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Oxidative Stress and Antioxidant in Pregnancy Women Conceived by in Vitro Fertilization and Intrauterine Insemination

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Abstract

In vitro fertilization (IVF) is a popular kind of assisted reproduction. This method is among the most popular treatments for infertility, to those who cannot have a baby in normal way. One alternative for treating infertility is intrauterine insemination (IUI), which is simple, safe, inexpensive, and does not involve any intrusive procedures. Oocyte senility and other reproductive issues in females may be caused by oxidative stress (OS), which is the result of high amounts of reactive oxygen species (ROS). Antioxidants have long been used to treat subfertility because they can balance out the elevated levels of ROS that induce OS. Serum levels of superoxide dismutase, catalase, glutathione, reactive oxygen species, and malondialdehyde were measured in relation to age, body mass index (BMI), and the various forms of assisted reproductive technology used by pregnant women in this research. Enzyme-linked immune sorbent assay (ELISA) based on for the detection of SOD , CAT , ROS levels in the serum of pregnant women in the first trimester of pregnancy, while GSH measured by using amino acid analyzer. Results demonstrated a statistically significant drop in serum SOD, CAT, and GSH levels in the IVF, IUI, and SP groups compared to the NP group throughout pregnancy. Even if reactive oxygen species (ROS) and malondialdehyde (MDA) levels rise dramatically. The age and body mass index groups, as well as the three pregnancy groups (IVF, IUI, and SP), did not vary significantly from one another. There is significant decreases in serum GSH, CAT, and SOD during pregnancy corresponding significant increases in serum of ROS and MDA because pregnant women were more capable to oxidative damage than the non-pregnant. There is no significant effect among the groups of pregnant (IVF, IUI, and SP), perhaps because they are similar in age and BMI.

Keywords: *Superoxide dismutase, Catalase, Glutathione, Reactive oxygen species, Malondialdehyde*

Tüp Bebek ve İntrauterin İnseminasyon Yöntemleriyle Gebe Kalan Kadınlarda Oksidatif Stres ve Antioksidan

Özet

Tüp bebek (*IVF*), popüler bir yardımcı üreme türüdür. Bu yöntem, normal şekilde çocuk sahibi olamayanlar için en popüler kısırlık tedavileri arasındadır. Kısırlığı tedavi etmek için bir alternatif, basit, güvenli, ucuz olan ve herhangi bir müdahaleci prosedür içermeyen rahim içi tohumlamadır (IUI). Kadınlarda oosit yaşlılığı ve diğer üreme sorunları, yüksek miktarda reaktif oksijen türlerinin (ROS) sonucu olan oksidatif stresten (OS) kaynaklanabilir. Antioksidanlar, OS'yi indükleyen yüksek ROS seviyelerini dengeleyebildikleri için uzun zamandır kısırlığı tedavi etmek için kullanılmaktadır. Bu araştırmada, süperoksit dismutaz, katalaz, glutatyon, reaktif oksijen türleri ve malondialdehit serum seviyeleri, yaş, vücut kitle indeksi (VKİ) ve hamile kadınların kullandığı çeşitli yardımcı üreme teknolojisi biçimleriyle ilişkili olarak ölçüldü. Gebe kadınların serumunda SOD, CAT, ROS seviyelerinin tespiti için enzim bağlantılı immün sorbent testi (ELISA), GSH ise amino asit analizörü kullanılarak ölçülür. Sonuçlar, gebelik boyunca IVF, IUI ve SP gruplarında NP grubuna kıyasla serum SOD, CAT ve GSH seviyelerinde istatistiksel olarak anlamlı bir düşüş olduğunu göstermiştir. Reaktif oksijen türleri (ROS) ve malondialdehit (MDA) seviyeleri önemli ölçüde yükselse bile. Yaş ve vücut kitle indeksi grupları ile üç gebelik grubu (IVF, IUI ve SP) birbirlerinden önemli ölçüde farklılık göstermemiştir. Gebelik sırasında serum GSH, CAT ve SOD'da önemli düşüşler vardır ve buna karşılık serum ROS ve MDA'da önemli artışlar vardır çünkü hamile kadınlar hamile olmayanlara göre oksidatif hasara daha yatkındır. Gebe grupları (IVF, IUI ve SP) arasında önemli bir etki yoktur, muhtemelen yaş ve BMI açısından benzer oldukları içindir.

Anahtar kelimeler: *Süperoksit dismutaz, Katalaz, Glutatyon, Reaktif oksijen türleri, Malondialdehit*

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1. Introduction

In vitro fertilisation (IVF) is a popular kind of assisted reproduction. This method is the only option for infertile couples trying to have a family, and it's a popular prescription for infertility. *In vitro* fertilisation (IVF) might be a good option for people who have tried other therapies for endometriosis without success, including medical or surgical procedures, conservative methods of reproduction that have not worked, or unexplained infertility [1]. The two forms of antioxidants that can be found in the body normally are enzymatic antioxidants and non-enzymatic antioxidants. Catalase (CAT), the glutathione peroxidase (GSH-Px), the glutathione reductase (GSH-R), and the superoxide dismutase (SOD), are the most prominent enzymatic antioxidants. Because glutathione shields eggs from oxidative stress during folliculogenesis, it is essential for egg quality. The oocytes with higher intracellular glutathione levels create embryos that are more robust and healthy. Oxidative stress clarify to be one of the main causes of IVF failure, among other factors [2].

2. Experimental Procedure

2.1. Workpiece material

Venous blood was collected by disposable syringes, after left few minutes then centrifuged to seperate serum of blood that use for tests.

2.2. Test procedure, test parameters and tooling

Enzyme-linked immune sorbent assay (ELISA) based on for the detection of SOD, CAT, ROS levels in the serum of pregnant women in the first trimester of pregnancy, while GSH measured by using amino acid analyzer.

2.3. Experimental design and analysis

The case study include 150 pregnant women in the first trimester of the pregnancy these women divided in to three type: women that pregnant by *in vitro* fertilization-embryo transfer technique (IVF), b. women that pregnant by intrauterine insemination technique (IUI), and c. women that pregnant spontaneously (SP). The study take 50 apparently healthy women without pregnant (non-pregnant women) considered as a control groups (NP). Sample collection and work carried out in Taiba Center for Infertility Treatment, in Babylon province/Iraq For the period from March 2022 to January 2023. The study was confirmed by the University of Babylon/College of the Medicine Ethical Committee, According to document number 11-1 and permission form on April 27, 2023. Informed of admission was obtained from all women that share before the data collection.

3. Results and Discussion

The present study showed that the serum SOD, CAT, and GSH showed a significant decrease in IVF, IUI, SP pregnant groups in comparison with NP. There were a non- significant that difference present between the different pregnant groups ($p<0.05$) regarding the serum level of SOD, CAT, and GSH as shown in the (Table1). Statistical analysis also showed a non-significant difference present between the different age and BMI groups regarding the serum levels of all studied SOD, CAT, and GSH (Table 2 and 3). Statistical analysis show there is a significant increase in serum of reactive oxygen species and Malondialdehyde in groups of SP, IUI, and IVF comparison to non-pregnant ($p<0.05$), while a non- significant the difference present between the different pregnant groups (Table 4). There is increase but not significant effect in serum of ROS and MDA in SP group, there is no statistical effect in IVF and IUI groups (Table 5). In BMI groups, there is no significant analyses in serum of ROS and MDA in all groups of pregnant as show on in (Table 6).

Table 1. The relation of serum levels of SOD, CAT, and GSH between different groups.

Group	SOD Mean±SD	P value	CAT Mean±SD	P value	GSH Mean±SD	P value
IVF	58.202±8.502	0.991	42.095±4.451	0.557	4.780±0.20	0.667
IUI	57.365±7.317		42.842±8.737		4.665±0.85	
IVF	58.202±8.502	0.717	42.095±4.451	0.546	4.780±0.20	0.345
SP	58.227±9.176		41.456±6.022		4.635±0.59	
IVF	58.202±8.502	0.048*	42.095±4.451	0.047*	4.780±0.20	0.018*
NP	62.105±9.665		44.234±7.615		4.808±0.58	
IUI	57.365±7.317	0.705	42.842±8.737	0.836	4.665±0.85	0.640
SP	58.227±9.176		41.456±6.022		4.635±0.59	
IUI	57.365±7.317	0.034*	42.842±8.737	0.012*	4.665±0.85	0.030*
NP	62.105±9.665		44.234±7.615		4.808±0.58	
SP	58.227±9.176	0.025*	41.456±6.022	0.024*	4.635±0.59	0.015*
NP	62.105±9.665		44.234±7.615		4.808±0.58	

P > 0.05: Non-Significant; * p<0.05 Significant; **p<0.01: Highly Significant
In vitro fertilization-embryo transfer (IVF), intrauterine insemination (IUI), Spontaneous pregnancy (SP), Non-pregnant (NP).

Table 2. Means ±SD of serum levels of SOD, CAT, and GSH in the different pregnant groups according to the age.

Biochemical Test	Age group (Year)	(SP) Mean ±SD	P value	(IUI) Mean ±SD	P Value	(IVF) Mean ±SD	P value
SOD	18-25	60.291±8.11	0.627	60.63±7.194	0.626	62.337±8.275	0.286
	26-33	58.80±10.31		60.146±7.823		59.922±8.863	
	Above 34	57.134±9.725		58.219±6.94		57.213±7.981	
CAT	18-25	43.807±7.907	0.175	42.063±3.332	0.667	44.042±4.139	0.250
	26-33	40.081±2.758		42.887±2.870		43.365±5.130	
	Above 34	42.457±4.878		42.169±2.876		41.417±3.408	
GSH	18-25	4.992±0.73	0.02*	4.917±0.29	0.10	4.843±0.17	0.16
	26-33	4.545±0.49		4.861±0.68		4.850±0.24	
	Above 34	4.506±0.37		4.553±0.28		4.730±0.14	

P > 0.05: Non-Significant; * p<0.05 Significant; **p<0.01: Highly Significant

Table 3. Means ±SD of serum levels of SOD, CAT, and GSH in the different pregnant groups according to the BMI.

Biochemical Test	BMI	(SP) Mean ±SD	P Value	(IUI) Mean ±SD	P value	IVF Mean ±SD	P value
SOD	Normal weight	60.634±6.88	0.591	58.959±6.95	0.743	61.781±8.172	0.166
	Overweight	59.091±9.98		60.539±7.54		61.401±9.778	
	Obese	56.884±10.1		58.869±7.77		56.961±7.233	
CAT	Normal weight	44.893±9.28	0.062	42.811±2.96	0.771	43.501±4.869	0.280
	Overweight	42.261±3.83		42.463±3.12		43.956±4.499	
	Obese	39.475±2.31		41.984±2.79		41.732±4.071	
GSH	Normal weight	4.952±0.65	0.132	5.012±0.71	0.076	4.837±0.31	0.443
	Overweight	4.719±0.68		4.695±0.40		4.739±.031	
	Obese	4.463±0.28		4.610±0.26		4.713±0.21	

Non-Significant; * p<0.05 Significant; **p<0.01: Highly Significant

Table 4. The relation of serum levels of ROS and MDA between different groups.

Group	ROS Mean±SD	P value	MDA Mean±SD	P value
IVF	323.209±49.07	0.853	1.67±0.95	0.314
IUI	321.35±42.29		1.73±0.41	
IVF	323.209±49.07	0.873	1.67±0.95	0.348
SP	312.05±34.27		1.65±0.51	
IVF	323.209±49.07	0.048*	1.67±0.95	0.041*
NP	282.70±65.45		1.47±0.37	
IUI	321.35±42.29	0.965	1.73±0.41	0.967
SP	312.05±34.27		1.65±0.51	
IUI	321.35±42.29	0.026*	1.73±0.41	0.018*
NP	282.70±65.45		1.47±0.37	
SP	312.05±34.27	0.021*	1.65±0.51	0.049*
NP	282.70±65.45		1.47±0.37	

P > 0.05: Non-Significant; * p<0.05 Significant; **p<0.01: Highly Significant

Table 5. Means ±SD of serum levels of ROS and MDA in the different pregnant groups according to the age.

Biochemical Test	Age group (Year)	(SP) Mean ±SD	P value	(IUI) Mean ±SD	P Value	(IVF) Mean ±SD	P value
ROS	18-25	314.07±25.63	0.702	323.04±38.51	0.973	320.17±50.25	0.584
	26-33	319.90±39.17		319.84±39.18		309.31±54.00	
	Above 34	325.18±33.91		319.54±50.97		328.34±41.92	
MDA	18-25	1.53±0.41	0.435	1.67±0.27	0.804	1.90±1.31	0.630
	26-33	1.62±0.40		1.61±0.27		1.64±0.25	
	Above 34	1.73±0.4		1.63±0.26		1.68±0.26	

P > 0.05: Non-Significant; * p<0.05 Significant; **p<0.01: Highly Significant

Table 6. Means ±SD of serum levels of SOD, CAT, and GSH in the different pregnant groups according to the BMI.

Biochemical Test	BMI	(SP) Mean ±SD	P Value	(IUI) Mean ±SD	P value	IVF Mean ±SD	P value
ROS	Normal weight	325.57±41.44	0.420	323.39±38.77	0.397	321.51±43.49	0.508
	Overweight	314.40±33.40		326.30±45.15		307.18±52.08	
	Obese	330.06±35.33		306.06±41.44		326.02±50.61	
MDA	Normal weight	1.43±0.25	0.916	1.65±0.27	0.681	2.11±1.65	0.252
	Overweight	1.70±0.42		1.59±0.28		1.66±0.29	
	Obese	1.72±0.43		1.67±0.24		1.63±0.23	

P > 0.05: Non-Significant; * p<0.05 Significant; **p<0.01: Highly Significant

In the current research, it was found that there were significant decreases in serum GSH, CAT, and SOD in pregnant women when compared to non-pregnant women ($P < 0.05$). These results come in agreement with other research; they found that the GSH and CAT levels underwent slight but significant decreases in the healthy pregnant women when compared with that of healthy non-pregnant women [3]. Bassi found a highly significant decrease in SOD in pregnant in the first trimester than that of non- pregnant [4]. In addition, Singh found a significant decrease in CAT during pregnancy period [5]. The present research showed a significant increase of in serum MDA in healthy pregnant women when compared with that of healthy non-pregnant women. According to some studies reported that the MDA was increased during normal pregnancy [6]. During normal pregnancy, it was found that a little increase in the oxidative stress could occur, even in the presence of the antioxidant systems [7]. According to the findings, there was a statistically significant difference in the MDA levels between women who underwent IVF and those who did not become pregnant. MDA levels were significantly higher in IVF pregnant women than in non-pregnant ones, which may be related to the weak positive correlation between MDA and the quantity of grade A embryos and fertilization rate, two key indicators of successful IVF outcomes. These findings concur with those of Pasqualotto, who discovered that lipid peroxidation levels were higher in pregnant women [8]. The present research showed a non-significant increase of serum ROS in healthy pregnant women in the first trimester when compared with that of healthy non-pregnant women. During the time of pregnancy, the numerous physiological and the metabolic changes that occur in the mother's body which help the production of ROS, particular in the second half of the pregnancy. The primary causes of this are an increase in basic metabolism and oxygen "consumption" as well as the predominant use of fatty acids as an energy source by the majority of maternal retro placental tissues. Pregnancy's third trimester is a unique time when insulin resistance, fat catabolism, and the release of free fatty acids all increase. The third trimester of the pregnancy is a special time when free fatty acid release, fat catabolism, and insulin resistance all rise. The level of oxidative stress was slightly significantly in women with IUI pregnant comparison to NP its maybe increase endometrial content of ROS [9]. The present study show increase in serum of ROS in IVF pregnant women some data indicate that an increase in ROS linked to rising maternal age may have an impact on oocyte quality, where it found ROS demonstrated to negatively affect oocyte maturation, but they also play a critical function in cellular signaling for the activation of meiosis in the oocyte [10].

4. Conclusion

The study found significant decrease in serum SOD, CAT, and GSH levels in serum pregnant groups compared to the NP group during pregnancy. These decrease corresponding significant increases in serum of ROS and MDA because pregnant women were more capable to oxidative damage than the non-pregnant as show by the decreased antioxidants. There is no significant effect among the groups of pregnant (IVF, IUI, and SP), perhaps because they are similar in age and BMI.

Symbols

IVF	*In vitro* fertilization-embryo transfer
IUI	Intrauterine insemination
SP	Spontaneous pregnancy
NP	Non-pregnant
OS	Oxidative Stress
RO	Reactive Oxygen Species
SOD	Superoxide dismutase
CAT	Catalase
GSH	Glutathione
MDA	Malondialdehyde
BMI	Body Mass Index
ELISA	Enzyme-linked immune sorbent assay
GSH-Px	Glutathione peroxidase
GSH-R	glutathione reductase

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Declarations and Ethical Standards

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. The author(s) of this article declare that the materials and methods used in this study do not require ethical committee permission and/or legal-special permission.

Author Contributions

Author 3 conceived of the presented idea. Author 1 developed the theory, performed the computations and carried out the experiments. Author 2 supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

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