Oxidative Stress Parameters on Embryo's Culture Media Effectiveness of Embryo Quality and Ultimately of Predict Intracytoplasmic Sperm Injection Outcome

Sami R. Al-Katib*, Basima Sh. AlGhazali** and Ban J. Edan***

*Department of Physiology-Collage of Medicine-University of Kufa, Iraq  
** Department of Obs. & Gyn.-Collage of Medicine-University of Kufa, Iraq  
***Department of Physiology-Collage of Medicine-Babylonian University, Iraq  
*Correspondence email: sami.alkatib@uokufa.ed.iq

Abstract

Oxidative stress during embryonic, fetal and placental development may affect outcome of pregnancy. The objective of this study was to determine the effectiveness of oxidative status of the culture media before transfer may predict the embryo quality. This study was carried out on 75 infertile women aged between 22-45 years (31.43 ± 5.38 years), referred to the fertility clinic in Al-Sadder teaching hospital and undergone intracytoplasmic sperm injection throughout period from March 2013 to January 2014. Malondialdehyde (MDA), Glutathione (GSH) and Catalase enzyme (CAT) in culture media was measured by spectrophotometer. The results of this study showed that the pregnancy rate was 20%. For embryos transferred after 48-72 hours, MDA level in culture media had a significant positive correlation with grad IV embryo at p<0.05 and insignificant negative correlation with grad I and II embryo at p>0.05. GSH level in culture media had a significant positive correlation with the cleavage rate at p<0.05 and insignificant negative correlation with the grade III and IV embryos at p>0.05. CAT level in culture media was significantly higher in pregnant women when compared with non-pregnant women at P<0.05 and had a significant positive correlation with the grade II embryo at p<0.05 respectively. The best cut off point of CAT associated with pregnancy was 0.67 U/min.

Keywords: Oxidative stress, Malondialdehyde, Glutathione, Catalase enzyme Culture media.


Introduction

Oxidant and antioxidants have been concerned in the regulation of reproductive processes in both animal and human, such as cyclic luteal and endometrial changes, follicular development, ovulation, fertilization, embryogenesis, embryonic implantation, and placental
differentiation and growth (Agarwal et al., 2011). Imbalance between ROS manufacture and antioxidant systems induces oxidative stress that harmfully impacts reproductive processes (Al-Gubory et al., 2010). High level of reactive oxygen species (ROS) during embryonic, fetal and placental development may affect outcome of pregnancy (Al-Gubory et al., 2010). A number of biomarkers indicative of oxidative stress (OS) status, including superoxide, glutathione, glutathione peroxidase, catalase, lipid peroxides, and nitric oxide, have been identified within the ovary, endometrium, fallopian tubes, embryo, placenta, and the peritoneal fluid of women (Al-Gubory et al., 2010). Throughout, assisted reproduction technologies (ART) and ROS might initiate from multiple oocytes in a dish, large cumulus cell mass, or the spermatozoa used for insemination and from embryos (Agarwal et al., 2012). The existence of metallic cations, light exposure and oxygen concentration are all factors in the culture media that can augment embryo production of ROS (Levente, 2012). Researchers found that measuring ROS levels in ART culture, embryos, follicular fluid or semen may be useful in counseling patients concerning unsuccessful IVF/ICSI and in planning future attempts (Aydin et al., 2013). The ROS in culture media may impact post-fertilization progress, i.e. cleavage rate, blastocyst yield, and embryo quality (indicators of ART outcome) (Agarwal et al., 2011; Aydin et al., 2013). The ROS level in embryo culture medium was reported to negatively influence embryo cleavage and could even help to predict clinical pregnancies (Bedaiwy et al., 2010). Considering these findings, the ROS level observed as an embryonic metabolic marker in embryo culture medium can be used as one of the criteria for embryo selection, and more clinical pregnancies can be attained by single embryo transferred (ET), which can be performed based on this approach (Aydin, et al., 2013). The objective of this study was to determine the effectiveness of oxidative status of the culture media before transfer may predict the embryo quality.

Materials and methods

Study Population

This study was carried out on 75 infertile women aged between 22-45 years (31.43 ± 5.38 years), referred to the fertility clinic in Al-Sadder teaching hospital and undergone intracytoplasmic sperm injection throughout period from March 2013 to January 2014. Malondialdehyde (MDA), Glutathione (GSH) and Catalase enzyme (CAT) in culture media was measured by spectrophotometer. This prospective cohort study was approved by approved by the Ethics Committee of the University of Kufa and informed consent was obtained from all participants. All of them were recruited according to the following criteria:
Non-smokers, free from hepatitis and HIV (by screening test), absence of any metabolic or endocrine system-associated diseases, or any other associated condition which could alter the level of free radicals like malignancy and antioxidant therapy. All of these women underwent ovulation induction with either long (N=24) or short (N=51) protocols based on timing, hormonal conditions and ovarian reserve status of the women on the discretion of the clinician. An hCG injection was given to trigger the final stages of oocyte maturation and ultrasound-guided oocyte pick-up was performed 34–36 hours later. The embryos were classified according to their morphology and percentage of fragmentation (Hazlet, 2011). After removal of embryo from culture media, the reminder of culture media was collected and kept at−4 °C. Measurement of MDA was based on the calorimetric reaction with thiobarbituric acid (TBA) to form pink color product, which could be measured by spectrophotometer (Lunec, 1990). Determination of GSH depends on the action of sulfhydryl groups (Boyer, 2000). Sulfhydryl group of GSH could reduce disulfide chromogen of 5,5′-Dithiobis 2.nitrobenzoic acid (DTNB) and change it to an intensely yellow compound which could measure its absorbance directly by spectrophotometer at 412 nm and it was directly proportional to the GSH concentration (Burtis and Ashwood,1999). Catalase activity was determined by the decrease in absorbance due to H₂O₂ conception (Abi, 1974).

Statistical analysis was performed in this study using SPSS (Statistical Package for Social Science; Version 17) program. Independent t-test was used to estimate differences between groups in continuous variables. Pearson’s correlation analysis was used for correlation. The binary logistic regression analysis used to determine the odds ratio for pregnancy as dependent variable. Receiver operating characteristic (ROC) curves were generated to investigate the predictability of OS biomarkers for pregnancy. The sensitivity, specificity were calculated for the optimal OS cut-off levels determined by ROC curve analysis. Results are reported as (mean ±SD). P<0.05 was considered statistically significant (Daniel, 1999).

**Results**

Malondialdehyde level in culture media showed insignificantly lower in pregnant women when compared with non-pregnant women at P>0.05. Also, its levels was insignificantly lower in pregnant women when compared with non-pregnant women at P>0.05 (Table 1).
Table (1): MDA level in culture media in µM in Pregnant and non-pregnant women in each cause of infertility

<table>
<thead>
<tr>
<th>Causes</th>
<th>Pregnant (Mean ± SD) µM</th>
<th>Non pregnant (Mean ± SD) µM</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male factors</td>
<td>1.96 ± 1.04</td>
<td>1.64 ± 0.31</td>
<td>NS</td>
</tr>
<tr>
<td>Female factors</td>
<td>2.37 ± 0.77</td>
<td>3.46 ± 1.53</td>
<td>NS</td>
</tr>
<tr>
<td>Unexplained factors</td>
<td>-</td>
<td>3.26 ± 2.13</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>2.13 ± 0.72</td>
<td>2.98 ± 1.50</td>
<td>NS</td>
</tr>
<tr>
<td>P value among groups</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

NS: No significant differences at P<0.05

Levels of C.GSH were insignificantly higher in pregnant women when compared with non-pregnant women (P>0.05). Also, its levels was insignificantly higher in pregnant women when compared with non-pregnant women in respect to infertility cause (P>0.05) in (Table 2).

Table (2): C. GSH level in µM in pregnant and not pregnant women in each infertility factor

<table>
<thead>
<tr>
<th>Causes</th>
<th>Pregnant (Mean ± SD) µM</th>
<th>Non pregnant (Mean ± SD) µM</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male Factors</td>
<td>21.33 ± 9.10</td>
<td>20.59 ± 7.44</td>
<td>NS</td>
</tr>
<tr>
<td>Female Factors</td>
<td>31.33 ± 4.27</td>
<td>26.92 ± 6.06</td>
<td>NS</td>
</tr>
<tr>
<td>Unexplained Factors</td>
<td>-</td>
<td>17.98 ± 3.82</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>26.42 ± 8.61</td>
<td>22.89 ± 6.94</td>
<td>NS</td>
</tr>
<tr>
<td>P value among groups</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

NS: No significant differences at P<0.05, *S: significant differences at P<0.05

Levels of C.CAT were significantly higher in pregnant women when compared with non-pregnant women at P<0.05 but its levels were insignificantly higher in pregnant women when compared with non-pregnant women in respect to infertility cause at P<0.05 (Table 3).

Table (3): CAT level in culture media in U/min in pregnant and not pregnant women in each infertility factor

<table>
<thead>
<tr>
<th>Causes</th>
<th>Pregnant (Mean ± SD) U/min</th>
<th>Non pregnant (Mean ± SD) U/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male factors</td>
<td>0.92 ± 0.16</td>
<td>0.80 ± 0.16</td>
</tr>
<tr>
<td>Female factors</td>
<td>0.82 ± 0.29</td>
<td>0.53 ± 0.20</td>
</tr>
<tr>
<td>Unexplained factors</td>
<td>-</td>
<td>0.60 ± 0.08</td>
</tr>
<tr>
<td>Total</td>
<td>0.87 ± 0.22</td>
<td>0.63 ± 0.21*</td>
</tr>
<tr>
<td>P value among groups</td>
<td>NS</td>
<td>S</td>
</tr>
</tbody>
</table>

NS: No significant differences at P<0.05, *S: significant differences at P<0.05
The best cut off point of C.MDA associated with pregnancy was 2.80 µ which detected from Receiver operating characteristic (ROC) curve as shown in figure (1 a). The best cut off point for C.GSH was 27.00µ which detected from ROC curve as shown in figure (1 b). The best cut off point for C.CAT was 0.67 U/min which detected from ROC curve as shown in figure (1 c). Receiver operating characteristic ROC curve analysis revealed that area under the curve for CAT was 0.808 for predicting pregnancy followed by glutathione (AUC=0.641) (Figure 1). Less level of C.MDA and more level of C.CAT and C.GSH associated with increase pregnancy success. Increase level of MDA is better at predicting unsuccessful pregnancy outcome (Table 4).

![Figure (1): ROC curve analysis of culture media Malondialdehyde, glutathione and Catalas in predicting pregnancy outcome](image)

<table>
<thead>
<tr>
<th>Area</th>
<th>Cut off point</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>P value</th>
<th>Asymptotic 95% Confidence Interval</th>
<th>NS: No significant differences at P&lt;0.05, *S: significant differences at P&lt;0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.MDA</td>
<td>0.650</td>
<td>2.80</td>
<td>83%</td>
<td>60%</td>
<td>NS</td>
<td>0.377 - 0.923</td>
</tr>
<tr>
<td>C.GSH</td>
<td>0.641</td>
<td>27.00</td>
<td>67%</td>
<td>80%</td>
<td>NS</td>
<td>0.387 - 0.894</td>
</tr>
<tr>
<td>C.CAT</td>
<td>0.808*</td>
<td>0.67</td>
<td>88%</td>
<td>64%</td>
<td>S</td>
<td>0.553 - 1.00</td>
</tr>
</tbody>
</table>
Correlation analyses between oxidative stress markers and ICSI parameters showed that C.MDA level had a significant positive correlation with grad IV embryo at P<0.05 and had an insignificant negative correlation with grad I and II embryo at P>0.05 (Table 5). C.GSH level had a significant positive correlation with the cleavage rate at P<0.05 (Table 5) and had insignificant negative correlation with the grade III and IV embryos at P>0.05. C.CAT had a significant positive correlation with the grade II embryo at P<0.05 respectively) (Table 5). CAT level had an insignificant negative correlation with the grade III and IV embryos at P>0.05 (Table 5).

Table 5: Relation of oxidative stress marker in culture media with ICSI outcome

<table>
<thead>
<tr>
<th>ICSI Characteristics</th>
<th>C.MDA</th>
<th>C.GSH</th>
<th>C.CAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>PN</td>
<td>0.086</td>
<td>NS</td>
<td>0.437</td>
</tr>
<tr>
<td>Fertilization rate (%)</td>
<td>0.064</td>
<td>NS</td>
<td>0.203</td>
</tr>
<tr>
<td>Cleavage rate</td>
<td>0.140</td>
<td>NS</td>
<td>0.389*</td>
</tr>
<tr>
<td>Total embryo</td>
<td>0.012</td>
<td>NS</td>
<td>0.117</td>
</tr>
<tr>
<td>Embryo grading</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grad I</td>
<td>-0.061</td>
<td>NS</td>
<td>0.176</td>
</tr>
<tr>
<td>Grad II</td>
<td>-0.132</td>
<td>NS</td>
<td>0.058</td>
</tr>
<tr>
<td>Grad III</td>
<td>0.103</td>
<td>NS</td>
<td>-0.371</td>
</tr>
<tr>
<td>Grad IV</td>
<td>0.108*</td>
<td>S</td>
<td>-0.211</td>
</tr>
</tbody>
</table>

* S Correlation is significant at the 0.05 level (2-tailed). NS: No significant differences at P<0.05, r correlation coefficient

The relationship of measured factors with the positive pregnancy using a binary logistic regression analysis was performed. In this analysis C.CAT was significantly associated with the positive pregnancy. Increased C.CAT was associated with increased odds ratio (OR) for the positive pregnancy (OR = 1.410, [1.330-1.596]). Also, increased C.GSH was insignificantly associated with increased odds ratio for the positive pregnancy, while increased C.MDA was insignificantly associated with decreased odds ratio for the positive pregnancy (Table 6).
Table (6): Binary logistic regression analysis for positive pregnancy as the dependent variable

<table>
<thead>
<tr>
<th>Oxidative stress Parameters</th>
<th>P value</th>
<th>OR</th>
<th>95% Confidence Interval for Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower Bound</td>
</tr>
<tr>
<td>Positive Pregnancy</td>
<td>C.MDA</td>
<td>NS</td>
<td>0.637</td>
</tr>
<tr>
<td></td>
<td>C.GSH</td>
<td>NS</td>
<td>1.068</td>
</tr>
<tr>
<td></td>
<td>C.CAT</td>
<td>S</td>
<td>1.410*</td>
</tr>
</tbody>
</table>

*S Correlation is significant at the 0.05 level (2-tailed), NS: No significant differences at P<0.05

Discussion

Malondialdehyde level in culture media showed insignificantly lower in pregnant women when compared with non-pregnant women at P>0.05 as shown in (table 1). Also, its levels was insignificantly lower in pregnant women when compared with non-pregnant women in respect to infertility cause at P>0.05. This result agreed with du Plessis et al., (2008).

Pervious study found that the pregnant women showed significantly lower ROS levels in culture media than non-pregnant women in patient undergo IVF - ICSI (du Plessis et al., 2008). C.GSH levels showed insignificantly higher in pregnant women when compared with non-pregnant women at P>0.05. Also, its levels were insignificantly higher in pregnant women when compared with non-pregnant women in respect to infertility cause at P>0.05, this result agreed with Kawamura et al., (2010).

Oxidative stress in culture media can partly diminish oocyte GSH content, enhancing the effect of sustained OS and thus, risking oocyte fertilization and viability. The unwanted effects of sustained OS and resulting loss of oocyte antioxidant content were shown to be improved by addition lipophilic and hydrosoluble antioxidants to the culture media to lessen OS (Kawamura, et al., 2010).

Some studies found that antioxidant capacity were positively correlated with the pregnancy rate (Velthut et al., 2013). Pervious study found that the results of five antioxidant activity assays and antioxidant scores of pregnant groups had slightly superior than not pregnant group, but no significant difference was obtained between them (Huang et al., 2014).
The levels of CAT showed significantly higher in pregnant women when compared with non-pregnant women at P<0.05 but its levels was insignificantly higher in pregnant women when compared with non-pregnant women in respect to infertility cause at P<0.05, this results agreed with (Aydin, et al., 2013), the total antioxidant levels in patients with clinical pregnancy were significantly higher than non-pregnant women (Aydin, et al., 2013).

The demonstration of intracellular antioxidant enzymes activity could be a prospective biomarker for ART success (Ivailo et al., 212), the OS is correlated with negatives ART outcomes (Gupta et al., 2014). Previous studies reported that increasing in the production and accumulation of ROS; lead to postponed embryonic progress, embryonic fragmentation, apoptosis or health impairment during pregnancy (Kang et al., 2011; Arias et al., 2011; Lee et al., 2012). High oxidative stress appears to slow down meiotic spindle formation and subsequently affects embryo quality (Rajani et al., 2012). The ROC curve analysis indicated that the Catalase level in culture media showed higher sensitivity in predicting successful pregnancy outcome.

References


