**Original Research Article**

# Evaluation of oxidative stress markers in infertile women

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## Abstract

Reproductive failure is a significant public health concern. Recently, ROS have been shown to have an important role in the normal functioning of the reproductive system and in the pathogenesis of infertility in females. Oxidative stress affects both natural and assisted infertility. Treatment that reduces OS may help infertile women with the disease that is caused by imbalance between free radicals and antioxidants. MDA is an end product of lipid peroxidation which can be used as a biomarker to measure the level of oxidative stress. TAC measures the low molecular weight non enzymatic antioxidants. Increase in MDA and decrease in TAC indicates an increase in oxidative stress. In the present study, it was observed that there was a statistically significant increase in serum MDA levels in female factor infertility and unexplained infertility when compared to controls. There was a statistically significant decrease in serum TAC in female factor infertility and unexplained infertility when compared to controls and statistically significant increase in serum LDH in infertile women with unexplained infertility compared to the control group and female factor infertility signifying the apoptotic damage in the unexplained infertility.

**Key words**

ROS - reactive oxygen species, OS – oxidative stress, MDA- Malondialdehide, TAC- Total antioxidant capacity, LDH – Lactate dehydrogenase.

## Introduction

Infertility is a disease defined as "the inability to conceive following 12 or more months of unprotected sex before an investigation is undertaken unless the medical history and physical findings dictate earlier evaluation and treatment [1]. Approximately 15-20% of couples of reproductive age is infertile which can be attributed equally to both male and female factors. ROS are involved in physiological functions in female reproduction such as are oocyte maturation, ovarian steroidogenesis, ovulation, implantation, and formation of fluid filled cavity, blastocyst, luteolysis and luteal maintenance in pregnancy. ROS acts as mediators of various signaling path ways. Elevated or sustained generation of free radicals lead to imbalance in the intracellular redox homeostasis. Excess levels of free radicals and ROS can be neutralized by antioxidants. Any imbalance between ROS and antioxidants can cause oxidative stress [2]. Oxidative stress involved in various pathologies of female reproductive tract like (PCOS) polycystic ovarian syndrome, endometriosis, tubal factor infertility, unexplained infertility, fibroids, recurrent pregnancy loss, spontanious abortions. OS also involved in hypothyroidism, hyperthyroidism, fetal teratogenicity, IUGR (intrauterine growth retardation), hydatiform mole, oligospermia, asthenospermia, azospermia . All of which leads to immense burden on maternal, fetal morbidity and mortality [3]. The effects of ROS result from damage to lipids, nucleic acids, proteins and other cellular materials. ROS also causes depletion of ATP levels. Lipid peroxidation is a self propagating reaction unless it is counteracted by antioxidants. Malondialdehyde (MDA) is a stable end product formed from peroxidation of polyunsaturated fattyacids of cell membrane. As hypoxia intensifies the peroxidation and cell membrane disruption, increase in extra cellular activity of Lactate dehydrogenase (LDH). LDH can be used as a hypoxia marker [4]. The male and female reproductive tracts are rich in both enzymatic and non-enzymatic antioxidants. As it is practically impossible to measure all the biologically active antioxidants in human samples, the concept of a “global” assessment of antioxidant capacity has proved attractive. Total antioxidant capacity (TAC) mainly measures non enzymatic antioxidants [5].

## Materials and methods

This study was done in a group of 70 women aged 20-40 years with primary infertility. The entire group was further divided in to 2 groups based on the etiology of infertility: Infertility due to female factor (Group 2) and Unexplained infertility (Group 3). The results were compared and correlated with controls. 35 age matched females with history of at least one childbirth were included in this study as controls (group 1). In our study, we excluded infertile women with male factor infertility and other systemic disorders .We collected blood in plain vacutainer tubes and following parameters were analyzed, serum MDA - TBARS (thiobarbituric acid reactive substance) assay, serum LDH - Optimised DGKC, kinetic assay, serum FRAP as a marker of total anti oxidant capacity – Benzie and Strain method [6] . The data was analyzed using SPSS (Statistical package for social science). Unpaired t-Test was used to assess the significance of difference of mean values of different parameters in between the cases and controls.

## Results

In the present study, we found that there was significant rise in serum MDA, decrease in TAC and less significant rise LDH in case group 2 when compared to controls. There was significant rise in serum MDA, serum LDH and decrease in TAC in case group 3 when compared to controls. There was significant rise in serum

LDH and decrease in TAC in case group 3 when compared to case group 2 (**Table – 1 to 3**).

## Discussion

Infertility is a common problem experienced by many couples. Numerous treatments are available for infertility. However in some cases, the treatment is empirical in nature because the etiology is not fully understood. Oxidative stress has been implicated in embryo fragmentation, DNA damage, apoptosis and poor pregnancy outcome. Oxidative stress is involved in various gynecological disorders like PCOS, endometriosis, PID, tubal factors, other ovulatory disorders, hyperprolactenemia and hypothyroidism. It has been suggested that cyclical ROS production may, over time, contribute to oophoritis associated with autoimmune premature ovarian failure, and exacerbated by diminished antioxidant status [7-9]. Macrophages, neutrophils and granulose cells in the graphian follicle are the source of reactive oxygen species, during follicular maturation oocytes are well protected against toxic injury due to oxidative stress by important antioxidants. Total antioxidant capacity measures the low molecular weight non enzymatic antioxidants. Increase in MDA and decrease in TAC indicates an increase in oxidative stress. Results obtained from the present study regarding oxidative stress in female infertility are in agreement with earlier reports of several studies [2, 10-18].

**Table – 1:** Mean±SEM, SD, p and t values of analyzed parameters in controls and female factor infertility cases.

| Parameter | Mean±SEM controls | SD controls | Mean±SEM Cases | SD Cases | t value | P Value |
|---|---|---|---|---|---|---|
| MDA | 203.83 ± 8.72 | 53.43 | 701.26±28.6 | 169.09 | 17.2 | < 0.0001 |
| TAC | 1154±34.5 | 204.43 | 785.48±23.8 | 141.33 | 8.78 | < 0.0001 |
| LDH | 223.15± 9.65 | 54.14 | 231.15±8.28 | 48.86 | 4.62 | 0.56 |
| BMI | 21.90±0.32 | 1.87 | 23.5±0.29 | 2.5 | 0.32 | 0.0001 |

**Table – 2:** Mean±SE, SD, p and t values of analyzed parameters in controls and unexplained infertility cases.

| Parameter | Mean±SEM Controls | SD controls | Mean±SEM Cases | SD Cases | t value | P Value |
|---|---|---|---|---|---|---|
| MDA | 203.83 ± 8.72 | 53.43 | 722.63±21.98 | 131.89 | 17.06 | < 0.0001 |
| TAC | 1154±34.5 | 204.43 | 526.57±13.2 | 78.17 | 16.96 | < 0.0001 |
| LDH | 223.15±9.65 | 54.14 | 272.14±7.17 | 42.46 | 7.44 | 0.0001 |
| BMI | 21.20±0.32 | 1.87 | 22.43±0.4 | 2.37 | 1.85 | <0.001 |

**Table – 3:** Mean±SEM, SD, p and t values of analyzed parameters in female factor infertility and unexplained infertility cases.

| Parameter | Mean±SEM Case 2 | SD Case 2 | Mean±SEM Case 3 | SD Case 3 | t value | p value |
|---|---|---|---|---|---|---|
| MDA | 704.26±28.6 | 169.09 | 722.63±21.98 | 131.89 | 2.5 | 0.6 |
| TAC | 785.48±23.8 | 141.33 | 526.57±13.2 | 78.17 | 9.4 | 0.0001 |
| LDH | 231.15±8.28 | 48.86 | 272.14±7.17 | 42.46 | 0.15 | 0.0006 |
| BMI | 23.15±0.29 | 2.5 | 22.43±0.4 | 2.37 | 2.13 | 0.06 |

Veena Bhaskar, et al. reported that elevated MDA levels in serum of infertile women, than in fertile women signifies that the oxidative damage in infertile women. Gonzalez et al in their study observed that there is generation of reactive oxygen species from mononuclear cells which occurred in response to hyperglycemia in women with PCOS. This increase in reactive oxygen species was seen both in obese and lean PCOS when compared to matched controls and was independent of obesity [19]. Yilmaz, et al. where significantly lower total antioxidant status was measured in the serum of PCOS women in comparison to healthy controls. Serum malonyl-dialdehyde, was significantly increased in these subjects. Elevated lipoprotein complexes and lowered antioxidants contribute to excessive growth of endometrial stromal cells implicating the role of OS in infertility associated with endometriosis [14]. Osman Hussein G, et al. proved that the levels of total antioxidant capacity, Catalase and superoxide dismutase were significantly lower in both serum and peritoneal fluid of infertile endometriotic women compared with controls [16].

Hypothyroidism is one of the common endocrine disorder causing female infertility. Hypothyroidism can affect fertility due to anovulatory cycles, luteal phase defects, hyperprolactinemia, and sex hormone imbalance [20, 21]. Das, et al. study found that an overall negative correlation between reactive oxygen species in follicular fluid and embryo quality and the association of lower total antioxidant capacity with decreased fertilization potential [22]. In the present study mean value of serum total antioxidant capacity levels is significantly low in unexplained infertility compared to women with female factor infertility. Polak, et al. reported that total anti oxidant status was found to be lower in peritoneal fluid of women with idiopathic infertility compared to fertile controls and individuals with tubal infertility, they hypothesized that peritoneal fluid diffuses into the fallopian tubes where it may cause damage to sperm, which are known to be sensitive to oxidative stress [23]. In the present study it was observed that statistically significant increase in serum LDH in infertile women with unexplained infertility compared to the control group and female factor infertility signifying the apoptotic damage in the unexplained infertility. Hypoxia intensifies the lipid peroxidation and cell membrane disruption, increase in extra cellular activity of lactate dehydrogenase. In the present study, it was observed that statistically significant increase in mean values of BMI in infertile women with female factor infertility and unexplained infertility compared to controls .Obesity affects ovulation, response to fertility treatment, pregnancy rates and outcome A.M. Clark observed that weight reduction improves the chances of conception, fertility rates in infertile women irrespective of their infertility diagnosis [24 -32]. Robker, et al. [17] observed that adipocyte hypertrophy can increase cytokine, adipokine production, hypoxia and oxidative stress which can contributes to infertility in obese women.

## Conclusion

Reactive oxygen species appears to have both physiological and pathological role in the female reproductive tract. Oxidative stress can affect female fertility potential in a number of ways. It may affect ovulation, fertilization, implantation and embryo development. The present study aims to assess the predictive values and correlation between oxidative stress markers (malondialdehide, total antioxidant capacity, lactate dehydrogenase). Hence the present study confirms that presence of increased lipid peroxidation and oxidative stress in infertile women. Thus administration of antioxidant vitamins and minerals like vitamin C, vitamin E, Zn, Se, taurine, hypotaurine, glutathione and beta-carotene may benefit in the treatment of infertility.

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