

1 **EVALUATION OF SERUM α -TOCOPHEROL AND ANEMIA AMONG INFERTILE**
2 **PATIENTS ATTENDING SPECIALISTS HOSPITAL, SOKOTO**

3
4 **ABSTRACT**

5 Infertility is the inability of a couple to achieve pregnancy over an average period of one year
6 despite adequate, regular and unprotected sexual intercourse. Avitaminosis E has been
7 implicated in the development of infertility and hemolytic anemia in animals. There is, however,
8 little evidence that man is ever scanty in vitamin E. The aim of this study is to evaluate serum α -
9 tocopherol levels and anemia among infertile patients attending Specialists Hospital, Sokoto. A
10 total of fifty (50) infertile patients and fifty (50) apparently healthy fertile married as control
11 were recruited for this study. The blood samples collected were analyzed for α -tocopherol using
12 Hashim and Schuttringer (1996) method, and PCV using hematocrit reader. The data obtained
13 were analyzed using the Student's T-independence test. The result shows that the level of serum
14 α -tocopherol was significantly lower ($p < 0.05$) in infertile patients (0.65 ± 0.04) compared to
15 controls (1.38 ± 0.02). The result, however, shows no statistically significant difference of PCV
16 and BMI (37.58 ± 0.4 and 21.14 ± 0.34 respectively) in infertile patients when compared to the
17 controls (38.61 ± 0.4 and 22.05 ± 2.64 respectively). A reduced serum α -tocopherol level among
18 infertile patients was observed in this study. The α -tocopherol has been described to be a potent
19 antisterility factor on account of the development of the sterility in its absence. We, therefore,
20 commend the incorporation of α -tocopherol in the diagnosis and treatment of infertility in
21 human.

22 **Key-words:** Infertility, α -tocopherol, anemia.

23 **1.0 Introduction**

24 Infertility is the inability of a couple to achieve pregnancy over an average period of one year (in
25 a woman under 35 years of age) or 16 months (in a woman above 35 years of age) despite
26 adequate, regular (3-4 times per week), and unprotected sexual intercourse (1). According to
27 American Pregnancy Association, infertility is defined as trying to get pregnant for at least a year
28 without success (2). Contraception is normally achieved within 12 months in 80 to 85% of
29 couples using no contraceptive measures (3). In primary infertility, the woman has never
30 conceived despite combination and exposure to sexual intercourse for at least two years, while in
31 secondary infertility, the woman has previously conceived but subsequently unable to conceive
32 despite combination and exposure to sexual intercourse for a period of two years (1). Tocopherol
33 was derived from two Greek words: tokos (=offspring) and phenol (=to bear). Tocopherol,
34 therefore, literally means to bear children. This satisfactorily suggests the involvement of
35 tocopherol in fertility (4). Alpha-tocopherol, a potent antioxidant vitamin that protects cells
36 damage from free radicals was designated as antisterility factor on account of the development of
37 sterility in its deficiency (5). The characteristic symptoms of experimentally-induced vitamin E
38 deficiency vary from animals to animals. In mature female rats, sterility develops because of
39 absorption of a fetus after conception. While in males, the germinal epithelium of the testes
40 degenerates and spermatozoa become non-motile. Avitaminosis E has also been implicated in the
41 development of hemolytic anemia in monkeys (5).

42 Global estimates suggested that nearly 72.4 million couples experience fertility problems (6).
43 World Health Organization (7), estimated that between 8% and 12% of couples experienced
44 some form of infertility during their reproductive lives. Thus, affecting 50 to 80 million people
45 worldwide, out of which 20 to 35 million couples in Africa are expected to experience this

46 problem. This can be extrapolated to 3 to 4 million Nigeria couples suffering from infertility (8).
47 In Africa, the prevalence of infertility is higher particularly in Sub-Sahara Africa ranging from
48 20% to 60% (9). An estimate of 19% infertile couples in Ile-Ife (10), and 15% from Usmanu
49 Danfodiyo University Teaching Hospital, Sokoto has been reported (11). There is, however, little
50 evidence that man is ever deficient in the α -tocopherol. The aim of this study is to determine the
51 serum levels of α -tocopherol and anemia among infertile patients attending Specialists Hospital,
52 Sokoto.

53 **2.0 Materials and Methods**

54 **2.1 Chemicals and Equipment**

55 All chemicals and equipment used are of analytical grade. The chemicals used include xylene,
56 ethanol, α -dipyridyl and ferric chloride. The equipment used include bench-top universal
57 centrifuge, spectrophotometer, Bench-top micro-hematocrit centrifuge, and hematocrit reader.

58 **2.2 Study Population**

59 A total of one hundred (100) subjects were recruited for this study. They consist of fifty (50)
60 apparently healthy married fertile subjects as control and fifty (50) infertile patients attending
61 Specialist Hospital, Sokoto.

62 **2.3 Ethical Consideration and Clearance**

63 The approval for this study was sought and granted from the ethics and research committee of
64 the Specialists Hospital, Sokoto prior to the commencement of the study. The ethical clearance
65 number of the study is SHS/HREC/2016/446.

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67 **2.4 Sampling Techniques**

68 The arrangement was made with the clinicians where those that satisfy the study inclusion
69 criteria were selected. The nature and reasons for the study were explained fully to the subjects
70 in the appropriate language. Subjects consent was our priority and was obtained with their full
71 history. Specimen collection was made and findings were documented in the proforma.

72 **2.5 Anthropometric Measurements**

73 The standard procedure (12) was employed for anthropometric measurements. Body mass index
74 (BMI) was determined using the weight in kilogram (kg) divided by the square of the height in
75 meters. The values of 20-25, <30 but >25, >30 and <20 were considered as normal, overweight,
76 obese and underweight respectively.

77 **2.6 Analytical Techniques**

78 Serum α -tocopherol was estimated using the Hashim and Schuttringer method (13), while the
79 pack cell volume (PCV) was read using a microhematocrit reader (14).

80 **2.7 Statistical/Data Analysis**

81 The analysis of the data obtained was treated accordingly using Grap and Instat3 © (2008)
82 Statistical package. The results were expressed as Mean \pm SEM. Paired comparisons were
83 carried out using the Student's T-independence test. A P-value ≤ 0.05 were considered
84 statistically significant.

85 **3.0 Results**

86 The Socio-demographic characteristics of the study subjects are represented in Table1. They
 87 consist of fifty (50) infertile patients and fifty (50) apparently healthy married fertile as controls,
 88 with mean age and BMI of (36.93±0.57 and 21.14±0.34) and (34.08±0.15 and 25.25±2.64) for
 89 patients and controls respectively. Table 2 shows serum α -tocopherol and BMI among patients
 90 and control. The result shows that the serum α -tocopherol of the patients were significantly lower
 91 (P<0.05) (0.65±0.04) than the controls (1.38±0.02). Table 3 presents pack cell volume (PCV)
 92 and body mass index (BMI) among patients and controls. The result showed no statistically
 93 significant difference (p>0.05) in PCV of infertile patients when compared to controls. Table 4
 94 correlates α -tocopherol and PCV of the study subjects. Statistically, significant difference was
 95 observed (P<0.05) between the serum level of α -tocopherol of the patients when compared with
 96 controls.

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98 **Table1: Demographic and clinical characteristics (Mean±SEM) of the study subjects**

Subjects	N	Age(yrs)	BMI(kg/M ²)
Control	50	34.08±0.15	25.25±2.64
Male	30	34.30±0.36	23.54±0.60
Female	20	33.94±0.10	26.31±4.26
Patients	50	36.93±0.57	21.14±0.34
Male	22	35.75±1.21	20.47±0.64
Female	28	37.31±0.65	21.36±0.40

99 N= Number of subjects, BMI= Body mass index, Yrs= years, Kg= kilogram, M= meter.

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101 **Table 2: Serum α -tocopherol and BMI (Mean \pm SEM) of the study subjects**

Subjects	N	Age(yrs)	α -tocopherol(μ mol/L)	BMI(kg/m ²)
Control	50	34.08 \pm 0.15	1.38 \pm 0.02	22.03 \pm 2.64
Patients	50	36.03 \pm 0.57	0.65 \pm 0.04	21.14 \pm 0.34
P-Value		>0.05	<0.05	>0.05

102 N= Number of subjects, BMI=Body mass index, α -Alpha, Kg= kilogram, M=meter, μ mol/L= micromole per liter,
 103 yrs=Years.

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105 **Table 3: Pack cell volume and BMI (Mean \pm SEM) of the study subjects.**

Subjects	N	Age(yrs)	PCV (%)	BMI(kg/m ²)
Control	50	34.08 \pm 0.15	38.61 \pm 0.4	22.03 \pm 2.64
Patients	50	36.03 \pm 0.57	37.58 \pm 0.4	21.14 \pm 0.34
P-Value		>0.05	>0.05	>0.05

106 N= Number of subjects, BMI=Body mass index, %= percentage, α = Alpha, μ mol/L=Micromole per liter,
 107 PCV=Pack cell volume.

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112 **Table4: Serum α -tocopherol and pack cell volume (PCV) (Mean \pm SEM) of the study**
 113 **subjects.**

Subjects	N	Age(yrs)	α -tocopherol(μ mol/L)	PCV (%)
Controls	50	34.08 \pm 0.15	1.38 \pm 0.02	38.61 \pm 0.4
Patients	50	36.03 \pm 0.57	0.65 \pm 0.04	37.58 \pm 0.4
P-Value		>0.05	<0.05	>0.05

114 N=Number of Subjects, yrs =Years, PCV= Pack Cell Volume, α =Alpha, %= Percentage.

115 **4.0 Discussion**

116 Many studies have demonstrated the damaging effects of elevated free radicals (reaction oxygen
 117 species) to sperm function. Increasing vitamin E supplement in the hope to prevent the oxidative
 118 damage was a proposed solution. In the past decade, a number of studies were undertaken to
 119 ascertain whether such a proposal is truly helpful. Unfortunately, the few studies, the small
 120 sample size, and conflicting data have made it difficult for clinicians and researchers to agree on
 121 a recommendation. However, the existing studies do seem to be encouraging (15).

122 Oxidative stress, decreases antioxidant capacity and impaired sperm mitochondrial functions are
 123 the main factors contributing to infertility (16). In this study, a significantly lower level of serum
 124 α -tocopherol was observed in infertility compared to control. This is similar to the study
 125 conducted by Serena *et al*, (17), who shows a significant decrease in serum α -tocopherol level in
 126 infertile patients when compared to control. This is, however, in contrary to the study of
 127 Sasikumar *et al*, (18), who reported an increased level of serum α -tocopherol in the test group in
 128 comparison with the controls.

129 Over the last decade, intensive research has been focused on various antioxidants and their
130 optimal doses and combinations, for more effective and safe treatment of human fertility
131 disturbances (15). Although, reaction oxygen species (ROS) have been shown to be required for
132 sperm capacitation, hyperactivation, and sperm-oocyte fusion (19), excessive levels of ROS can
133 negatively impact sperm quality (20). Improvement of sperm parameters after antioxidant
134 therapy of infertility may result in higher pregnancy rate (21).

135 In a number of studies, it has been shown that oral supplement of Vitamin E Significantly
136 improved sperm motility (22). There has been existing evidence that suggests a relationship
137 between daily antioxidant intake and better semen quality among healthy men. Semen analysis
138 was performed on 97 healthy male volunteers and results were correlated with the results of a
139 dietary assessment questionnaire (2). Higher levels of vitamin E intake were associated with
140 higher levels of progressive sperm motility (2).

141 In conclusion, a reduced serum α -tocopherol in infertile patients was observed in this study. The
142 α -tocopherol has been described to be an antisterility factor on the account of the development of
143 sterility in its absence. We, therefore, commend the incorporation of serum α -tocopherol for both
144 diagnosis and treatment of infertility in humans.

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146 **Consent disclaimer: NA**

147 **References:**

- 148 1. Cooper, T.G., Noonan, E. and Von Eckardstein, S. WHO reference values for human
149 semen characteristics. Human Reproduction. 2010, 16(3): 231-245.

- 150 2. . Eskenazi, B., Kidd, S.A., Marks, A.R., Slotter, E., Block, G., and Wyrobek, A.J.
151 Antioxidant intake is associated with semen quality in healthy men. *Hum. Reprod.* 2005,
152 20(4): 1006- 1012.
- 153 3. Griffit, J.E. and Wilson, J.D. Disorders of the testes. In: Isselbacher, K.J. *et al.*, (editors).
154 Harrison's Principles of Internal Medicine. New York. McGraw Hill. 1994, 2006- 2017.
- 155 4. Murray, M.M. and Michael, T. Encyclopaedia of Nutritional Supplements. The Essential
156 Guide for Improving Your Health Naturally. Three River Press. 1996, 34-38
- 157 5. Jain, J., Sunjay, J. and Nitin, J. Fat soluble Vitamins: in Fundamentals of Biochemistry.
158 S. chand & company ltd. 2009, 959-987.
- 159 6. Boivin, J., Bunting, L., Collins, J.A, and Nygren, K.G. International estimates of
160 infertility and treatment seeking potential need and demand for infertility medical care.
161 Human Reproduction. 2007, 22:1506-1512.
- 162 7. World Health Organization (WHO). Infertility: A tabulation of available data on the
163 prevalence of primary and secondary infertility. Programme on maternal and child health
164 and family planning. Division of Family Health. World Health Organization. Genera.
165 1991.
- 166 8. Thomas, K.D., Adeoye, J. and Olusanya, O.O. Biochemical Markers in Seminal plasma
167 of Sub-fertile Nigerian men. *Tropical Journal of Obstetrics and Gynaecology.* 1995,
168 15:19.
- 169 9. Oguniyi, S.O., Makinde, O.O. and Dare, F.O. Abortion-related deaths in Ile-Ife, Nigeria.
170 African Journal of Medicine and medical services. 1999, 19(4): 271-274.
- 171 10. Okonofua, F.E. Case against new productive technologies in developing countries.
172 British Journal of Obstetrics and Gynaecology. 1996, 103:957-962.

- 173 11. Panti, A.A. and Sannu, Y.T. The profile of infertility in a teaching hospital in north-west,
174 Nigeria. *Sahel Medical Journal*. 2014, 17:7-11.
- 175 12. Abdoulrazaq, M.Y. Anthropometric and Biochemical profile of malnourished and well-
176 fed children in Magarya District, Niger Republic. A research proposal submitted to the
177 Department of Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria.2010, 17.
- 178 13. Hashim, S.A. and Schuttringer, G.R. Rapid determination of tocopherol in macro and
179 micro quantities of plasma. *American Journal of Clinical Nutrition*. 1966, 19(137), 3-9.
- 180 14. Baker, F.J., Silverton, R.E. and Pallister, C.J. The Full Blood count in: Baker and
181 Silverton's Introduction to Medical Laboratory Technology, Bounty Press. Ltd. 2010,
182 354-373.
- 183 15. Gharagozloo, P. and Aitken, R.J. The role of sperm oxidative stress in male fertility and
184 the significance of oral antioxidant therapy: *Human Reproduction*. 2011, 26(7):1628-
185 1640.
- 186 16. Sheweita, S.A., Tylmisany, A.M. and Al-Sawaf, H. "Mechanism of male infertility: the
187 role of antioxidants". *Current Drug Metabolism*. 2005, 6(5): 495-501.
- 188 17. Serena, B., Maria, C.T., Simona, C., Silvia-De, S., Carlo, B. Differences in blood and
189 semen oxidative status in fertile and infertile men and their relationship with sperm
190 quality. *Reproductive Biomedicine online*. 2012, 25:300-306.
- 191 18. Sasikumar, S., Sunder, J.S., Madhankumar, E.K., Amburu, P., Kalaiselvi,S., Gopinath,
192 P.M., Chitra, S. and Uma, R. A study on the significant biochemical changes in the serum
193 of infertile women. *Internation Journal of Current Research and Academic Review*. 2014,
194 2: 967-115.

- 195 19. Aitken, R.J., Clarkson, J.S., Hargreave, T.B., Irvine, D.S. and Wu, F.C. Analysis of the
196 relationship between defective sperm function and the generation of ROS in cases of
197 oligozoospermia. *J. Androl.* 1989, 10(3): 214-220.
- 198 20. Eskenazi, B., Wyrobek, A.J. and Slotter, E. The association of age and semen quality in
199 healthy men. *Hum. Reprod.* 2003, 18(2): 447-454.
- 200 21. KO, E.Y. and Sabanegh, E.S. "The role of neutraceuticals in male fertility." *Urologic*
201 *Clinics of North America.* 2014, 41(1):181-193.
- 202 22. Suleiman, S.A. Lipid peroxidation and human sperm motility: Protective role of vitamin
203 E. *Journal of Andrology.* 1996, 17(5): 530-537.

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