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

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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.

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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 H_2O_2 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

Causes	C.MDA		P value
	Pregnant (Mean ± SD) µM	Non pregnant (Mean ± SD) µM	
Male factors	1.96 ± 1.04	1.64 ± 0.31	NS
Female factors	2.37 ± 0.77	3.46 ± 1.53	NS
Unexplained factors	-	3.26 ± 2.13	-
Total	2.13 ± 0.72	2.98 ± 1.50	NS
P value among groups	NS	NS	

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

Causes	C.GSH		P value
	Pregnant (Mean ± SD) µM	Non pregnant Mean ± SD) µM	
Male Factors	21.33 ± 9.10	20.59 ± 7.44	NS
Female Factors	31.33 ± 4.27	26.92 ± 6.06	NS
Unexplained Factors	-	17.98 ± 3.82	-
Total	26.42 ± 8.61	22.89 ± 6.94	NS
P value among groups	NS	NS	

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

Causes	C.CAT		P Value
	Pregnant (Mean ± SD) U/min	Non pregnant (Mean ± SD) U/min	
Male factors	0.92 ± 0.16	0. 80 ± 0.16	NS
Female factors	0.82 ± 0.29	0.53 ± 0.20	NS
Unexplained factors	-	0.60 ± 0.08	-
Total	0.87 ± 0.22	0.63 ± 0.21*	S
P value among groups	NS	S	

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).

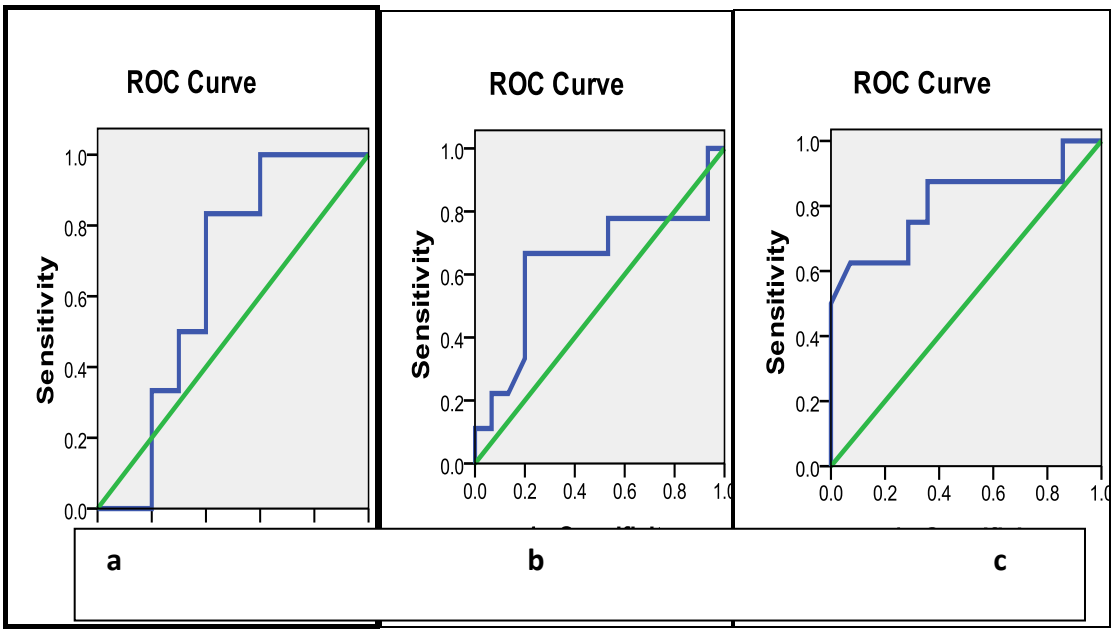

Figure (1): ROC curve analysis of culture media Malondialdehyde, glutathione and Catalas in predicting pregnancy outcome

Table (4): Coordination of the ROC curve for oxidative stress markers

	Area	Cut off point U/min	Sensitivity	Specificity	P value	Asymptotic 95% Confidence Interval	
						Lower Bound	Upper Bound
C.MDA	0.650	2.80	83%	60%	NS	0.377	0.923
C.GSH	0.641	27.00	67%	80%	NS	0.387	0.894
C.CAT	0.808*	0.67	88%	64%	S	0.553	1.00

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

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

ICSI Characteristics		C.MDA		C.GSH		C.CAT	
		r	p	r	p	r	p
PN		0.086	NS	0.437	NS	0.078	NS
Fertilization rate (%)		0.064	NS	0.203	NS	0.128	NS
Cleavage rate		0.140	NS	0.389*	S	0.308	NS
Total embryo		0.012	NS	0.117	NS	0.076	NS
Embryo grading	Grad I	-0.061	NS	0.176	NS	0.246	NS
	Grad II	-0.132	NS	0.058	NS	0.553*	S
	Grad III	0.103	NS	-0.371	NS	-0.094	NS
	Grad IV	0.108*	S	-0.211	NS	-0.073	NS

* 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

Oxidative stress Parameters		P value	OR	95% Confidence Interval for Odds Ratio	
				Lower Bound	Upper Bound
Positive Pregnancy	C.MDA	NS	0.637	0.261	1.555
	C.GSH	NS	1.068	0.950	1.201
	C.CAT	S	1.410*	1.330	1.596

*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.

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