1 ***Original Research Article***

2 # EVALUATION OF OXIDATIVE STRESS MARKERS AND HORMONAL PROFILES
3 # IN WOMEN DIAGNOSED WITH INFERTILITY IN PORT HARCOURT

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6 ## ABSTRACT

7 **Aim**: The study evaluates the contribution of oxidative stress and fertility hormones to female infertility in Port
8 Harcourt.

9 **Methodology**: A total of 140 women aged 15 – 49 years consisting 70 apparently healthy infertile women attending
10 diagnostic fertility clinics in Port Harcourt as test subjects and 70 age-matched healthy fertile women as control
11 were recruited. Subjects were recruited using structured questionnaires after given their informed consent. The
12 levels of Malondialdehyde, total antioxidant capacity, lipid peroxidation index, follicle stimulating hormone,
13 luteinizing hormone, prolactin, progesterone and estrogen of infertile and the fertile (control) subjects were
14 determined by standard procedures.

15 **Result**: there was statistically significant increase in lipid peroxidation index in the test subjects than in the fertile
16 group ($p<0.05$). Total antioxidant capacity showed a statistically reduced value in the test subjects than in the
17 control at $p<0.05$. Lipid peroxidation index was significantly increased in test subjects exposed to oxidant agents
18 like alcohol, infections and ulcer than their counterparts who were not exposed to any of the agents ($p<0.05$). Also
19 significantly elevated lipid peroxidation was observed in test subjects with normal hormone levels compared to
20 those with hormone imbalance ($p<0.05$).

21 **Conclusion**: The outcome of this study suggests that the infertility being experienced by some of the infertile
22 women in Port Harcourt are due not only to endocrine dysfunction, but some order conditions that induce oxidative
23 stress. Thus investigation of oxidative parameters is highly suggested as an adjunct for effective management of
24 unexplained infertility in women. Further studies on estimation of antioxidant enzymes including superoxide
25 dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GHR) are recommended to validate
26 reliability.

27 Keywords: Infertility, fertility, oxidative stress, hormones, peroxidation index, intercourse

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29 ## INTRODUCTION

30 Infertility is the incapability to attain gestation within after one year of unprotected, non-
31 contraceptive regular sexual intercourse. Infertility could be primary (when couples have never
32 conceived in their lifetime) or secondary (when another child could not be achieved after a year
33 when one or both partners have previously had a child or children [1]. Over time, infertility has
34 been on steady increase in Nigeria compared with what was observed in the past [2]. It has been

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35 reported that about 8-12 out of every 100 couples in diverse nationalities are affected by
36 infertility [3-5]. According to the report of Giwa-Osagie [6], there are over twelve million
37 infertile people in Nigeria. In African states, subfertility is projected at 10-25%, the female
38 factors are responsible for the greater percentage of the causes (55%) while the male factors are
39 responsible for 30–40% of causes. Idiopathic infertility accounts for 5–15% [6]. The burden of
40 infertility in our environs is so high that almost half of women seeking consultation with
41 gynaecologists complain of inability to get pregnant [7].

42 Oxidative stress is the term generally used to describe a state of imbalance between pro-
43 oxidant (free radicals) and antioxidants [8]. The free radicals (reactive oxygen species (ROS) and
44 reactive nitrogen species (RNS)) are products of cellular metabolism constantly taking place in
45 the body. They are needed in a certain quantity for normal cell functions [9]. The body usually
46 responds to excess amount of free radicals produced through an organized system known as
47 antioxidant defense system. This system helps the living organisms to combat the radicals and
48 reduce their toxic effects on cells and tissues. Antioxidants are the many substances that
49 markedly slow down or hinder oxidation of substances. They are usually smaller relative to the
50 amount of those oxidizable substrates like DNA, proteins, lipids, and, carbohydrates [10]. The
51 major work antioxidant defense does is to shield the cells and tissues from the damaging effects
52 of reactive species. The reactive species are either produced in living organisms through
53 processes involving inflammation of cell and tissues, disease conditions or normal metabolism
54 (interior sources). Otherwise they are produced from sources like irradiation, food, drugs etc.
55 (exterior sources). In any case, an increased generation of free radicals may instigate oxidative
56 damage [10].

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57 Moreover, alteration in rate at which reactive species are generated as well as the
58 effectiveness of the antioxidant defence mechanisms in living cells may result to oxidative stress
59 (OS), giving rise to development of some pathological conditions. When there is increase in
60 production of ROS/RNS or there is a reduced antioxidant status (or both), the natural antioxidant
61 defence mechanisms of the body may be overpowered, thereby creating an unfavourable
62 environment for the normal functioning of the various systems of the body including
63 reproductive system in the females. This could lead to development of some reproductive disease
64 conditions including endometrioses, polycystic ovary syndromes (PCOS) and unexplained
65 infertility. Also associated with this state of oxidative disturbance are pregnancy complications
66 including preeclampsia, abortion, intrauterine growth restriction (IUGR) and repeated pregnancy
67 loss [8, 11-13]. The degrading effect of oxidative stress (OS) on quality of ova has been
68 previously described in mouse. Hence, fertilization as well as gestation rates in humans are
69 adversely affected by OS [14]. Sterility could suffer as a result of reduced antioxidant status in
70 the human body. Thus antioxidant therapy or consumption of antioxidant-containing food can be
71 of great help in management or even prevention of sterility [15].

72 Several current studies have linked excessive free radical productions with some
73 controllable lifestyle factors like alcohol consumption, smoking of cigarette, use of some
74 recreational drugs and exposure to irradiations [16]. The substances have ability to generate high
75 volume of reactive species. Exposure to some occupational and environmental factors such as
76 heavy metals like lead can also promote ROS/RNS generation. Hence women exposed to these
77 factors may possibly experience disturbed reproductive system, resulting in infertility.

78 The peroxidative action of oxidants on polyunsaturated fatty acids (PUFAs) leads to the
79 production of malondialdehyde (MDA) alongside many other secondary products. Because

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80 MDA is relatively stable it is often used as a marker of OS. The gamete as well as the genital
81 tracts is rich in enzymatic antioxidants (superoxide dismutase (SOD), glutathione peroxidase,
82 glutathione reductase, and catalase) as well as non-enzymatic antioxidants (glutathione, vitamins
83 E, and C and uric acid) [17].

84 It will be an almost impossible task to measure one by one all the antioxidants present in
85 a living organism. Hence the more convenient way of accessing the antioxidant status of an
86 individual is to determine the total antioxidant capacity (TAC). The amount of the overall
87 activities of non-enzymatic antioxidants taking place in an organism is referred to as total
88 antioxidant capacity [18]. Lipid peroxidation index (LPI) being MDA: TAC ratio can be a useful
89 indicator of oxidative stress and may be used to monitor antioxidant therapy [19].

90 Although subfertility is a major challenge confronting couples in Nigeria, there is dearth
91 of reports on the role and implication of oxidative stress in the etiology of infertility in Nigeria.
92 This study is the first recorded report involving the use of oxidative stress markers in the
93 investigation of infertility in infertile women in Port Harcourt. This study was, therefore, aimed
94 at evaluating the impact of oxidative stress markers and hormonal profiles in women diagnosed
95 with infertility in Port Harcourt, Nigeria.

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98 **MATERIALS AND METHODS**

99 **Study Area**

100 This work was done in Port Harcourt, Rivers State of Nigeria

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101 **Subjects' selection**: A total of 70 infertile female subjects, under reproductive ages (15 – 49
102 years), who willingly consented to participate in the study were randomly selected among
103 patients attending diagnostic centers and fertility clinics in Port Harcourt including Rivers State
104 University Teaching Hospital (RSUTH) and Image Diagnostic Center, Port Harcourt. Ethical
105 approval for the study was obtained from the Rivers State Ministry of Health, Port Harcourt. A
106 forced-choice (closed ended) questionnaire was used to collect relevant information required for
107 inclusion or exclusion of subjects. The well-structured questionnaires were given to each
108 participant and they were guided by a trained laboratory staff to fill the forms. Also a total of
109 seventy (70) healthy and fertile female subjects, who were within the reproductive ages of 15 –
110 49 years were recruited as controls using the questionnaire.

111 **Study Design:** This research is designed as a case controlled, and the sampling technique used
112 was random and convenience sampling techniques [20]. The sample size was obtained by using
113 the formula for calculation of sample size in a case-control design as described by Jaykaran &
114 Tamoghna [21].

115 **Inclusion Criteria:**

116 a) **Case group:** Women included in this group were those :

117 i. Married for at least 12 months, and have been having regular, unprotected sexual
118 intercourse for at least 12 months without achieving conception.

119 ii. within the ages 15-49 years [22].

120 iii. Not under any contraceptive use for at least one year.

121 iv. Whose male partners has been investigated for fertility and found fertile with normal
122 seminal fluid parameters.

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123 b) **Control group**: those included in this group were:

124 i. Fertile women having at least a child in the past one year and are not under any

125 contraceptive drug.

126 ii. Those within the fertility ages of 15-49 years.

127 **Criteria for Exclusion as Controls:**

128 Women under any of the following conditions were excluded from the study:

129 i. Those who have suffered from serious illness or hospitalized in the past 3months.

130 ii. Chronic illnesses like cancer, hypertension, asthma and diabetes mellitus which could

131 interfere with result obtained.

132 iii. Those with history of recurrent/untreated genital tract infections within 1 year

133 iv. Those with history of ulcer for the past one year

134 v. Persons under drugs for infertility

135 vi. All regular alcohol consumers and cigarette smokers were excluded.

136 **Blood sample collection**: The blood samples were collected on the day 21 of menstrual cycle of

137 the subjects by venepuncture, dispensed into plain bottles and centrifuged after clotting using

138 bench centrifuge. The serum separated and frozen at -20 ^{0}C till assay

139 **Determination of Serum Fertility Hormone Concentrations**

140 Human follicle stimulating hormone (FSH), luteinizing hormone (LH) and prolactin (PRL) levels

141 were determined using Solid Phase enzyme-linked immunosorbent assay (ELISA) method of

142 Engvall & Perlmann [23]. Estrogen as well as progesterone was determined using competitive

143 binding Enzyme immunoassay (EIA) method of Van-Weemen and Schuurs [24]. No special

144 pretreatment was necessary for this assay as all grossly hemolyzed, lipaemic, or turbid samples

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145 were excluded in the assay. It was also ensured that no sample containing sodium azide was
146 used.

147 **Determination of MDA Concentration**

148 Thiobabituric acid reactive substance (TBARS) colorimetric assay technique of Bernheim *et al*.
149 [25] was used. This assay is based on the reaction of a chromogenic reagent, 2-thiobarbituric
150 acid, with MDA at 25^0C. One molecule of MDA reacts with two molecule of 2-thiobarbituric
151 acid via a knoevenagel-type condensation to yield a chromophore with absorbance maximum at
152 532nm. The intensity of the stable pink color formed is proportional to the amount of MDA
153 present in the sample.

154 **Determination of TAC Concentration**

155 Serum total antioxidant capacity (TAC) levels were determined spectrophotometrically using
156 CUPRAC-BCS assay method of Campos *et al.* [26]. This assay evaluates the capacity of the
157 antioxidants of a sample to reduce the Cu^{2+} to Cu^{+} in the presence of a chelating agent. These
158 chelators form colored stable complexes with Cu^{+} that have a maximum absorption at 450 – 490
159 nm. The CUPRAC assay measures the thiol-group antioxidants and other plasma antioxidants
160 such as ascorbic acid, α-tocopherol, β-carotene, uric acid, albumin, and bilirubin. The reduction
161 potential of antioxidants in the sample/standard effectively reduces Cu^{+2} to Cu^{+}, thus changing
162 the ion's absorption characteristics. This reduced form of copper will selectively form a stable
163 2:1 complex with the chromogenic agent (the Chelator- bathocuproinedisulfonic-acid disodium
164 salt (BCS)) with absorption maximum at 450 nm. A known concentration of trolox is used to
165 create a calibration curve, from which the TAC concentration in samples is extrapolated. The
166 concentrations are expressed as mM/L Trolox equivalent.

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167 Lipid peroxidation index (LPI) was calculated as the ratio of MDA to TAC.

168 **RESULTS**

169 MDA, TAC and LPI were measured in a total of 70 infertile women (case) and 70 fertile women
170 (control). The frequency and percentage distribution of the observed clinical characteristic of the
171 studied population (case group) is shown in table 1. Out of the seventy (70) infertile women
172 recruited, 13 (18.6%) were between 20-29 years, 41 (58.6%) were between 30 -39 years, while
173 16 (22.8%) were within 40-49 years. 16 (22.9%) of the subjects were affected by primary
174 infertility, while 54 (77.1%) were affected by secondary infertility. Also, 53 (75.7%) of the
175 women have suffered childlessness for less than five years, while 17 (24.3%) of the women have
176 stayed childless for at least 5 years but not more than ten (10) years. Similarly, a total of 16
177 (22.9%) subjects had pelvic inflammatory diseases as a result of urinary/genital tract infections,
178 5 (7.1) were alcohol drinkers, 10 (14.3) had ulcer, 3 (4.3) had infections and also drink alcohol,
179 while 36 (51.4) were not exposed to any of the aforementioned oxidant agents.

180 **Table 1: Demographic Characteristics of the Case Subjects**

Characteristics	**Group**	**Percentage (%)**	**Total**
Ages (years)	20 – 29 (13)	18.6	100
	30 - 39 (41)	58.6	
	40 – 49 (16)	22.8	
Types of Infertility	Primary (16)	22.9	100
	Secondary (54)	77.1	100
Duration of infertility	1 – 5 (53)	75.7	

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(Years)	6 – 10 (17)	24.3	100
Hormonal factor	Normal (23)	32.9	100
	Ovarian insufficiency (8)	11.4	
	Hyperprolactinaemia (35)	50.0	
	Hypogonadotrophic hypogonadism (4)	5.7	
Exposure to oxidants agents	Not exposed (36)	51.4	100
	Exposed to infection (16)	22.9	
	Alcohol (5)	7.1	
	Ulcer (H. Pylori) (10)	14.3	
	Infection and alcohol (3)	4.3	

Hormonal Characteristics of Case and Control

Table 2 presents the mean ± SEM of fertility hormones (LH, FSH, prolactin, progesterone and estradiol in the studied population. The mean ± SEM of FSH, LH, and Prolactin were found to be higher in the infertile women with values of 10.72 ±2.32 mIU/mL, 12.62 ± 2.09 mIU/mL and 30.3 ± 3.04 ng/ml respectively than in the control group who are fertile women with values: 6.30 ± 0.28 mIU/ml, 9.32 ± 1.53 mIU/mL and 21.87 ± 4.13 ng/mL respectively. However, the increased values were not statistically significant (p>0.05). Estradiol and progesterone levels were lower in the case group of 38.02 ± 3.87pg/mL and 3.50 ± 0.39ng/ml respectively than in the

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control group with values of 75.59 ± 2.73pg/mL and 7.37 ± 0.70 ng/mL respectively. These differences were statistically significant ($p<0.05$).

Table 2: **Hormonal Characteristics of Case and Control Groups ((Mean ± SEM)**

Parameters	Controls N= 70	Tests N= 70	t-value	P-value	Remarks
Age (years)	34.01 ± 0.72	35.79 ± 0.66	0	>0.9999	NS
FSH (mIU/ml)	6.30 ± 0.28	10.72 ± 2.32	1.892	0.0606	NS
LH (mIU/ml)	9.32 ± 1.53	12.62 ± 2.09	1.272	0.2057	NS
Estradiol (pg/ml)	75.59 ± 2.73	38.02 ± 3.87	7.905	<0.0001***	S
Progesterone (ng/ml)	7.37 ± 0.70	3.50 ± 0.39	4.847	<0.0001***	S
Prolactin (ng/ml)	21.87 ± 4.13	30.3 ± 3.04	1.642	0.0116*	S

Key: FSH-follicle stimulating hormone, LH-leutinizing hormone, NS – not significant, S – statistically significant, * p<0.05, *** p<0.0001

Levels of Fertility Hormones and Oxidative Parameters in the Test and Control Subjects According to Age Group.

Table 3 presents the mean concentrations (mean ± SEM) of hormonal and oxidative parameters according to age groups. The infertile subjects were classified into three age groups (20 – 29 years, 30 -39 years and 40 -49 years) respectively. The mean values of LH and FSH were highest in the 40 – 49 years category. The mean ±SEM of LH and FSH for the 40 – 49 years age group were 22.3 ± 6.31mIU/ml and 26.09 ± 8.42mIU/ml respectively while for the 30 -39 years age group the LH and FSH value were 8.46 ± 1.77mIU/ml for LH and 6.66± 1.53mIU/ml for FSH respectively. The mean values of LH and FSH for the 20 – 29 years were 13.83 ± 5.21mIU/ml

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205 for LH and 4.61± 1.00mIU/ml for FSH. There were statistically significant difference between
206 the means of the LH and FSH among the three age categories ($p<0.05$). Prolactin level was
207 highest among the 20 -29 years age group (43.15± 12.66ng/ml) and lowest among the 40 -49
208 years age group (25.29 ± 3.94ng/ml) but the difference was not significant p=0.1211.
209 Progesterone and eostrogen levels were lowest among the 40 -49 years group (2.41 ± 0.49ng/ml
210 (progesterone) and 29.36 ± 5.88pg/ml for estrogen and the differences in means were not
211 statistically significant ($p>0.05$) among the three age groups. The level of oxidative peroxidation
212 was highest among the 30 – 39 years age group (LPI_{30-39} = 49.10 ± 13.96, LPI_{20-29} =32.39 ± 8.90,
213 LPI_{40-49}= 26.61 ± 6.98) respectively. However, no significant difference ($p>0.05$) was found in
214 the level of oxidative peroxidation index among the three groups.

215

216 **Table 3: The Mean ±SEM of Fertility Hormones and Oxidative Stress Markers in the**
217 **Infertile Population by Age Group.**

Ages (years)	LH (mIU/ml)	FSH (mIU/ml)	PRL. (ng/ml)	Prog. (ng/ml)	E2 (pg/ml)	MDA (µM/L)	TAC (mM/L)	LPI
20 – 29	13.83± 5.21[a]	4.61± 1.00[a]	43.15± 12.66	3.29± 0.98	53.05 ± 12.05	8.45± 1.57	0.69± 0.24	32.39± 8.90
30 – 39	8.46± 1.77 [a]	6.66± 1.53[a]	28.19± 2.84	3.99± 0.55	36.63 ± 4.77	15.83± 3.10	0.64± 0.11	49.10± 13.96
40 – 49	22.3± 6.31[b]	26.09± 8.42[b]	25.29 ± 3.94	2.41± 0.49	29.36± 5.88	9.64± 1.50	0.80± 0.17	26.61± 6.98
P-value	0.0241	0.0008	0.1211	0.2533	0.1343	0.2181	0.2797	0.6794
F-value	3.942	7.892	2.179	1.402	2.069	1.558	0.7569	0.5104
Remarks	S	S	NS	NS	NS	NS	NS	NS

218 Mean with different superscripts (on each column) are statistically different from each other. LH-leutinizing
219 hormone, FSH-follicle stimulating hormone, PRL-prolactin, Prog.-progesterone, E2-Estradiol, MDA-

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malondialdehyde, TAC- total antioxidant capacity and LPI-lipid peroxidation index, NS – not significant, S – significant, * – statistically significant and ** – very significant.

Oxidative Characteristics of Case and Control Groups.

Table 4 provides the mean concentrations (Mean ± SEM) of oxidative parameters (MDA, TAC and LPI) of infertile and fertile (control) groups in the studied population. The mean concentrations (Mean ± SEM) of MDA and LPI were higher in the infertile group (13.05 ± 1.90 µM/L and 40.85 ± 8.52 respectively) than in the fertile group (9.34 ± 0.92 µM/L and 16.21 ±2.50). Whereas the difference was not statistically significant ($p>0.05$) for MDA, it was LPI ($p<0.05$). The total antioxidant capacity (TAC) was lower in the infertile group (0.69 ± 0.09 mM/L) when compared with the fertile control group (1.33 ± 0.14 mM/L) and the difference was statistically significant ($p<0.05$).

Table 4: Oxidative Characteristics of Case and Control Groups (Mean ± SEM)

Parameters	Control group N= 70	Infertile group N= 70	T-value	P-value	Remarks
Age (years)	34.01 ± 0.72	35.79 ± 0.66	0	0.9999	NS
MDA (µM/L)	9.34 ± 0.92	13.05 ± 1.90	1.754	0.0816	NS
TAC (mM/L)	1.33 ± 0.14	0.69 ± 0.09	3.897	0.0002***	S
LPI	16.21 ± 2.5	40.85 ± 8.52	2.774	0.0063**	S

KEY: S – Significant, NS – not significant, ** – very Significant, *** – highly significant

Mean Levels of MDA, TAC and LPI in the Infertile Group According to Normal Hormone Levels and Abnormal Hormone Levels Compared

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The mean concentrations of MDA, TAC and LPI according to normal hormone levels and abnormal hormone levels in the infertile subjects are represented in table 5. The oxidative parameters (MDA, TAC and LPI) were determined for the infertile women with abnormal hormone levels and the infertile women with normal hormone levels. The values were compared with control group of normal fertile women with normal hormone levels. The mean concentration of MDA in the infertile women with abnormal hormone levels was 14.04 ± 2.48µM/L compared to its lower value of 11.88 ± 2.85 µM/L in the infertile women with normal hormone level and both values were higher than that for the control group and the variation did not show any significance ($p=0.1375$). TAC mean concentrations were 0.84 ±0.12 mM/L in infertile women with abnormal hormone levels group and 0.33 ± 0.06mM/L (lower) in infertile women with normal hormone level group; both values were lower than the value for the fertile women with normal hormone group of 1.33 ± 0.14mM/L and the difference in the mean concentrations was statistically significant ($p <0.0001$). The LPI mean concentrations were higher in the infertile women with normal hormone group (59.36 ± 23.34) than in the infertile women with abnormal hormone group (32.71 ± 5.36). Both values were higher than the value for the fertile women with normal hormone (16.21 ± 2.50) but no significant difference ($p>0.05$) between the means of LPI of the infertile women with abnormal hormone group and LPI of the fertile women with normal hormone group, however, significant ($p<0.05$) variation between means of LPI of infertile women with normal hormone group, infertile women with abnormal hormone levels and the fertile women with normal hormone group was seen.

Table 5: Mean Levels of Oxidative Markers (MDA, TAC & LPI) in the Infertile Group According to Normal Hormonal Levels and Abnormal Hormonal Levels Compared.

Group	**MDA** (µM/L)	**TAC (mM/L)**	**LPI**

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Normal fertile women with normal hormone levels (control)	9.34 ± 0.92	1.33 ± 0.14^{a}	16.21 ± 2.50^{a}
Infertile women with normal hormone levels	11.88 ± 2.85	0.33 ± 0.06****[b]	59.36 ± 23.34**[b]
Infertile women with abnormal hormone levels	14.04 ± 2.48	0.84 ± 0.12*[c]	32.71 ± 5.36^{c}
P-value	0.1375	<0.0001	0.0027
F-value	2.013	10.29	6.188
Remark	NS	S	S

Mean with different superscript (on each columns) are statistically different from each other. NS – not significant, S – statistically significant, * –significant, ** – very significant, *** – highly significant.

DISCUSSION

Infertility is now a global problem facing every population of all societies, both developed and developing countries are been increasingly affected [3,5]. Effective treatment and management of this menace requires a holistic approach born out of a comprehensive understanding of factors affecting the disease. Infertility has been often related to endocrine disorder affecting the hypothalamo-pituitary-ovarian axis, eliciting imbalance in the female hormonal profile. Researchers are currently linking infertility with oxidative stress [8,12].

The result of this study showed that there was a significantly higher induction of oxidative stress in the infertile women when compared with the fertile control subjects. The LPI and TAC were significantly ($p=0.0063$ and $p<0.0002$) higher in the infertile women when

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275 compared with the fertile control. This result is in agreement with studies of Agawal *et al*. [8],
276 Attaran *et al.* [27] and Oyewole *et al*. [17].

277 The mean concentration of MDA in this study was insignificantly ($p>0.05$) higher while
278 the LPI was significantly ($p<0.05$) higher in the infertile group compared to the fertile group. A
279 strong positive correlation of MDA with the lipid peroxidation index (LPI) ($r = 0.661$) was also
280 observed. The study also showed a significantly ($p<0.05$) lower level of total antioxidant
281 capacity (TAC) in the infertile than the fertile women, and the LPI was negatively correlated
282 with TAC in the infertile women ($r= -0.30$, $p= 0.014$). Since LPI was used as index of oxidative
283 stress, a rise in MDA and fall in TAC levels elicited an increase in oxidative stress [28]. This
284 study showed that there was significant oxidative stress in the infertile compared to the fertile
285 women. And that the overall activity of antioxidant system was weaker in the infertile women
286 than in the fertile women. The weaker antioxidant system may have being responsible for the
287 observed oxidative stress expressed in the infertile group as shown by the raised value of the
288 lipid peroxidation index. This result is in agreement with Oyewoye *et al.* [17] who estimated the
289 total antioxidants capacity (TAC) levels in the follicular fluid of women undergoing IVF and
290 found that the TAC level in follicular fluids that produced oocytes which become fertilized
291 where significantly higher compared to those whose oocytes did not get fertilized. The Oyewoye
292 *et al*. [17] proved that fertile gametes contain strong antioxidants. In the present study the
293 diminished TAC observed may have occurred as a result of increased oxidant activities (an
294 elevated oxidant level infers fatigued antioxidant defense), thereby weakening the scavenger'
295 ability to defuse the oxidants' toxic effects [13]. Therefore, the diminished TAC may be
296 responsible for the oxidative stress experienced by the infertile women in the studied population.
297 Hence, antioxidant supplementation therapy may be of help in management of infertility in this

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area. These findings are also supported by the earlier work of Tripathi *et al*. [15] who proved that antioxidants could be helpful in treatment of infertility.

The comparison of the level of oxidative stress in the infertile subjects based on hormone classification showed significant ($p<0.05$) increase in mean LPI value among infertile women with normal hormone levels above those with abnormal hormone levels (imbalance) when compared with the fertile women (control group). Mean TAC level was significantly ($p<0.0001$) lower in the infertile subjects with normal hormone levels than those with abnormal hormone levels compared with control fertile women with normal hormone levels. This suggests that the infertility being experienced by some of the subjects may not be due to endocrine dysfunction; rather some other conditions that induce oxidative stress may be responsible. This position is in agreement with the reports of Tarin *et al*. [14] and Huang *et al.* [16].

The present study further compared the oxidative parameters in the infertile women with normal hormone profile based on exposure to oxidant agents with the fertile control group. The result showed a significant decrease in TAC level in those (infertile women with normal hormone profile) exposed to oxidants agents (infections, alcohol, and ulcer) and those who were not exposed to any of the aforementioned agents (but are infertile with normal fertility hormone levels) when compared with control subjects ($p<0.05$). The LPI was significantly ($p<0.05$) higher in the exposed subgroup than the non-exposed when compared with control. This result suggests that there may be a significant state of oxidative stress in the exposed subgroup than the non-exposed, which may have resulted to their infertility. This observation is in agreement with reports of several researchers who have demonstrated the roles of the aforementioned oxidant agents in induction of oxidative damage [16, 29-30].

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320 Alcohol is primarily eliminated from the body through an oxidative mechanism occurring
321 in the liver. Alcohol hepatic metabolism produces acetaldehyde which upon further
322 dehydrogenation yields acetic acids with acetyl and methyl radicals. These metabolites generate
323 a high amount of oxidants [31]. The overproduced ROS promotes lipid peroxidation, decrease
324 antioxidant enzyme activities (SOD), and deplete GSH concentration, thereby introducing
325 oxidative stress [31]. Alcohol induced OS can initiate the oxidation steps of the Maillard reaction
326 which promotes AGE (advanced glycation end products) formation. Accumulation of the toxic
327 product, AGE, is linked with the upregulation of antioxidant activities. The binding of AGE to its
328 receptor (RAGE) induces a state of inflammation through activation of NF-Kappa B (a
329 transcription factor) and then cytokine expression [29]. Thus, alcohol use can speed up oxidative
330 stress through some mechanisms that involved enhancement of apoptosis, alteration of cell
331 structures and damaging of tissues. A study showed that when mouse embryo was exposed to
332 ethanol, it experienced an increased oxidants generation, lipid peroxidation, apoptosis and in
333 vitro deformation. The study also reported that when SOD and/or vitamins were administered
334 simultaneously, the effect of oxidative stress was reduced [30].

335 Tubal infertility has been largely related to infections of the genital tract and
336 consequently oxidative stress [29]. Tubal damage has been reported as the most common cause
337 of secondary infertility in our environment [32]. Augusta *et al*. [33] observed an association
338 between reproductive hormones and oxidative markers in infertile women infected with
339 chlamydia and reported a moderate increase in LH with a significant low TAC level in
340 chlamydia positive infertile women compared with chlamydia negative fertile control subjects.
341 Macrophages and polymorphonuclear leukocytes are inducted through the inflammatory
342 response to infections of the genital tract. The activities of macrophages and cytokines result in

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greater ROS generation and consequently oxidative-induced cell destruction [34]. Similarly, a strong positive correlation of MDA with LPI (r= 0.964) in the oxidant-exposed subgroup was also observed in this study. Since LPI is used as the index of oxidative stress in this study, it then implies that the increased oxidative stress in this subgroup could be due to increased MDA production that is linked with increased free radical generation occurring through the metabolic processes of the oxidant molecules that subdued the antioxidant defense system as previously reported [35]. Therefore interventions that eliminate exposure to oxidant sources including infections, alcohol, irradiations, cigarettes and ulcer (H-pylori) may be of help in infertility managements.

CONCLUSION

There is a significant increase in oxidative stress markers in women diagnosed with infertility in Port Harcourt metropolis which has been caused by exposure to antioxidant agents. Thus, evaluation of oxidative stress parameters should form part of the panel of analysis used in the investigation of infertility in women in the studied population. Further studies on estimation of antioxidant enzymes including superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GHR) is recommended to validate reliability.

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