyte quality remains a subtle problem for female infertility, and the investigation of factors that affect IVF/ICSI outcome may help to improve success rates. FF is a serum transudate, which contains metabolism products by granulosa and theca cells and provides the micro-environment of the grown oocyte, directly influences on the oocyte quality and implantation1. Several studies have focused on the microenvironment surrounding the oocyte, such as ROS and antioxidants found in FF1-2. Oxidation stress has been suggested as one of the most important factors that negatively affect assisted reproduction outcome 3, in order to protect the follicles from oxidative insult, follicular fluid is naturally provided with an efficient antioxidant system 4. Total anti-oxidants status (TAS) is composed of antioxidant capacity of total protein (85%; mainly albumin), uric acid, bilirubin, carotenoids, tocopherol and ascorbic acid 5. Indeed, an imbalance between ROS and the antioxidant defense system in the FF could be responsible for abnormal oocyte development, causing damage to the DNA, cytoskeleton and cell membrane, which would result in lower egg quality and lead to decreased fertilization potential.
of the oocytes in ART cycles. Most of losses of anti-
oxidant in human reproduction take place even before
the implantation as up to 50% of losses occur during that
time. Also, the environment is influenced by endocrine
signaling and by the type of gonadotrophin the follicle is
exposed to during the follicular phase leading to reduced
protection against oxidation. Therefore, the objective
of this study was to determine the association between
total antioxidant capacity and implantation rate, both in
serum and FF of women who had successful implantation
compared to those in women with implantation failure.

Materials and Method

Subjects

The study included 117 women (mean age 31.1
± 5.7 years) admitted at Center of infertility diagnosis
and assisted reproductive technology/Kamal ALSamarai Hospital. This cross-sectional study of non-
reproductive women who underwent intra-cytoplasmic
sperm injection consisting of 21 women with successful
implantation and 96 women with implantation failure
were recruited. Patients with endometriosis, endometrial
polyps, fibroid in uterus and diabetes mellitus and any
systemic disease were excluded. Indication for ICSI in
non-reproductive women was tubal obstruction or male
factor infertility.

Ovarian Stimulation Protocol: All of the patients
received gonadotropin releasing hormone antagonist
(GnRH-ant) protocol for ovarian stimulation and were treated with recombinant follicle-stimulating
hormone (rFSH) (Gonal-F, Merckserono, Switzerland)
per day from the 2nd day of spontaneous or induced
menstruation. The dose of gonadotropins was adjusted
according to ovarian response, as detected by ultrasound
examination. As soon as the dominant follicle reached
14 mm in diameter, (GnRH) antagonist cetrotrelix
at 0.25 mg (Cetrotide®, Serono, Switzerland) was
administered daily, until the day of ovulation
triggering which was obtained by hCG injection (Ovitrelle at 250 μg; Merck- Serono, Geneva: Switzerland), when
atleast three follicles of size >18 mm were present in
the ovaries, oocyte puncture was performed at the 36th
hour after hCG injection. After FF aspiration oocytes
were separated and transferred into culture media, then,
FF was located into a 15-ml plane tube and centrifuged
at 300 g for 5 min and supernatant was stored at -80°C
until further analysis; also for each patient, at the day of
embryo transfer, blood serum samples for comparative
analysis was collected.

ICSI Producers: Oocyte denudation and ICSI
were performed 3 hours after retrieval, and the in vitro
culture was carried out in cleavage Gain medium
(Fertipro/Belgium) under mineral oil until day 2 (2–5
cells stage) in automated incubators with 6% CO2 at
37 °C, the growth of all the embryos from each patient
(n=117) was continuously monitored. Embryo quality
was assessed before embryo-transfer, and a maximum
of three embryos transferred to all patients. Pregnancies
were diagnosed by serum positive b-HCG levels (>100
miu/ml) 14 days after embryo transfer.

Parameters Analyses: Age, duration of non-
reproductive, body mass index (BMI), serum E2 were
assessed as possible confounders. E2 were measured
sandwich enzyme immunoassay ELISA method based
on a human monoclonal antibody (Biomerieux/France)
according to the manufacturer’s instructions. TAC was
measured in serum and FF using a test kit (Omnignostix
GmbH & CoKH, Austria) by spectrophotometric
quantification. Briefly, it is based on the reaction of
peroxides with peroxidase followed by a color reaction of
the chromogenic substrate tetra-methyl-benzidine in the
presence of biological antioxidants. Its blue colour turns
to yellow complex after addition of the stop solution
which had a maximum absorbance at the wavelength of
450nm.

Statistical Analysis: Statistical analysis carried out
by using Vassar Stats Web Site for Statistical Computation
(Lowry, 2013). Qualitative data expressed as percentage
values, whereas measurable data expressed as (M ±
SE). However; the difference between two independent
samples analyzed by t-test, while comparison of
categorical data between the different groups carried-out
by using Chi square test. The significance of differences
estimated at two-tail P level less than 0.05.

Results

Demographic and clinical characteristic parameters
of the subjects are presented in Table 1. The ICSI cycle
characteristics of our patients are shown in Table 2. The
total number of retrieved oocytes was 1094 (range 1-28),
at the time of the ICSI procedure, the nuclear maturity
of the intact oocytes revealed 801 oocytes in metaphase
II and ranged from 1-23, embryo obtained was 537.
The mean percentage of efficiency of fertilization rate
was 69%, the mean ranged of embryo transfer was 1-5,
regarding implantation status, only twenty one of women
has revealed successful implantation whereas ninety six women has implantation failure. The clinical ongoing implantation rate per transferred embryo was 17.9%. Table 3 shows the antioxidant profile in serum and FF of two groups of women’s. TAC were higher in serum of women who had implantation failure than successful but, did not show significant differences (2.24 ± 0.52 versus 1.96 ± 0.42mmol/L, respectively, P=0.051). In contrast, TAC were decreased in FF of women who had implantation failure (0.55 ± 0.42mmol/L) compared to women of successful implantation (1.08 ± 0.64mmol/L) and show highly significant (P=0.002).

Table 1: Demographic and clinical characteristic of the patients.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Range</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>117</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>20–45</td>
<td>31.1±5.7</td>
</tr>
<tr>
<td>Infertility duration (Years)</td>
<td>2–24</td>
<td>7.8±4.3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>48–108</td>
<td>73.1±10.5</td>
</tr>
<tr>
<td>Length (m)</td>
<td>1.43-1.78</td>
<td>1.59±0.06</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.9-36.3</td>
<td>28.6±3.6</td>
</tr>
<tr>
<td>E2 level (pg/ml)</td>
<td>255–4023</td>
<td>1583±895</td>
</tr>
</tbody>
</table>

BMI: body mass index; E2: estradiol.

Table 2: ICSI cycle characteristics of patients.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total</th>
<th>Range</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retrieved oocyte</td>
<td>1094</td>
<td>1 – 28</td>
<td>9.3</td>
</tr>
<tr>
<td>MII801</td>
<td>801</td>
<td>1-23</td>
<td>6.8</td>
</tr>
<tr>
<td>Embryo obtained</td>
<td>537</td>
<td>0-18</td>
<td>4.6</td>
</tr>
<tr>
<td>Efficiency of fertilization 69%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Embryo transferred</td>
<td>--</td>
<td>1-5</td>
<td>--</td>
</tr>
<tr>
<td>Implantation status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Successful 21</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Failure 96</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Implantation rate</td>
<td>17.9%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Comparison of antioxidant status between successful and failure implantation groups

<table>
<thead>
<tr>
<th>Total anti-oxidant capacity (mmol/L) (M±SD)</th>
<th>Implantation Group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Successful (n=21)</td>
<td>Failure (n=96)</td>
</tr>
<tr>
<td>In serum</td>
<td>1.96 ± 0.42</td>
<td>2.24 ± 0.52</td>
</tr>
<tr>
<td>In follicular fluid</td>
<td>1.08 ± 0.64</td>
<td>0.55 ± 0.42</td>
</tr>
</tbody>
</table>

TAC< 1 (low); 1-1.3 (borderline); > 1.3 (sufficient)

Values are mean ± SD; TAC: Total antioxidant capacity.

In order to confirm these data, we evaluated the frequency of TAC category in FF as shown in Fig 1, and revealed highly significant differences (P=0.003). The majority of failure groups (84.4%) had low TAC compared with (40%) in successful groups, while a significant increase of sufficient TAC in successful than failure groups (50% versus 6.1% respectively); in addition to that, the borderline TAC were (9.1% versus 10%) in failure and successful groups.
**Discussion**

In spite of the total number of oocyte retrieved and efficiency of fertilization was high, it appears that failure to achieve implantation with ICSI in this study was very high. Research about antioxidants status appears to be in strict relation with assisted reproduction outcome. In the present study, total TAC levels were lower in FF of patients who had implantation failure after ICSI. However, follicular fluid forms the biochemical micro-environment of the oocyte before ovulation and assists in estimating the developmental competence of female gametes. FF contains proteins, sugars, ROS, antioxidants, and hormones which have a direct impact on the maturation ability and the quality of oocytes, also rich in low molecular weight metabolites that are direct or indirect regulators of oxidative stress and antioxidant production. Rupture of the follicular wall during ovulation can be modeled as a short inflammatory process. An increase in various substances in the follicle near the time of ovulation, which can induce oxidative stress. Free radical-generating agents include histamine, bradykinin, angiotensin, prostaglandins (PG), eicosanoids, proteolytic enzymes, nitric oxide, and superoxide. The ROS are produced within the follicle during the ovulation process, imbalance between antioxidants factors and ROS production in ovarian FF could adversely influence on the quality of the oocyte, fertilization, and embryo development. Elevated ROS levels in patients with unexplained infertility imply reduced levels of antioxidants such as vitamin E and glutathione, resulting in a reduced ability to scavenge ROS and neutralize its toxic effects. On the other hand, ROS could induce inflammatory response accompanied by the releasing of pro-inflammatory cytokines such as, IL-6 decreases aromatase activity within follicles, which lead to reduction in intra-follicular estradiol concentration, fertility and fertilizing capacity.

Inflammation and oxidative stress have been implicated in the pathogenesis of several chronic disorders. Although ovarian stimulation also induces ROS production, disrupts the oxidant–antioxidant balance and leads to oxidative stress. Our observation is an agreement with the literature reports have shown that women who became pregnant after IVF therapy had a tendency toward higher levels of TAC in their follicular fluid compared to those who did not achieve pregnancy.

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**Figure 1. Frequency of TAC categories in follicular fluid of women with successful and failure implantation.**
Several research groups have concluded that the oxidant–antioxidant balance in the oocytes environment can have a significant impact on IVF outcome in women with endometriosis. On the other hand, obtained in a study by Attaran et al. who investigated FF levels TAC in women undergoing IVF; but these authors did not observe a difference in TAC levels between patients who became pregnant and those who did not. In contrast, high TAC level has been reported as a marker for poor response to ovulation induction in women with polycystic ovarian syndrome. On the basis of the etiology of infertility, women with male factor infertility, which can be considered as healthy control subjects, presented the best follicular antioxidant profile in comparison to those with female or unexplained infertility, confirming the presence of oxidation stress and reduced antioxidant capacity in FF from women with reproductive diseases. In accordance with this study, previous reports have shown that follicular total antioxidant capacity is positively correlated with pregnancy rate; at the same time, a previous study demonstrated that elevated blood plasma antioxidant status was favorable for achieving clinical pregnancy. In short, both systemic and local antioxidant status appears to be in strict relation with assisted reproduction outcome. The results may be help physicians on the treatment of IVF/ICSI, as well as scientists in clarifying the etiology of ICSI.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Ministry of Education and all experiments were carried out in accordance with approved guidelines.

References
14. Biswas SK. Does the interdependence between oxidative stress andinflammation explain the antioxidant paradox?. Oxidat Med Cell Longev.


