



RESEARCH ARTICLE

Oxidative Stress (OS) Impaired Female Fertility**Hazim Ismail Abdulbari ALL-Ahmed¹, Hussein Jassim Obade², Rasha Abdul-Hussein Mahood³**

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Key words:***Corresponding Author****Hazim Ismail ALL-Ahmed****Abstract**

Elevation of oxidative stress suggested cause infertility in female with unexplained infertility. Aim of this project to study who the excess of oxidative stress cause impaired female infertility by effects on antioxidants enzymes activity. Blood samples collected from 40 females with unexplained infertility and from 20 female fertility to measured oxidative stress by protein peroxidase (PPO), lipid peroxidase (LPO) and activities of antioxidant enzymes: glutathione peroxidase (GPX), superoxide dismutase (SOD). Significant elevation in levels of oxidative stress biomarkers in female with unexplained infertility represented by increased levels of PPO and LPO (82.63 ± 10.73 ; 63.84 ± 9.51) when compared with fertile females (61.52 ± 8.66 ; 42.67 ± 7.05) respectively. While the results show significant decrease in activity of antioxidants enzymes represented by GPX and SOD in female with unexplained infertility (9.47 ± 1.56 ; 14.33 ± 3.58) compared with fertile females (12.65 ± 3.02 ; 20.09 ± 5.42) respectively. We concluded from this study that the elevation of oxidative stress in female with unexplained infertility cause disturbances in activity of antioxidants enzymes and may be cause infertility in females, recovery this relationship help to treatment the female with unexplained infertility and with increase the Oxidative stress by administration of antioxidants as drugs or plants extracts.

*Copy Right, IJAR, 2015,. All rights reserved***INTRODUCTION**

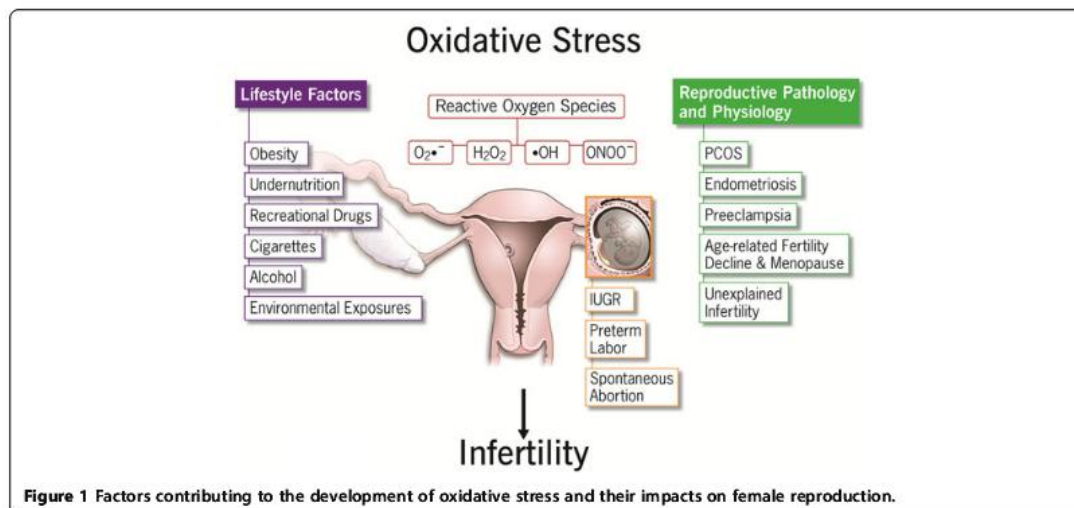
Oxidative stress (OS) occurred as results of imbalance between pro-oxidants and antioxidants [1]. The alteration in this ratio may be lead to elevated levels of reactive oxygen species ROS or/and reactive nitrogen species RNS, or cause reduce in mechanisms of antioxidant defense [2,3].

Unexplained infertility is defined as the inability to conceive after 12 months of unprotected intercourse in couples where known causes of infertility have been ruled out. Pathophysiology causes remains unclear, although the literature suggests a possible contribution by increased levels of ROS, especially shown by increased levels of protein peroxidase (PPO), lipid peroxidase (LPO) [4,5] in comparison to antioxidant concentration [6]. The increased amounts of ROS in these patients are suggestive of a reduction in antioxidant defenses, including GSH and vitamin E [7]. The low antioxidant status of the peritoneal fluid may be a determinant factor in the pathogenesis of idiopathic infertility.

Increase ROS production may be overcome the antioxidants defense system in the body and lead to unstable in physiological reaction in normal female [1] **Figure 1**. This disturbance can lead to many reproductive diseases including: polycystic ovary system (PCOS), endometriosis and female unexplained infertility.

ROS when elevated can cause another complication especially during pregnancy may lead to recurrent spontaneous abortion and intrauterine growth restriction (IUGR) [8].

Mechanisms of antioxidants defense carried out when increased ROS levels and antioxidant are act scavenger against it, to maintain oxidants/antioxidants ratio balance in the body, by two types of antioxidants:



- 1- **Non enzymatic:** consist of dietary supplements and synthetic antioxidants such as vitamin C, GSH, taurine, hypotaurine, vitamin E, Zn, selenium (Se), beta-carotene, and carotene [9].
- 2- **Enzymatic:** endogenous antioxidants enzymes include SOD, catalase, GPX and glutathioneoxidase.

Materials and methods:

Samples and patients:

Blood samples collected from 40 females with unexplained infertility and from 20 female fertility to measured oxidative stress by protein peroxidase (PPO), lipid peroxidase (LPO) and activities of antioxidant enzymes: glutathione peroxidase (GPX), superoxide dismutase (SOD).

Oxidative stress biomarkers measurement:

1. Lipid Peroxidation

Estimated of lipid peroxidation was according to the method of Ohkawa *et al.* [10]:

- 1- The amount of malondialdehyde (MDA) produced was used as an index of lipid peroxidation.
- 2- The samples were treated with 600 μ l of 10% *Trichloroacetic Acid* (TCA) solution, mixed gently and centrifugation 5000 rpm for 10 min.
- 3- After 5 min was added 150 μ l of Thiobarbituric acid (TBA) reagent to 200 μ l of the supernatant and mixed with 100 μ l of distilled water.
- 4- A blank tube without the sample was used as reference.
- 5- Both the sample and the blank tubes were placed in a boiling water bath for 10 min and allowed to equilibrate with room temperature.
- 6- Absorbance of the samples and blank were measured at 532 nm. All values were expressed as nmoles of MDA/ml present in it.

Protein Peroxidation

Estimated of protein peroxidation according to the method of Ohkawa *et al.* [10]:

- 1- 10 μ l of samples were treated with 600 μ l of 10% *Trichloro acetic Acid* (TCA) solution, mixed gently and centrifugation for 10 min at 5,000 rpm after 5 min.
- 2- The precipitate was washed two times with distilled water and then dissolved in 0.6 ml of 0.5 N NaOH solution.
- 3- 200 μ l of this sample solution is again mixed with 150 μ l of Thiobarbituric acid (TBA) reagent and 100 μ l of distilled water.
- 4- A blank tube without the sample was used as reference.
- 5- Both the sample and the blank tubes were kept for equilibration similar to lipid peroxidation and absorbance's were measured at 532 nm.
- 6- The protein peroxide is expressed as Nano moles of thiobarbituric acid reactive substance producing MDA per ml of sample.

2. Antioxidants enzymes measurement:

Determination of SOD

Estimated activity of SOD was using done by using Superoxide Dismutase diagnostic ELISA kit (Biovision, Inc. USA) according to the manufacturer’s instructions.

Determination of GPX Activity

Estimated activity of GPX was using done by using Superoxide Dismutase diagnostic ELISA kit (Biovision, Inc. USA) according to the manufacturer’s instructions.

Statistical analysis:

Data were analyzed by one-way analysis of variance (ANOVA- test), by SPSS version 13 data are presented as means \pm SD. The level of significance was $P < .05$.

Results and Discussion:

Elevation of oxidative stress represented by protein peroxidase (PPO), lipid peroxidase (LPO) (82.63 ± 10.73 ; 63.84 ± 9.51) in females with unexplained infertility compared with fertile females (61.52 ± 8.66 ; 42.67 ± 7.05) respectively. **Figure2.** While the results of activities of antioxidant enzymes represented by: glutathione peroxidase (GPX), superoxide dismutase (SOD) (9.47 ± 1.56 ; 14.33 ± 3.58) showed significant decrease in females with unexplained infertility compared with fertile females (12.65 ± 3.02 ; 20.09 ± 5.42) respectively. **Figure 3.**

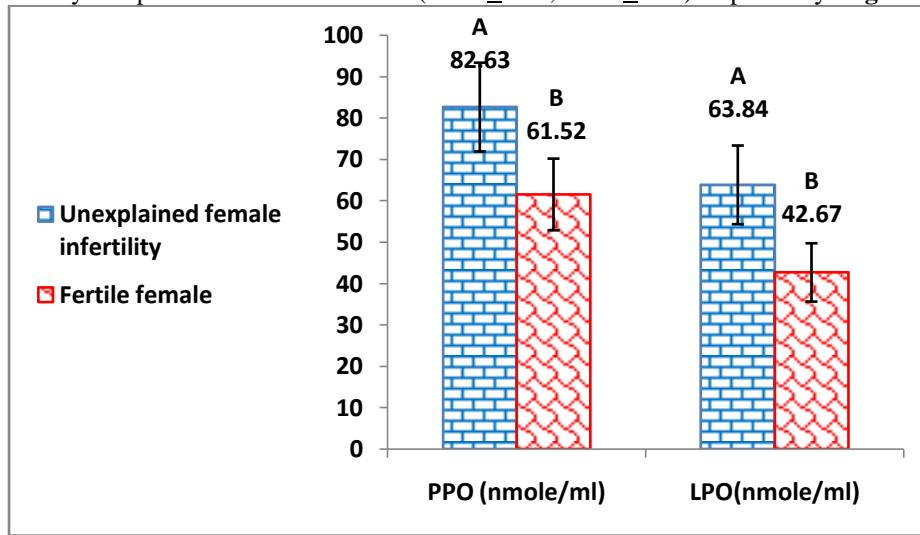


Figure2. Levels of oxidative stress biomarker: protein peroxidase (PPO), lipid peroxidase (LPO) in patients and control

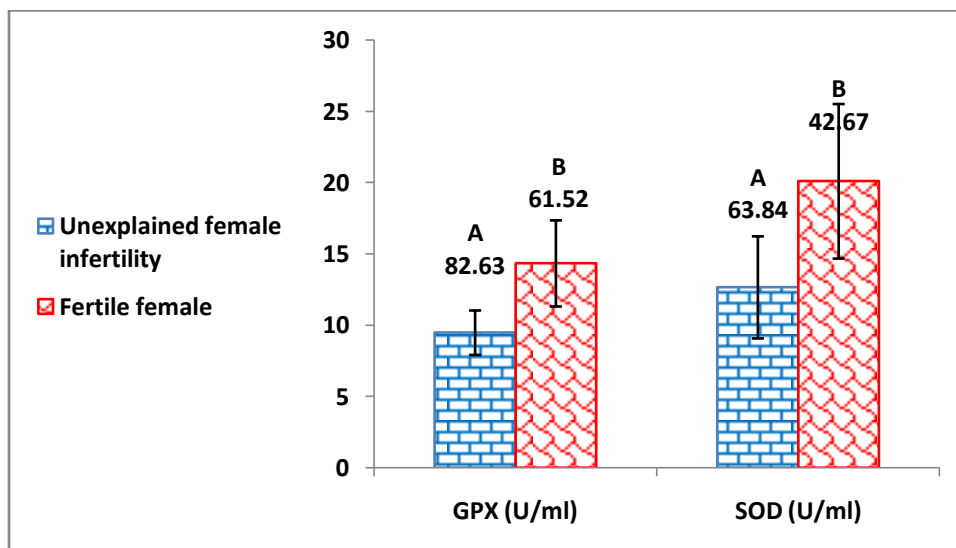


Figure3. Levels of antioxidant enzymes: glutathione peroxidase (GPX), superoxide dismutase (SOD) in patients and control

The increased of oxidative stress was caused decrease in activities of antioxidant enzymes which play important roles in scavenger process against elevation of oxidative stress in organisms and the defect in this process lead to infertility in males and females.

Many studies suggested that found positive relationship between excess oxidative stress and decrease the antioxidant enzymes activities which lead to impaired infertility in females [11-13] this results agreement with my results obtained from this project.

Normally oocytes grow and developed in ovary every month, but first meiosis resumes in one only of them (dominant oocyte). This process cause elevated in ROS levels and inhibition by antioxidant. While progression of meiosis II is promoted by antioxidants [14].

Behrman *et al.*, [14] reported that the present complication relationship between ROS and antioxidants in ovary. Elevated production of steroid in follicle growing lead to increase P450, resulting formation ROS, which produced by pre-ovulatory follicle have important role to inducer ovulation process. [15].

The oxygen which considered important for growth and development of ovarian follicles by prevent stimulation follicle angiogenesis.

ROS Follicles raise apoptosis, while Glutathione (GSH) and follicular stimulating hormone (FSH) necessary for growing follicle. Increases in the levels of estrogen as results to FSH stimulation, triggering the generation of catalase in the dominant follicle, and thus avoiding apoptosis [14].

There are many factor have relationship ROS and reproduction in female such as: Maternal age, oocyte quality decreases when increasing maternal age. The low quality of oocytes contain increased mtDNA damage and chromosomal aneuploidy, secondary to age-related dysfunctions. These mitochondrial changes may arise from elevated ROS, which occurs through the opening of ion channels (e.g. loss of Ca²⁺ homeostasis) [15].

Menses stopping cause increase iron levels in body which lead to elevated iron stores in body and could be induce oxidative imbalance. Menopause also leads to a decrease in estrogen and the loss of its protective effects against oxidative damage to the endometrium [16].

Oxidative stress is suggested to and pathologically physiologically have negative relationship with the number of reproductive processes including in female: folliculogenesis, oocyte maturation, endometriosis in male: sperm DNA damage, necrozoospermia, asthenospermia, and cause defective embryo development [17,18]. Oxidative stress overwhelmingly cause damages in the cellular membrane, and delay development of embryo [19], and cause cellular apoptosis which can yield fragmented embryos with a limited ability to implant [20].

The OS have different roles to effective on infertility according to female factor infertility including women with tubal or endometriosis has been evaluated, Women with endometriosis often have elevated macrophage levels that may produce excessive ROS in the peritoneal environment [21]. Increased ROS levels in the tubal and peritoneal environments negatively alter fertilization capabilities and embryonic development [19].

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