# Oxidative stress and antioxidant in pregnancy women conceived by In Vitro fertilization and Intrauterine Insemination

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**Abstract**

One of the most widely used methods of assisted reproduction is in vitro fertilization (IVF). This approach is a most used infertility curative and frequently represents the sole chance for the infertile couples to become parents. The Intrauterine Insemination (IUI) is a straightforward, affordable, non-invasive, and secure therapy option for the management of infertility. Oxidative stress (OS) is the outcome of significant reactive oxygen species (ROS) causing oocyte senility and many reproductive problems in females, while antioxidant can balance out the increased levels of ROS that cause a high state of OS, they have long been used in the treatment of subfertility. This study was to detect the serum of superoxide dismutase, catalase, glutathione, reactive oxygen species, and Malondialdehyde levels of pregnant with different types of assisted reproductive techniques in relation with the age group, body mass index (BMI). 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. 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. While ROS and MDA significant increase. There were a non- significant difference present between the different pregnant groups (IVF, IUI, and SP) also in ages and BMI groups. 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 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.

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

**Introduction**

One of the most widely used methods of assisted reproduction is in vitro fertilization (IVF). This approach is a widely used infertility medication and frequently represents the sole way for infertile couples to become parents. IVF may be beneficial for patients with a history of endometriosis who have undergone unsuccessful medical or surgical treatment as well as those whose conservative reproductive treatments have failed or who have 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].

**Patients and Methods**

The case study include 150 pregnant women in the first trimester of the pregnancy these women divided in to three types a. women that pregnant by IVF technique, b. women that pregnant by IUI technique, and c. women that pregnant spontaneously. The study take 50 apparently healthy women without pregnant considered as a control groups. 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. Informed of admission was obtained from all women that share before the data collection.

**Results**

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 (Table 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 in serum of ROS in SP and IVF groups. While MDA increase and not significant in SP group only, 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.

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

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

**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 |

**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 |

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

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

 **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 |

 **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 |

**Discussion**

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].

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