

Oxidative Stress in Primary Infertility of Women

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Abstract

The current study was designed to investigate the changes of oxidative stress (OS) in primary infertility of females. To achieve the intended aim, 84 infertile women of ages 28.66 ± 6.29 years (mean $\pm$ SD) and 30 healthy fertile women of ages 30.3 ± 6.45 years (mean $\pm$ SD) were enrolled. The levels of malondialdehyde (MDA), catalase (CAT) and glutathione-S-transferase (GST) were determined by spectrophotometric methods. Serum follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels were measured by an enzyme linked fluorescent assay (ELFA). The results indicated a significant ($p < 0.001$) increase of MDA concentration and significant ($p < 0.001$) decreases of CAT and GST activities in the infertile women when compared with those of the control group. The linear regression analysis demonstrated significant ($r = 0.27, p < 0.05$) positive correlation for MDA levels and significant negative correlations for CAT ($r = -0.24, p < 0.05$) and GST levels ($r = -0.26, p < 0.05$) with the age of infertile women. Changes of oxidative stress was observed to be dependent on the body mass index (BMI) and the duration of infertility of the enrolled women. The changes of MDA, CAT and GST levels seem to be independent on etiology of infertility and the menstruation pattern. The 2 linear regression analysis revealed significant ($r = 0.28, p < 0.05$) positive correlation for MDA levels with the FSH concentration and significant ($r = -0.29, p < 0.05$) negative correlation with the LH concentration. CAT exhibited significant ($r = 0.30, p < 0.05$) positive correlation with the FSH concentration, while GST activity demonstrated significant ($r = 0.24, p < 0.05$) positive correlation with the LH concentration. These results suggest that oxidative stress is involved in the pathophysiology of primary infertility in females, in particular through the directing of gonadotrophin changes in these patients.

Index terms—

1 Introduction

Oxidative stress (OS) is a common condition caused by biological systems in aerobic conditions such that antioxidants cannot scavenge the reactive oxygen species (ROS). This causes an excessive generation of ROS, which damages cells, tissues, and organs (1). Evidence suggests that OS induced by ROS such as superoxide anion ($O_2^{\cdot-}$), hydroxyl radicals ($OH^{\cdot}$) and a range of lipid peroxyl radicals produced in vascular cells is involved in the pathogenesis of a wide range of diseases of the reproductive system such as endometriosis and infection (2).

OS impacts fertilization and can further induce apoptosis, resulting in embryo fragmentation, implantation failure, or abortion. In the fallopian tubes, OS may induce damaging effects in an embryo. The endometrium, which facilitates embryo implantation and development, can become defective when the female reproductive tract experiences an ROS-antioxidant imbalance (3). OS may hinder the support required for the continuation of a pregnancy by causing luteal regression and insufficient luteal hormone levels (4). Several other known causes of infertility, such as endometriosis, hydrosalpinx, polycystic ovarian disease, unexplained infertility, and recurrent

pregnancy loss (RPL) may be attributed to O in the environment (5). OS induces infertility in women through a variety of mechanisms. Ovarian follicles experiencing OS can lead to direct damage to oocytes. Oocytes and spermatozoa can also experience direct damage, which can lead to impaired fertilization due to an environment of OS in the peritoneal cavity. Even when fertilization occurs, apoptosis leading to embryo fragmentation, implantation failure, abortion, or congenital abnormalities in offspring can occur. OS in the fallopian tubes can cause direct adverse effects on the embryo defects in the endometrium, which normally supports the embryo and its development, can arise when there is an ROS antioxidant imbalance in the female reproductive tract (3). ROS-antioxidant imbalance is also implicated in luteal regression and insufficient luteal hormonal support for the continuation of a pregnancy (4). OS has been implicated in many other causes of infertility, such as endometriosis, hydrosalpinx, polycystic ovarian disease, unexplained infertility, and recurrent pregnancy loss (6).

2 II. Patients And The Control Groups

A total of eighty four women with primary infertility of age's 18-41 years with a mean $\pm$ SD 28.66 $\pm$ 6.29 years attending the fertility center in the AL-Sadder Teaching Hospital in Najaf city from October 2008 to May 2009 were included in the study. To compare the results, thirty healthy age matched (mean $\pm$ SD 30.3 $\pm$ 6.45 years) females with history of at least one child birth were also enrolled. Subjects suffered from diseases (hypertension, asthma and diabetes mellitus) interfere with the data obtained were excluded.

Disposable syringes and needles were used for blood collection. Venous blood samples, about 10 ml were collected from patients and healthy volunteer on day-2 of their menstrual cycle in tubes. After allowing the blood to clot at room temperature for 15 min, blood samples were centrifuged at 3000 xg for 15 min. Sera were separated and divided into five aliquot samples stored at -17°C , two for determination of the hormonal profile, the three others were used for estimation of oxidative stress parameters.

3 III. Determination of Malondialdehyde,

Catalase and Glutathione -S -Transferase Levels

The level of malondialdehyde was determined by modified procedure described by Guidet B. and Shah S.V. (7).

Catalase (CAT) activity was determined by the measurement of the decrease in the absorbance due to hydrogen peroxide (H_2O_2) consumption as described by Aebi H. (8).

GST activity was analyzed by measuring the conjugation of glutathione (GSH) and 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate, as described by Habig WH. et al (??)

4 IV. Determination of Serum FSH And LH Concentration

VIDAS® FSH and LH are an automated quantitative test for use on the VIDAS instruments for the determination of human follicle stimulating hormone (FSH) and human luteinizing hormone (LH), in human serum or plasma using the Enzyme Linked Fluorescent Assay (ELFA) technique. (10) V.

5 Results

6 a) Level of Malondialdehyde, Catalase and

Glutathione -S-Transferase in Infertile Women and the Control Group Malondialdehyde (MDA), catalase (CAT) and glutathione-S-transferase in females with primary infertility and thirty healthy women. The results were analyzed using student's t-test. There were significant ($p < 0.001$) decreases in CAT and GST activity level in females with primary infertility when compared with those of the control group. In contrast, MDA levels were found to increase significantly ($p < 0.001$) in females with primary infertility when compared with those of the control group (Table 3.1). The results indicated significant ($p < 0.001$) elevation of MDA levels in the three groups of patients when compared with those of the control group. On the other hand, CAT and GST activities exhibited significant ($p < 0.01$ and < 0.001 respectively) decreases in the three groups of patients with respect to those of the control groups. The linear regression analysis demonstrated significant ($r = 0.27$, $p < 0.05$) positive correlation for MDA levels and significant negative correlations for CAT ($r = -0.24$, $p < 0.05$) and GST levels ($r = -0.26$, $p < 0.05$) with the age of infertile women (Table 3). To understand the effect of body mass index (BMI) on the levels of serum MDA, CAT and GST in female infertility, patients were categorized into three groups. Group 1 consisted of 26 patients who had BMI values $< 25 \text{ Kg/m}^2$ (normal females). Group 2 comprised 28 patients who had BMI $> 25\text{--}30 \text{ Kg/m}^2$ (overweight) and Group 3 comprised 30 patients who had BMI $> 30 \text{ Kg/m}^2$ (obese).

The results pointed out a significant ($p < 0.001$) increase of MDA and significant decreases of CAT ($p < 0.01$) and GST ($p < 0.001$) levels in the three groups of infertile women when compared with the control group (Table 3.4). The linear regression analysis stated significant ($r = 0.23$, $p < 0.05$) positive correlation for MDA and significant ($r = -0.26$, $p < 0.05$) negative correlation for CAT levels with BMI values in the infertile women (Table 3.5). To verify the impact of infertility causes on MDA, CAT and GST values in infertile women, patients were categorized into 3 groups. Group A consisted of 37 females of ovulatory factor, group B consisted of 14 females of tubal and uterine factors and group C consisted of 33 females of unexplained factor. As shown in table 3.6, the three groups of infertile women showed a significant ($p < 0.001$) elevation of MDA and significant decreases of CAT ($p < 0.05$)

and GST ($p < 0.001$) levels when compared with those of the control group. To demonstrate the influence of duration of infertility on MDA, CAT and GST values in infertile women, the linear regression analysis was used to evaluate the data. Significant negative correlations were obtained for CAT ($r = -0.23$, $p < 0.05$) and GST ($r = -0.27$, $p < 0.05$) levels with the duration of infertility. MDA levels stated significant ($r = 0.30$, $p < 0.01$) positive correlation with duration of infertility (Table 3.7). To perceive the impact of menstruation pattern on the levels of MDA, CAT and GST in infertile women, patients were categorized into 2 groups. Those of regular cycle were 45 patients and those of irregular cycle were 39 patients. Their data were compared with the values of the control group by using the ANOVA analysis. A significant ($p < 0.001$) increase of MDA levels and significant decreases for CAT ($p < 0.01$) and GST ($p < 0.001$) activities were observed in the two groups of patients when compared with those of control group (Table 3). To verify the relevance of FSH and LH concentrations to the MDA, CAT and GST levels in infertile women, the linear regression analysis was used to evaluate the data. The results indicated significant ($r = 0.28$, $p < 0.05$) positive correlation for MDA levels with the FSH concentrations and significant ($r = -0.29$, $p < 0.05$) negative correlation with LH concentrations. CAT showed significant ($r = 0.30$, $p < 0.05$) positive correlation with FSH concentrations. GST activity exhibited significant ($r = 0.24$, $p < 0.05$) positive correlation with LH levels in infertile women (Table 3.9). Table 3.9 : Correlations of Serum Malondialdehyde (MDA), Catalase (CAT) and Glutathione-S-Transferase (GST) with FSH and LH Levels in Infertile Women VI.

7 Discussion

Successful pregnancy results from an interaction between myriad physiological processes in both men and women. Any disruption to this interactive system, whether in a man or woman, can result in an inability to have a biological child called infertility (4). ROS exert their cytotoxic effects by causing per oxidation of membrane phospholipids, which results in an increase in membrane permeability, loss of membrane integrity, enzyme inactivation, structural damage to DNA and cell death (11). Oxidative stress can have detrimental effects on female fertility by affecting ovulation, fertilization, embryo development, and implantation (4,12). Thus, OS is considered a cause of female infertility. This is particularly clear in cases of (14).

8 Global

The results indicate that infertile women have increased serum level of MDA and decreased serum levels of GST and CAT as compared to fertile women. They are in agreement with previous reports.

Veena Bhaskar S et al have found significantly higher concentration of MDA in serum of infertile women than in fertile women (15). Savita Setal have shown significantly high plasma levels of MDA in infertile women when compared to parous women and this is noticed from there ductions of levels of eicosapentaenoic acid (EPA), and more so indocosaheaxaenoic acid (DHA), they suggested that these changes are consequence of increased oxidative stress that mediate lipid per oxidation Product , i.e. MDA (16).

In the present study, MDA levels were found to be elevated with advancing age in infertile women suggesting raised lipid per oxidation in these patients. The rise seems to be developed as a consequence of declined production of antioxidant enzymes. Such decline was apparent for the activities of CAT and GST in association with the elevation of MDA levels as ages of the patients were advanced. Thus, aging could be considered as a risk factor for elevation of oxidative stress, and impaired fertility in aged females involves the imbalance in there do x potentials of these patients.

The present results were in a agreement with previous works in which follicular fluid aspirates from twelve young women aged 27-32 years and twelve older women aged 39-45 years undergoing IVF treatment were analysed for the activity and protein expression of catalase, SOD, G-PX, GST and G-Red. The specific activity of catalase was 60% lower in the older women when compared with the younger women. GST was also lower in the older women with respect to the younger patients (17). It is well known that germ cell membranes are particularly vulnerable to be attacked by ROS, being very rich in polyunsaturated fatty acids (18).

The current results illustrated elevated lipid peroxidation in association with depleted cytoprotective enzyme activity, i.e., CAT and GST, as weight of infertile women was raised. The reason of the dysregulation of the redox system may due to increased levels of adipose NADPH oxidase activity which raise the production of ROS in accumulated fat (19). This hypothesis was proved by experimental obeserats, in which rised lipid peroxidation has been observed (20).

The link between obesity and oxidative stress has been suggested in some studies. A good correlation between BMI and oxidative stress has been reported, indicating obesity as an independent risk factor for plasma lipid peroxidation (21,22). Obesity may induce systemic oxidative stress, which is, in turn, the underlying cause of selective increase in ROS, 16 dysregulation of adipocytokines and development of metabolic syndrome (23).

In the present investigation, 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 there productive systems in infertile patients. These evidences suggest that oxidative stress is an independent etiologic factor in female infertility. Such independency may relate to the activation of macrophages which are a source of generation of ROS (24).

In the present study, raised lipid peroxidation and decreased antioxidant enzyme activities are evident as the

duration of infertility was prolonged in the enrolled infertile women. The results suggest that prolonging of the duration of infertility exaggerate the implication of oxidative stress in the impairment of female infertility.

OS is involved in the modulation of cyclical changes in the endometrium. Altered SOD and ROS levels have been demonstrated in the endometrium during the late-secretory phase, just before menstruation. An elevated lipid peroxide concentration and decreased SOD concentrations have been reported in human endometrium in the latesecretory phase, and these changes may be responsible for the breakdown of the endometrium, implicating the involvement of OS in the process of menstruation (25). The expression of endothelial nitric oxide synthase (NOS) and inducible NOS have been demonstrated in the human endometrium and the endometrial vessels (26,27). Endothelial NOS is also thought to bring about changes that prepare the endometrium for implantation (25).

FSH was found to be positively correlated with MDA and CAT levels, suggesting oxidative effect in the reproductive system of the infertile women. In contrast, LH was ascertained to be correlated negatively with MDA level and positively with GST activity, suggesting antioxidative, i.e. protective role in the reproductive system of these patients. The oxidative effect of FSH may be induced through the action of progesterone, since this hormone has been documented to elicit oxidative stress in rats (28). Unfortunately progesterone and estradiol concentrations could not be measured in the studied patients due to technical limitations. The protective role of LH may be produced through the action of estradiol, the beneficial function of estradiol has been elucidated in rats (28).

Elevated endogenous LH concentration seems to be a powerful protective enzyme against oxidative stress, since it is correlated negatively with MDA level and positively with GST activity. These observations are essentially related to vitamin E. It was demonstrated that LH administration is associated with accumulation of ovarian vitamin E (29). The mechanism of LH stimulation of vitamin E accumulation is not clear, but may be due to increased lipoprotein accumulation by the corpus luteum. Vitamin E is transported by lipoproteins in plasma (30). LH is known to stimulate the accumulation of lipoproteins by the rat corpus luteum (31, 32). Hence, the accumulation of lipoproteins, may be the reason of elevated vitamin E and consequently the antioxidative function of LH in the reproductive tract.



Figure 1: Table 3 . 6 :

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1 : Levels of Malondialdehyde (MDA), Catalase (CAT) and Glutathione -S -
Transferase
(GST) in Infertile Women and Control Group

Figure 2: Table 3 .

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*[Note: : Levels of Malondialdehyde (MDA) , Catalase (CAT) and Glutathione -S-Transferase (GST) in Various
age Related Groups of Infertile Women Table 3.3 : Correlation Factors of Serum Malondialdehyde (MDA),
Catalase(CAT) and Glutathione-S-Transferase (GST) Levels with Age in Infertile Women c) Influence of Body
Mass Index on Malondialdehyde, Catalase and Glutathione -S Transferase Levels in Infertile Women]*

Figure 3: Table 3 . 2

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[Note: H]

Figure 4: Table 3 .

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5 : Correlation Factors of Serum Malondialdehyde (MDA) , Catalase (CAT) and Glutathione-S-Transferase (GST) Levels with Body Mass Index (BMI) in Infertile women
d) Relevance of Etiology of Infertility
Malondialdehyde, Catalase and Glutathione -S
Transferase Levels in Infertile Women

Figure 5: Table 3 .

Levels of Malondialdehyde(MDA), Catalase (CAT Table) and Glutathione -S-Transferase (GST) in Infertile with Regular and Irregular Cycle

Parameters	r	P-value
MDA	0.23	<0.05
CAT	-0.26	<0.05
GST	-0.12	NS

Parameters Group NO. Mean $\pm$ SD

MDA (μ M)	Contr	30	1.74 $\pm$ 0.75
	OF	37	4.23 $\pm$ 1.16
	TUF	14	4.20 $\pm$ 1.41

CAT (U/ml) g) Correlations of FSH and LH Concentrations with UF Control OF 33 30 37 3.93 $\pm$ 1.28 6.82

GST (U/L)	TUF	14	4.41 $\pm$ 2.09
	UF	33	4.43 $\pm$ 2.14
	Contr	30	1628.42 $\pm$ 284.86
	OF	37	751.53 $\pm$ 191.96
	TUF	14	678.59 $\pm$ 215.41
	UF	33	737.41 $\pm$ 201.30

O F: Ovulatory Factor

TUF: Tubal and Uterine Factor

U F: Unexplained Factor

Parameters	r	P-value
MDA	0.30	<0.01
CAT	-0.23	<0.05
GST	-0.27	<0.05

Figure 6:

endometriosis (13). It is suggested that OS is caused by ROS overproduction rather than antioxidant depletion

Parameters	Group	NO.	Mean $\pm$ SD	Range	P-value
MDA (μ M)	Control	30	1.74 $\pm$ 0.75	0.54 -3.10	
	RC	45	4.29 $\pm$ 1.31	2.29 -8.08	<0.001
	IRC	39	3.93 $\pm$ 1.14	2.42 -6.99	<0.001
CAT (U/ml)	Control	30	6.82 $\pm$ 4.72	1.74 -22.42	
	RC	45	4.32 $\pm$ 2.12	0.78 -8.59	<0.01
	IRC	39	4.73 $\pm$ 2.24	1.26 -9.68	<0.01
GST (U/L)	Control	30	1628.42 $\pm$ 284.86	1104.69 -2335.63	
	RC	45	740.67 $\pm$ 177.99	347.19 -1073.13	<0.001
	IRC	39	713.80 $\pm$ 207.93	284.06 -1136.25	<0.001

RC: Regular Cycle
IRC: Irregular Cycle

Parameters		r	H p-value
MDA	FSH	0.28	<0.05
	LH	-0.29	<0.05
	FSH	0.30	<0.01
CAT	LH	0.02	NS
	FSH	-0.07	NS
GST	LH	0.24	<0.05

Figure 7:

184 [Pasqualotto] , F Pasqualotto .
 185 [Sharma] , R Sharma .
 186 [Benjamin] , N Benjamin .
 187 [Peter] , N Peter .
 188 [Iborra] , A Iborra .
 189 [Agarwal] , A Agarwal .
 190 [Agarwal and Said] , A Agarwal , T M Said .
 191 [Habig] , W Habig .
 192 [Savita and Mehendalei] , S Savita , Mehendalei .
 193 [Anitha and Bams] , S Kilari Anitha , Bams .
 194 [Carbone and C; Tatone] , M Carbone , C C; Tatone .
 195 [Lenzi and Gandini] , A Lenzi , L Gandini .
 196 [Gower] , B A Gower .
 197 [Trevisan] , M Trevisan .
 198 [Browne] , R Browne .
 199 [Furukawa and Fujita] , S Furukawa , T Fujita .
 200 [Szczepanska and Kozlik] , M Szczepanska , J Kozlik .
 201 [Sugino] , N Sugino .
 202 [Takiguchi] , S Takiguchi .
 203 [Rosselli] , M Rosselli .
 204 [Ota] , H Ota .
 205 [Igarashi] , S Igarashi .
 206 [Raymond] , F Raymond .
 207 [Strauss] , J F Strauss .
 208 [Rajkumar] , K Rajkumar .
 209 [Aebi ()] H Aebi . *Methods of enzymatic analysis*, (New York) 1974. 2 p. 674.
 210 [Monache ()] 'Antioxidant enzymatic defences in human follicular fluid: characterization and age-dependent
 211 changes'. Delle Monache , S . *Molecular Human Reproductive* 2003. 9 p. .
 212 [Aten et al. ()] 'Behrman Regulation of Ovarian Antioxidant Vitamins, Reduced Glutathione, and Lipid Perox-
 213 idation by Luteinizing Hormone and Prostaglandin F2?'. Krishna M Aten , ; Duarte , R Harold . *Biology Of*
 214 *Reproduction* 1992. 46 p. .
 215 [Couture and Murphy ()] 'Binding of highdensitylipoproteins to luteal membranes: the role of prolactin.
 216 Luteinizing hormone and circulating lipoproteins'. R L Couture , B D Murphy . *Biology of Reproduction*
 217 1985. 32 p. .
 218 [Ram ()] 'Correlates of markers of oxidative status in the general population'. M Ram . *American Journal of*
 219 *Epidemiology* 2001. 154 p. .
 220 [Dobrian et al. ()] 'Development of hypertension in a rat model of diet induced obesity'. A D Dobrian , M J
 221 Davies , R L Prewitt . *Hypertension* 2000. 35 p. .
 222 [Kumtepe; Bunyamin Borekci and Mehmet Karaca ()] 'Effect of acute and chronic administration of proges-
 223 terone, estrogen, FSH and LH on oxidant and antioxidant parameters in ratgastric tissue'. Yakup Kumtepe;
 224 Bunyamin Borekci , ; Mehmet Karaca , ; . *Chemico-Biological Interactions* 2009. 182 p. .
 225 [Hatazawa ()] 'Endothelial nitric oxidsynthase in the endometrium during thEmenstrual cycle in patientswith
 226 endometriosis and adenomyosis'. J Hatazawa . *Fertility and Sterility* 1998. 69 (2) p. .
 227 [Guidet and Sv ()] 'Enhanced in vivo H2O2 generation by ratkidney in glycerol-induced renal failure'. B Guidet
 228 , Shah Sv . *American journal of physiology* 1989. 1257 p. .
 229 [Bhaskar and Upadhy; Satish Kumar Adiga ()] 'Evaluation of oxidative stress, antioxidants and prolactin in
 230 infertile women'. Veena Bhaskar , S; Sharmila Upadhy; Satish Kumar Adiga , ; . *Indian Journal of Clinical*
 231 *Biochemistry* 2008. p. .
 232 [Nagy and R; Goran ()] 'Fat distribution and plasma lipid-lipoprotein concentrations in pre-and postmenopausal
 233 women'. T Nagy , M R; Goran . *International Journal of Obesity and Related Metabolic Disorders*1998. 22 p.
 234 .

- [Halliwell B And Gutteridge ()] *Free radicals in biology and medicine*, J M Halliwell B And Gutteridge . 2007. Oxford, UK: Clarendon Press. (4th ed.)
- [Shimabukuro ()] 'Increased oxidativestress in obesity and its impact on metabolic syndrome'. M Shimabukuro . *Journal of Clinical Investigation* 2004. 14 p. .
- [Manual of FSH determination by VIDAS FSH Kit , Biomerieux-France, REF 30407.Halliwell B. Free radicals, antioxidants and human disease: curiosity, cause or consequence?'. *Lancet* 1994. 54 p. .
- [Olusi ()] 'Obesity is an independent risk factor for plasma lipid peroxidation and depletion of erythrocyte cytoprotective enzymes in humans'. S O Olusi . *International Journal of Obesity and Related Metabolic Disorders* 2002. 26 p. .
- [Agarwal and Allamaneni ()] 'Oxidants and antioxidants in human fertility'. A Agarwal , S Allamaneni . *Middle East Fertility Journal* 2004. 9 p. .
- [Palacio and Martinez ()] 'Oxidative stress and autoimmune response in the infertile woman'. J Palacio , P Martinez . *Chemical immunology and allergy* 2005. 88 p. .
- [Agarwal et al. ()] 'Oxidative stress and its implications in female infertility -a clinicians perspective'. A Agarwal , S Gupta , R Sharma . *Reproductive Biomedicine Online* 2005. 11 (5) p. .
- [Bedaiwy ()] 'Oxidative stress in an assisted reproductive techniques setting'. M A Bedaiwy . *Fertility and Sterility* 2006. 86 (3) p. .
- [Skrzypczak ()] 'Oxidative stress maybe a piece in the endometriosis puzzle'. J Skrzypczak . *Fertility and Sterility* 2003. 79 p. .
- [Deshmukal ()] 'Oxidative stress-mediated essential polyunsaturated fatty acid alterations in female infertility'. Chaya S Deshmukal . *Human Fertility* 2009. 12 (1) p. .
- [Kobayashi ()] 'Oxidativestress in normospermic men undergoing infertility evaluation'. H Kobayashi . *Andrology* 2001. 22 p. .
- [Bedaiwy and Falcone ()] 'Peritoneal fluid environment in endometriosis: Clinico pathological implications'. M Bedaiwy , T Falcone . *Minerva Ginecologica* 2003. 55 p. .
- [Lombardo (200265)] 'Polyunsaturated fatty acids of germ cell membranes, glutathione and blutathione-dependent enzyme-PHGPx: from basic to clinic'. F Lombardo . *Contraception* 200265. p. .
- [Agarwal A ()] 'Role of free radicals in female reproductive diseases and assisted reproduction'. Agarwal A , Allamaneni S . *Reproductive Biomedicine Online* 2004. 9 p. .
- [Keller and Dubey ()] 'Role of nitric oxide in the biology, physiology and pathophysiology of reproduction'. P J Keller , R K Dubey . *Human Reproduction Update* 1998. 4 (1) p. .
- [Gupta ()] 'Sikka The role of free radicals and antioxidants in reproduction'. S Gupta . *Current Opinion in Obstetric and Gynecology* 2006. 18 p. .
- [Kashida ()] 'Superoxide dismutase expression in the human corpus luteum during the menstrual cycle and in early pregnancy'. S Kashida . *Molecular Human Reproduction* 2000. 6 (1) p. .
- [Pabst and Glutathione S-Transferase ()] 'The First Enzymatic Step in Mercapturic Acid Formation'. M; Pabst , Jakoby W Glutathione S-Transferase . *Biological Chemistry* 1974. 22 (25) p. .
- [Macgregor ()] 'Uptake of high density lipoproteins by rat ovaries in vivo and dispersed ovarian cells in vitro. Direct correlation of high density lipoprotein uptake with steroidogenic activity'. C Macgregor , Gwvnnne Jr . *Journal of Steroid Biochemistry* 1982. 16 p. .
- [Rakesh ()] 'Varicocele is associated with elevated spermatozoal reactive oxygen species production and diminished seminal plasma antioxidant capacity'. K Rakesh . *Urology* 1999. 161 p. .