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Correlation of infertility with biochemical marker of lipid peroxidation (MDA) and antioxidant level (vitamin C and vitamin E) in young infertile women of reproductive age group

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Abstract
Aim and Objective: Biochemical marker of lipid peroxidation (MDA) and antioxidants level (vitamin C and vitamin E) in young infertile women of reproductive age group.
Introduction: Infertility is defined as the inability of couples to conceive a clinical pregnancy after 12 months or more of unprotected intercourse. It is estimated that 25% of couple will experience an episode of infertility in their reproductive life. Infertility is a medical problem that affect a vast proportion of the world's young population (10%-15%). Advances in assisted reproductive technologies, such as IVF, can offer hope to many couples where treatment is available, although barriers exist in terms of medical coverage and affordability.
Material and methods: Detailed data was collected about name, age, urban/rural residence, height, weight, medical history including obstetrics & gynaecological history were taken. Other information such as history of smoking, alcoholism, were noted. Informed consent was obtained from each patient before sample collection. The study was approved by PMCH ethical committee. Blood sample was investigated for various parameters like TBARS (MDA) and Vitamin C and Vitamin E.
Results and discussion: In our study there were all adult young females. None of the individuals were below the age of 15 years. In this study, a significant increase in plasma MDA level was observed in patient compared to control ($p< 0.001$). A significant decreased level of vitamin C and vitamin E was observed in patient group compared to control group ($p< 0.001$). The results indicate that infertile women have increased serum level of MDA and decreased serum levels of vitamin C and vitamin E as compared to fertile women.

Keywords: MDA, Vitamin C, Vitamin E

Introduction
Infertility is defined as the inability of couples to conceive a clinical pregnancy after 12 months or more of unprotected intercourse [1]. It is estimated that 25% of couple will experience an episode of infertility in their reproductive life [2]. Infertility is either classified as primary or secondary. Primary infertility refers to couples or patients who have had no previous successful pregnancies after regular unprotected intercourse for 12 months. Secondary infertility encompasses patients who have previously conceived, but are currently unable to conceive after regular unprotected intercourse for 12 months. Infertility is a medical problem that affect a vast proportion of the world's young population (10%-15%) [3]. Causes of female infertility include disturbances in hormonal or endocrine level (menstrual irregularity), tubal factors (occlusions, pelvic adhesions and other tubal abnormalities), acquired non-tubal factors (cervical or uterine disturbances), sexual dysfunction and congenital abnormalities [9].
Oxidative stress is essentially an imbalance between the production of Reactive oxygen species (ROS) and the ability of the body to counteract or detoxify their harmful effects through neutralization by antioxidants.
ROS can affect a variety of physiological functions in the reproductive tract, ROS within the follicular fluid plays a role in modulating oocyte maturation, folliculogenesis, ovarian steroidogenesis and luteolysis [10].

When the peritoneal cavity microenvironment is exposed to severe oxidative stress, the DNA of oocytes may be damaged, leading to defective fertilization. Even when fertilization is achieved, oxidative stress induced apoptosis may result in embryo fragmentation, implantation failure, abortion, impaired placentation and congenital abnormalities [11].

The antioxidants are compounds that dispose, scavenge and suppress the formation of free radicals or oppose their actions [12]. The male and female genital tracts are rich in both enzymatic and non-enzymatic antioxidants [13].

Ascorbic acid (vitamin C) is a redox catalyst which can reduce, and thereby neutralize, reactive oxygen species such as hydrogen peroxide. The concentration of ascorbic acid is reported to be much higher in human follicular fluid than in blood serum. This suggests active transport of ascorbic acid against the concentration gradient [14, 15] and that ascorbic acid may play a role as an antioxidant vitamin during folliculogenesis [16]. Levels of ROS are controlled and kept at physiological levels within the ovary by various antioxidant systems including vitamin C, which is known to have a protective effect within the follicle.

Vitamin E (α-tocopherol) is a lipid soluble vitamin with antioxidant activity. It consists of eight tocopherols and tocotrienols among these α-tocopherol form is the most important lipid soluble antioxidant, It plays a major role in antioxidant activities because it reacts with lipid radicals produced during lipid peroxidation [17] and protects membranes from oxidation. Vitamin E scavenges the oxygen radicals throughout the female reproductive tract. vitamin E has been reported to apparently be reduced in the peritoneal fluid of women with endometriosis, may block the onset of lipid peroxidation and primarily inhibit its propagation stage [18].

Aim and objective

Till date very few study of this type has been conducted in the southern part of Rajasthan so the aim of the study is:

- To investigate the oxidative stress by measuring the lipid peroxidation marker (MDA) in young infertile reproductive age group female.
- To study the role of various antioxidants like ascorbic acid (vitamin C), and tocopherols (vitamin E) in young infertile female of reproductive age group.
- To shed a light on the possible correlation between oxidant- antioxidant status in young infertile reproductive age group female

Material and Methods

Study area: study was conducted in department of obstetrics & gynaecology in Pacific Medical College & Hospital, Udaipur, Rajasthan.

Study population: Infertile female patients attended department of obstetrics & gynaecology in Pacific Medical & Hospital Udaipur (Rajasthan).

Sample size: 250 patients selected for the study and divided into two groups.

Group 1: It consist of 125 infertile female (mean age 20 to 45 years)

Group 2: It consist of 125 (precisely matched age.) healthy fertile female with no history of systemic illness.

Inclusion Criteria: Study included Infertile female patients attended department of obstetrics & gynaecology in Pacific Medical college & Hospital Udaipur (Rajasthan).

Exclusion Criteria: Male factor infertility, Female on oral contraceptive pills, Females suffering from chronic disease like chronic heart disease (CHD), hypertension, ischemic heart disease, diabetes mellitus (DM), chronic obstructive pulmonary disease(COPD), other major systemic illness in infertile female of reproductive age group were excluded from the study.

Methods of collection of data

Details about name, age, urban/rural residence, height, weight, medical history including obstetrics & gynaecological history were taken. Other information such as history of smoking, alcoholism, were noted. Informed consent was obtained from each patient before sample collection. The study was approved by PMCH ethical committee.

Blood collection, separation and storage of sample

10 ml blood sample was collected in a plain vial by vein puncture and allowed the blood to clot at room temperature and centrifuged at 3000 rpm for 10 min. The serum separated into proper aliquots and analyzed for test. Blood sample was investigated for various parameters like TBARS (MDA) and Vitamin C and Vitamin E. Thiobarbituric acid reactive substances (TBARS) (Buege and Aust, 1978).

Results and discussion

We have studied 125 infertile female patients (age between 20-45 years) with 125 (age, sex matched) healthy fertile females as controls. Out of 125 patients 76 females were primary infertile and 49 female patients having secondary infertility. The mean age of patients was (30.07 ± 5.87, n = 125) whereas in controls (29.01 ± 4.55, n = 125).

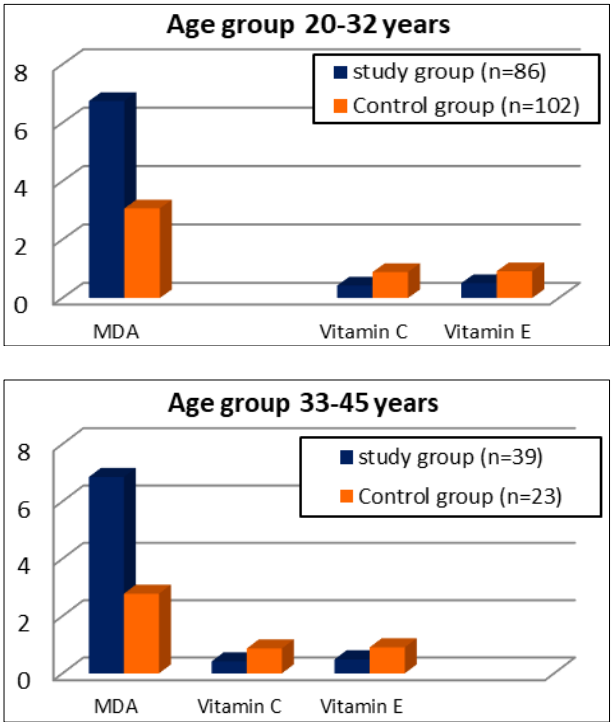
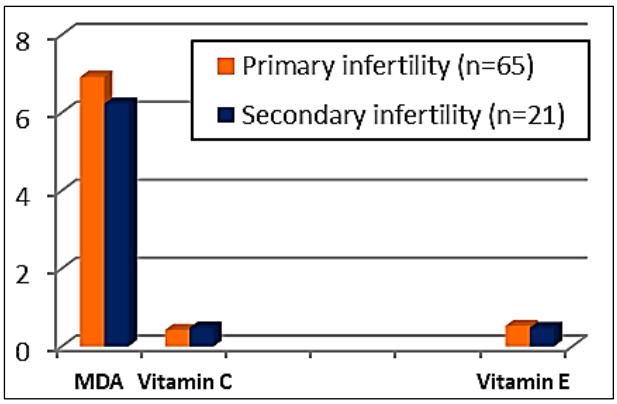
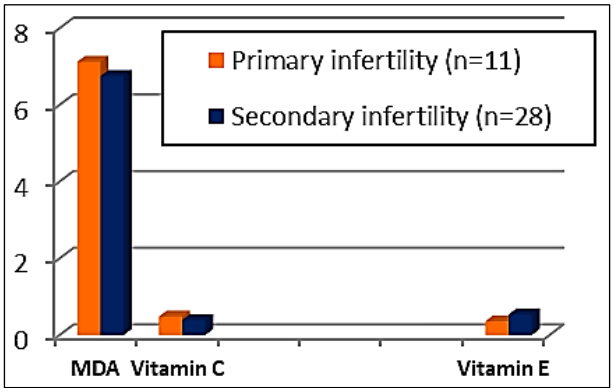


Fig 1: comparison of MDA, vitamin C and Vitamin E between study group and control group in the (a)age group 20-30 and (b)age group 33-45.



a) Age group 20-32 years



b) Age group 30-35 years

Fig 2: Comparison of MDA, Vitamin C and Vitamin E between primary and secondary infertile female patients

The present case control study was conducted on study population of 250 females. Out of which 125 are infertile females who attended department of obstetrics & gynaecology. Study subjects were taken from the outdoor clinic of department of obstetrics & gynaecology of our institution. 125 healthy subjects were selected over a period of 6 months in Pacific Medical college & Hospital, Udaipur Rajasthan. The results of patients were statistically compared with 125 healthy fertile females.

Group 1 (study group) - study group was consisted of 125 infertile female patients of different age groups (mean age 20 to 45 years) (n=125).

Group 2 (control group) - study group was consisted of 125 fertile female volunteers had same age & sex matched healthy controls (n=125).

- In study of "Biochemical marker of lipid peroxidation (MDA) and antioxidants level (vitamin C and vitamin E) in young infertile women of reproductive age group", we found elevated level of lipid peroxidation marker (MDA) in subjects as compared to control. The increase in MDA in our study is in agreement with the results of other studies.
- The comparison of mean ± SD of subjects of MDA (6.78 ± 1.73) and that of control (3.04 ± 1.04) is statistically significant with a p-value of 0.0001.
- The mean ± SD of subjects in case of vitamin C (0.43 ± 0.21) and that of control group was (0.857 ± 0.189) which is also statistically highly significant with a p-value of 0.0001. This demonstrated that the status of antioxidant vitamin C was significantly lower in cases as compared to control group.
- Similarly, the same trend was also observed in case of another antioxidant i.e. Vitamin E. From, mean ± SD of subjects (0.50 ± 0.25) and that of control (0.92 ± 0.14) it is observed
- Along with these antioxidants, blood glucose level was also measured in subjects and controls. It was observed that glucose level is elevated in infertile female patients than controls. The mean and sd of total leukocyte count for subject (9227 ± 1579) and control (7692 ± 1284) with a p-value of 0.0001, RBCs count for subject (4.05 ± 0.527) and control (4.53 ± 0.407) with a p-value of 0.0001, and comparison of Hb level (gm/dl) between control group and study group having mean ±SD of subjects (11.16 ± 1.35) and control (12.81 ±.96) with a p-value of 0.0001 shows that there is a statistically significant difference between subject and control.
- The mean ±SD of MDA, vitamin C and vitamin E for age group 20-32 years of subject (MDA 6.75 ± 1.70, vitamin C 0.43 ± 0.21, vitamin E 0.51 ± 0.23) and control (MDA 3.07 ± 1.02, vitamin C 0.89 ± 0.21, vitamin E 0.92 ± 0.14) shows that the level of MDA is elevated and that of vitamin E and vitamin c decreases in subject as compared to control. The p-value is also calculated for subject and control for this age group, which shows a statistically significant difference.
- The mean ±SD of MDA, vitamin C and vitamin E foe age group 33-45 years of subject (MDA 6.85 ± 1.82, vitamin C 0.42 ± 0.22, vitamin E 0.49 ± 0.28) and control (MDA 2.78 ± 0.95, vitamin C 0.87 ± 0.14, vitamin E 0.91 ± 0.15) shows that the level of MDA is elevated and that of vitamin E and vitamin c decreases in subject as compared to control.
- The p-value is also calculated for subject and control for this age group, which shows a statistically significant difference.
- From above result it is interpreted that the level of oxidative stress is significantly higher in females of age group 20-32 years and, similarly the level of antioxidants vitamin E and C is lower in females of this age group as compared to age group 33-45 years.
- The mean ±SD of MDA, vitamin C and vitamin E for age group 22-32 years for females of primary infertility (MDA 6.92±1.58, vitamin C 0.424±0.21, vitamin E 0.529±0.24) and secondary infertility (MDA 6.23±2.0, vitamin C 0.48±0.21, vitamin E 0.47±0.20) shows that the level of MDA is elevated and that of vitamin E and vitamin c decreases in primary infertility as compared to secondary infertility.
- The mean ±SD of MDA, vitamin C and vitamin E for age group 33-45 years for females of primary infertility (MDA 7.12±2.42, vitamin C 0.48±0.19, vitamin E 0.36±0.23) and secondary infertility (MDA 6.75±1.5, vitamin C 0.39±0.23, vitamin E 0.54±0.29) shows that the level of MDA is elevated and that of vitamin E and vitamin c decreases in primary infertility as compared to secondary infertility.
- The p-value is also calculated for subject and control for this age group, which shows a statistically significant difference.
- From above result it is interpreted that the level of oxidative stress is significantly higher in females of age group 33-45 years and, similarly the level of antioxidants vitamin E and C is lower in females of this age group as compared to age group 33-45 years

- In general when all groups were compared with controls, a significant difference was noticed nearly in all parameters. These results demonstrated the presence of an imbalance in oxidant- antioxidant system and provide further support for a free radical mediated damage in pathogenesis of infertility.

Thus we have concluded that primary infertility is more prevalent then secondary infertility in southern part of Rajasthan. We also found that female infertility is more in urban population then rural. It is may be because of life style and urbanization. We also observed that PCOD is the most prevalent cause of infertility among primary infertile patient while ovarian cyst is most prevalent among secondary infertility.

Several prospective and retrospective studies have demonstrated that serum Malondialdehyde (MDA) levels, marker for oxidative stress, were significantly higher in female infertile patients as compared to controls. In our study we also found increased level of MDA in infertile female patient as compared to controls. Studies on non-enzymatic antioxidants- Vitamin C, Vitamin E, in female infertile subjects have shown significantly decreased levels similarly we also concluded that infertile female patients have decreased level of vitamin C and Vitamin E as compared to controls. Infertility is the emerging public health problems in developing countries, When OS is diagnosed, treatment plans must focus on identifying and eliminating the source. When a specific cause is identified, medical and surgical management options should be considered to eliminate the source of free radicals. Unlike in the male, specific clinical conditions associated with OS have yet to be identified in women. This complicates the treatment of the primary cause of excessive reactive oxygen species production. Only after treatment of the primary etiology should patients be advised to take antioxidant supplementation.

Summary and Conclusion

The present case control study was conducted on 125 infertile female patient attended department of obstetrics & gynaecology in Pacific Medical college & Hospital, Udaipur (Rajasthan).

Onder and Gurer, 2001 [22] showed that ROS and other oxidants could also be formed in normal physiological process. Young and Woodside, 2001 [23] studied that H2O2 and other derivatives of peroxides increase in some conditions, diffuse into plasma. Here, antioxidant components of plasma overwhelm them, and they are simultaneously consumed. Agarwal A *et al.* 2005 [12] concluded that Successful pregnancy results from an interaction between myriad physiological processes in both men and women.

In this study, a significant increase in plasma MDA level was observed in patient compared to control ($p< 0.001$). A significant decreased level of vitamin C and vitamin E was observed in patient group compared to control group ($p< 0.001$). The results indicate that infertile women have increased serum level of MDA and decreased serum levels of vitamin C and vitamin E as compared to fertile women. They are in agreement with previous reports. Veena Bhaskar S *et al.* 2008 [24] have found significantly higher concentration of MDA in serum of infertile women than in fertile women in this study It was suggested that OS is caused by ROS overproduction rather than antioxidant depletion. Sane *et al.* 1991 [19] found that women undergoing induced or spontaneous abortions exhibited a maximum rise in serum MDA level.

Savita Setal *et al.* 2009 [25] have shown significantly high plasma levels of MDA in infertile women when compared to fertile women they suggested that changes are consequence of increased oxidative stress that mediate lipid per oxidation Product, i.e. MDA.

The elevation of lipid peroxidation and the depletion of antioxidant enzymes seems to vary similarly in the infertile women regardless to the etiology of infertility. The consequences appear to be equal precipitation in the pathophysiology of their productive systems in infertile patients. These evidences suggest that oxidative stress is an independent etiologic factor in female infertility. Lucky H. Sekhon, Sajal Gupta, *et al.* 2009 [26] also found increased level of MDA in serum of infertile female patients then healthy fertile females.

Zhang *et al.* 2007 [27] used methods of chemicalorimetry to measure and compare levels of serum lipid peroxides (LPO), MDA, vitamin E, and vitamin C in infertile females and normal fertile females. Levels of serum LPO and MDA in study group were significantly higher than those found in control group. Levels of vitamin E, vitamin C, and were lower in infertile patients than in the control group which is also in agreement with our study.

Surapaneni Krishna Mohan *et al.* 2009 and Joo Leon Lee *et al.* 2010 [28, 29] have demonstrated that serum Malondialdehyde (MDA) levels, marker for oxidative stress, were significantly higher in infertile female patients as compared to controls. Studies on non-enzymatic antioxidants- Vit C, Vit E, in infertile female subjects have shown significantly decreased levels.

Wilson CW MvL *et al.* 1973 and Aplin JD *et al.* 1986 has been suggested vitamin C as a regulator of female fertility. Our results were also similar to findings of investigator Sasikumar Sathiyanarayanan *et al.* 2011 [30] who also found decrease level of C in the test group on comparison with the control. randomized, controlled, multicenter study done by Another study done by Westphal LM *et al.* 2004 [31] demonstrated that women with a history of recurrent miscarriages and luteal phase defects had significantly lower concentrations of antioxidants than in healthy women. some previous studies showed that the serum vitamin C levels ($p< 0.001$) was lower in female patients having infertility as compared to control group which is in agreement with our study.

Naseer JH Al *et al.* 2014 [34] stated that Vitamin E is a major chain breaking antioxidant in membranes, located mainly in the ovary especially in follicular fluid. Our results are in accordance with studies of other investigators. Makinde KA and Adedeji *et al.* 1994 [35] found significant decreased level of vitamin E in serum of infertile women when compared with fertile controls this was corroborates with similar work done by Mehendale SS; KilariBams *et al.* 2009 [36] where it has been concluded that plasma vitamin E level is greater in fertile women than in infertile women. Savita *et al.* 2009 [37] suggest that the increased OS are associated with the decrease of antioxidants and associated with infertility. Ruder, EH; Hartman *et al.* 2009 [38] found decreased level of vitamin E in study group compared to controls and he concluded that Vitamin E directly neutralizes superoxide anion, hydrogen peroxide, and hydroxyl radical; so increase

these types of free radical may lead to depletion of vitamin E. Also vitamin E increases number of embryos developing to the expanded blastocysts and increases viability of embryos exposed to heat shock. So any change in its concentration may have a role in infertility. Bayer R. *et al* 1960 [39] Vitamin E may increase oocyte quality. In a human trial, infertile couples given vitamin E show a significant increase in fertility. Simsek *et al.* 1998 [20] evaluated the outcome of deficient antioxidant defense in women with infertility and demonstrated elevated OS and significantly decreased vitamin E in this population compared with the control group. Their findings confirm that OS may be involved in the pathogenesis of recurrent pregnancy loss.

A recent study by Kuscu *et al.* 2009 [21] demonstrated increased MDA levels in infertile female patients then controls.

Our study was different from the study done by Sasikumar Sathiyanarayanan *et al.* 2011 [30] who observed increased level of Vitamin E in the test group on comparison with the control while we have found significant decrease in serum vitamin E level in study group on comparison with control group while some studies found.

References

1. Definitions of infertility and recurrent pregnancy loss: a committee opinion. Practice Committee of American Society for Reproductive Medicine Fertil Steril [Pub med] 2013;99(1):63.
2. Kochen MM, Ittner E, Himmel W. Management of involuntary childlessness in general practice - patients' and doctors' views. Br. J Gen. Pract 1997;47:105-106.
3. World Health Organization. Infecundity, infertility, and childlessness in developing countries. DHS Comparative Reports No 9. Calverton, Maryland, USA: ORC Macro and the World Health Organization 2004.
4. Wu AK, Elliott P, Katz PP, Smith JF. Time costs of fertility care: the hidden hardship of building a family. Fertil. Steril 2013.
5. Chandra A, Martinez GM, Mosher WD, Abma JC, Jones J. Fertility, family planning, and reproductive health of US women: data from the 2002 National Survey of Family Growth. Vital Health Stat 2005;23;1-160.
6. Sharath KC, Najafi M, Malini SS. Association of Obesity with Male Infertility among Infertile Couples is not Significant in Mysore, South India. Advanced Studies in Biology 2013;5:319-325.
7. Chander PP, Indira H, Kusum Z. Need andfeasibility of providing assisted technologies for infertility management in resource poor settings. ICMR bulletin 2000;30:55-62.
8. Mascarenhas MN, Flaxman SR, Boerma T, Vanderpoel S, Stevens GA. National, Regional, and Global Trends in Infertility Prevalence since 1990: A Systematic Analysis of 277 Health Surveys. PLoS Med 2012;9:100-1356.
9. Jejeebhoy SJ. Infertility in India - levels, patterns and consequences: Priorities for social science research. Journal of Family Welfare 1998;44:15-24.
10. Benjamin N, Peter N, Rakesh K. Varicocele is associated with elevated spermatozoal reactive oxygen species production and diminished seminal plasma antioxidant capacity. Urology 1999;161:1831-1834.
11. Bedaiwy MA *et al.* "Differential growth of human embryos in vitro: role of reactive oxygen species", Fertil Steril 2004;82:593-600.
12. Agarwal A, Gupta S, Sharma R. Role of oxidative stress in female reproduction. Reprod Biol Endocrinol 2005;3.
13. Gavella M, Lipovac V, Vucic M, Rocic B. Superoxide anion scavenging capacity of human seminal plasma. Int J Androl 1996;19:82-90.
14. Luck MR, Jeyaseelan I, Scholes RA. Ascorbic acid and fertility. Biol Reprod 1995;52:262-6.
15. Paszkowski T, Clarke RN. The graafian follicle is a site of L-ascorbate accumulation. J Assist Reprod Genet 1999;16:41-5.
16. Veek L. Atlas of the human oocyte and early conception. Baltimore: Williams & Wilkins 1986.
17. Behrman HR, Kodaman PH, Preston SL, Gao S. Oxidative stress and the ovary. J Soc Gynecol Investing 2001;8:S40-S42.
18. Murphy AA, Santanam N, Morales AJ, Parthasarathy S. Lysophosphatidyl choline, a chemotactic factor for monocytes/T-lymphocytes is elevated in endometriosis. J Clin Endocrinol Metab 1998;83:2110-3.
19. Sane AS, Chokshi SA, Mishra VV, Barad DP, Shah VC, Nagpal S. Serum lipoperoxides in induced and spontaneous abortions. Gynecol Obstet Invest 1991;31(3):172-5.
20. Simsek M, Naziroglu M, Simsek H, Cay M, Aksakal M, Kumru S. Blood plasma levels of lipoperoxides, glutathione peroxidase, beta carotene, vitamin A and E in women with habitual abortion. Cell Biochem Funct 1998;16:227-31.
21. Kuscu NK, Var A. Oxidative stress but not endothelial dysfunction exists in non-obese, young group of patients with polycystic ovary syndrome. Acta Obstet Gynecol Scand 2009;88:612-7.
22. Rodgers RJ, Lavranos TC, Rodgers HF, Young FM, Vella CA. The physiology of the ovary: maturation of ovarian granulosa cells and a novel role for antioxidants in the corpus luteum. J Steroid Biochem Mol Biol 1995;53:241-246.
23. Young S, Woodside JV. "Antioxidants in Health and Disease". Journal of Clinical Pathology 2001;54(3):176-186.
24. Veena BS, Sharmila U, Satish KA *et al.* Evaluation of oxidative stress, antioxidants and prolactin in infertile women. Indian Journal of Clinical Biochemistry 2008, 23186-190.
25. Savita SM, Anitha SKB, Chaya SD. Oxidative stress mediated essential polyunsaturated fatty acid alterations in female infertility. Human Fertility 2009;12(1):28-33.
26. Lucky HS, Sajal G, Yesul K. Ashok Agarwal Center for Reproductive Medicine, Glickman Urological & Kidney Institute and Ob/Gyn & Women's Health Institute, Cleveland Clinic 9500 Euclid Avenue, Desk A19.1, Cleveland USA 2009;OH:44195.
27. Zhang D, Luo WY, Liao H, Wang CF, Sun Y. The effects of oxidative stress to PCOS. Sichuan Da Xue Xue Bao Yi Xue Ban 2008;39(3):421-3.
28. Surapaneni KM, Vishnu PV. Lipid peroxidation, glutathione, ascorbic acid, vitamin E antioxidant enzyme and serum homocysteine status in patients with polycystic ovary syndrome, biology and medicine 2009;1(3):44-49.

29. Joo LL, Chin-Kun B, Sajal G, Nabil A, Ashok A. Role of Oxidative stress in Polycystic Ovary Syndrome, current Women's Health Review 2010;6:96-107.
30. Sasikumar S, Shyam SJ, Madhankumar EK, Amburu P, Kalaiselvi1 S, Gopinath PM, Dakshayani D, Krithika JD, Chitra S, Uma R. A study on significant biochemical changes in the serum of infertile women. 1Nova IVI Fertility Centre, 41/42, MRC Nagar, RA Puram, Chennai-28, India 2Jaya College of Arts and Science, Tamilnadu, India 2011, 97.
31. Westphal LM *et al.* "A nutritional supplement for improving fertility in women: a pilot study", J Reprod Med 2004;49:289-293.
32. Fenkci V, Fenkci S, Yilmazer M, Serteser M. Decreased total antioxidant status and increased oxidative stress in women with polycystic ovary syndrome may contribute to the risk of cardiovascular disease. Fertil Steril 2003;80(1):123-7.
33. Archana S, Neela A, Prathmesh K. DY Patil University, School of medicine; Study of oxidative stress and antioxidant levels in polycystic ovarian disease. International J of Healthcare and Biomedical Research 2015;03(04):16-24.
34. Naseer JH, Mukhtar1 Al, Sahib YH, Morshidy2 Al. Ban J Edan3:Vitamin E and C Status In The Sera and Cervical Mucus Secretion of Infertile Female With Unexplained Infertility. 1, 3 College of Medicine, University of Babylon, Hilla, Iraq 2 College of Medicine, University of kufa, Al Najaf, Iraq Medical Journal of Babylon 2014;11(3).
35. Makinde KA, Adedeji OO. Comparative study of vitamin E levels of Nigerian men and age-matched fertile and infertile women. J. Nutr. Med 1994;4:39-42.
36. Mehendale SS, KilariBams AS, Deshmukh CS, Dhorepatil BS, Nimbargi VN, Joshi SR. Oxidative stressmediated essential polyunsaturated fatty acid alterations in female infertility. Hum. Fertil. J 2009;12(1):28-33.
37. Savita SM, Anitha SK, Chaya SD, Bharati A. Oxidative stress-mediated essential polyunsaturated fatty acid alterations in female infertility. Human Fertility 2009;12(1):28-33. 77 Bayer R. Treatment of infertility with vitamin E. Int. J Ferti 1960;5:70-78.
38. Ruder EH, Hartman TJ, Goldman MB. Impact of oxidative stress on female fertility. Curr. Opin. Obstet. Gynecol. Jun 2009;21(3):219-22.
39. Bayer R. Treatment of infertility with vitamin E. Int. J Ferti 1960;5:70-78.