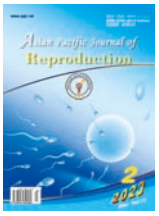

Original Article

Asian Pacific Journal of Reproduction

Journal homepage: www.apjr.net

doi: 10.4103/2305-0500.372379

Vitamin E modulates androgen receptor gene expression to attenuate ovarian dysfunctions in a rat model of dehydroepiandrosterone-induced polycystic ovary

Olugbemi T Olaniyan[1], Ayobami Dare[2✉], Charles O. Adetunji[3], Gloria E. Okotie[4], Joseph B. Dare[5], Bosun M. Adigun[6], Femi Adebayo[6]

[1]*Laboratory for Reproductive Biology and Developmental Programming, Department of Physiology, Edo University Uzairue, Edo State, Nigeria*

[2]*Discipline of Physiology, School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, Westville Campus, Durban, South Africa*

[3]*Applied Microbiology, Biotechnology and Nanotechnology Laboratory, Department of Microbiology, Edo University Uzairue, Edo State, Nigeria*

[4]*Department of Physiology, University of Ibadan, Ibadan, Nigeria*

[5]*Department of Anatomy, Osun State University Oshogbo, Nigeria*

[6]*Department of Physiology, Bingham University, Nasarawa State, Nigeria*

ABSTRACT

Objective: To investigate the protective effect of vitamin E in dehydroepiandrosterone (DHEA)-induced polycystic ovary in rats.

Methods: Premature female Wistar rats were randomly allocated into four groups, with 7 rats in each group. Group Ⅰ received corn oil (vehicle) and served as the control group; group Ⅱ received 0.2 mL of 0.06 mg/g DHEA in corn oil; group Ⅲ received 200 mg/kg vitamin E; group Ⅳ received DHEA plus vitamin E. All treatments lasted for 15 days, with DHEA administered subcutaneously, while vitamin E and corn oil were administered orally. After the experiment, serum samples and ovaries were harvested for biochemical, immunohistochemical, hormonal, and histological analysis. The ovarian mRNA expression of androgen receptor was analyzed by reverse transcriptase quantitative polymerase chain reaction (qPCR).

Results: The antioxidant and metabolic enzyme activity significantly decreased in the DHEA-treated rats compared to the control rats (P<0.05). Administration of vitamin E to DHEA-treated rats significantly decreased cytokines and malondialdehyde compared to the DHEA-treated rats. The histological analysis showed reduced atretic and cystic ovaries, increased *E*-cadherin and Bcl-2 expression, and reduced expression of Bax in the DHEA-treated rats co-treated with vitamin E. The mRNA expression of androgen receptor was upregulated in the DHEA-treated rats compared to the control rats.

Conclusions: Vitamin E ameliorates the hyperandrogenic effect of DHEA-induced polycystic ovaries *via* metabolic, antioxidant, and anti-apoptotic pathways.

KEYWORDS: Dehydroepiandrosterone; Metabolic markers; Polycystic ovarian syndrome; Vitamin E; Wistar rats

Significance

Polycystic ovarian syndrome (PCOS) contributes significantly to the increasing incidence of female infertility. Thus, the need for an effective regimen to reduce the incidence and progression of PCOS has attracted significant attention. Vitamin E is a flavonoid with potent therapeutic benefits *via* its anti-oxidant and anti-inflammatory activities. However, its effect on the pathogenic pathways implicated in PCOS has not been evaluated. In this study, we found that vitamin E supplementation in rats with polycystic ovary reduced oxidative injury, tissue inflammation, cell death, and downregulated androgen receptor mRNA expression, thereby ameliorating the hyperandrogenic effect of dehydroepiandrosterone in female rats.

✉To whom correspondance may be addressed. E-mail: 218084517@stu.ukzn.ac.za

This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial-Share Alike 4.0 License, which allows others to remix, tweak and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

©2023 *Asian Pacific Journal of Reproduction* Produced by Wolters Kluwer- Medknow.

How to cite this article: Olaniyan OT, Dare A, Adetunji CO, Okotie GE, Dare JB, Adigun BM, et al. Vitamin-E modulates androgen receptor gene expression to attenuate ovarian dysfunctions in a rat model of dehydroepiandrosterone-induced polycystic ovary. *Asian Pac J Reprod* 2023; 12(2): 81-89.

Article history: Received: 12 July 2022; Revision: 20 September 2022; Accepted: 19 October 2022; Available online: 30 March 2023

1. Introduction

Ovarian dysfunction accounts for about 10% of cases of human infertility, with polycystic ovary syndrome (PCOS) accounting for the increasing incidences of anovulatory infertility[1]. This heterogeneous disease is frequently characterized by hyperandrogenism, amenorrhea, and anovulation. Anovulatory infertility occurs when the ovaries do not release an oocyte, affecting 6%-20% of women of reproductive age[2]. Oxidative injury and inflammation have been correlated with increasing androgen levels[3]. Oxidative stress alters ovarian steroidogenic functions, increasing androgen production, altering follicular development, and promoting inflammation, culminating in infertility[4]. Cellular homeostasis relies on the complex interplay between free radicals and antioxidants. Excessive production of free radicals that overwhelm the antioxidant defense system results in oxidative stress[5] and damages vital cell components such as lipids, DNA, proteins, and carbohydrates. A major detrimental effect of the free radical attack is the oxidation of unsaturated fatty acids, known as lipid peroxidation. Thus, malondialdehyde (MDA), a significant end-product of lipid peroxidation, has been identified as a marker of oxidative stress and used to measure lipid peroxidation due to its stability[6]. Apoptosis is another risk factor that plays a central role in the pathogenesis and etiology of PCOS[7].

The ovarian cycle is characterized by widespread tissue remodeling where a more significant percentage of mammalian follicles fail to ovulate and become atretic by a hormonally controlled apoptotic process[8]. Dehydroepiandrosterone (DHEA) is the principal steroid secreted by the adrenal gland and ovary. It has been reported that DHEA promotes hyperandrogenization, which inhibits ovulation *via* oxidative stress and endocrine and immune dysfunction, thereby causing apoptosis and inflammation[3,9]. Animal models have demonstrated that polycystic ovary can be experimentally induced by daily subcutaneous injection of (6 mg/100 g in 0.2 mL of corn oil) DHEA for 15 days. This experimental model is characterized by significant ovarian oxidative and inflammatory injury, which starts before the cysts are developed and continues after cysts are formed[10]. Despite the giant stride to identify the cause of PCOS, its pathophysiology is yet to be fully elucidated. Thus, investigating the pathological mechanisms and probable treatment of PCOS has become an active area of research in reproductive medicine.

Generally, antioxidant therapies have shown numerous health benefits by significantly controlling lipid peroxidation in cellular membranes and reducing the detrimental effects of free radicals in the body. The antioxidant and anti-inflammatory activities of vitamin E have persuaded several researchers to evaluate its therapeutic potential in chronic diseases (such as cardiovascular diseases, infertility, and cancer), where oxidative stress has been highly implicated. Numerous experimental studies have shown that vitamin E reduces lipid peroxidation[11], reduces cell proliferation and protein kinase C activity, promotes phosphatase 2A activity, modulates alpha-tropomyosin gene expression[12], and reduces the expression of cell adhesion molecules in endothelial cells, thereby decreasing endothelial cell injury[12]. Literature has shown that vitamin E and C are potent antioxidants whose efficacy in female reproductive dysfunction has attracted clinical trials[13]. However, there is a paucity of data on the effects of vitamin E on female reproduction, polycystic ovaries, and pre-implantation embryonic development. Thus, this study aimed to evaluate the modulatory effects of vitamin E on ovarian oxidative metabolic status in DHEA-induced polycystic ovaries in female rats.

2. Materials and methods

2.1. Animals

Twenty-eight premature female Wistar rats [post-natal day-21, (18.0±3.0) g] were procured from the Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan. They were housed in a room with standard laboratory conditions [12-hour light–dark cycles; temperature (23±1) ℃, 40%–60% humidity] at Bingham University, Karu, Nasarawa State, Nigeria. The animals were allowed access to rat feed and water *ad libitum* for an acclimatization period of two weeks before the experiment.

2.2. Study design

The experimental animals were randomly divided into four groups (n=7 in each group) and treated as follows: Group Ⅰ received corn oil (10 mL/kg body weight, orally) and served as the control group; group Ⅱ received DHEA (6 mg/100 g in 0.2 mL corn oil subcutaneously daily) to induce polycystic ovary condition[7,14]; group Ⅲ received 200 mg/kg of vitamin E orally every day[15] to serve as an experimental control to the co-administration group, and group Ⅳ received both DHEA (6 mg/100 g body weight subcutaneously) and vitamin E (200 mg/kg body weight orally). All treatments were administered daily (between 8-10 a.m.) and lasted for 15 days, with DHEA administered subcutaneously, while vitamin E and corn oil were administered orally.

Twenty-four hours after the last administration, blood samples (2 mL at 10 a.m.) were collected through the retro-orbital venous sinus to obtain serum used to determine female reproductive hormones (progesterone and estrogen). After that, all animals were euthanized by cervical dislocation, and the ovaries were excised, adhering tissues were removed, and used for biochemical, reverse transcription-quantitative polymerase chain reaction (Rt-qPCR), and histopathological analysis.

2.3. Biochemical procedures

2.3.1. Lipid peroxidation and antioxidant assay

Lipid peroxidation assay was done using the method previously reported by Olaniyan *et al*[16] and expressed as nmol MDA per gram wet tissue. Superoxide dismutase (SOD) was analyzed as described by Beauchamp *et al*[17] with modification by Misra *et al*[18]. Catalase and glutathione-S-transferase (GST) activity were measured by the protocol reported by Sinha[19] and Habig *et al*[20], respectively. Reduced glutathione (GSH) level was estimated using the protocol reported by Jollow *et al*[21]. GSH level was presented in μmol GSH/g tissue, while the method described by Lowry *et al*[22] was used to measure protein concentration in the tissues.

2.3.2. Evaluation of inflammatory biomarkers

The concentration of vascular endothelial growth factor (VEGF) and tumour necrosis factor-alpha (TNF-α) was measured in the ovary using their specific enzyme-linked immunosorbent assay (ELISA) kit (Elabscience, USA) according to the Sandwich-ELISA principle described by the manufacturer as previously reported by Olaniyan *et al*[23].

2.4. Evaluation of hormonal concentration

The serum concentration of progesterone and estrogen was determined using their specific enzyme immunoassay (EIA) kit (Immunometrics Limited, UK), according to the protocol recommended by the manufacturer in accordance with the WHO standards for reproductive analysis as previously reported by Olaniyan *et al*[16].

2.5. Evaluation of proton pump (ATPase) activity in the ovary

Na^+/K^+-ATPase, Ca^{2+}-ATPase, and H^+-ATPase activities were analyzed using the protocol reported by Evans[24], with slight modifications. The ovarian homogenate was used to measure inorganic phosphate levels by spectrophotometry according to the method of Bonting[25].

2.6. Evaluation of androgen receptor mRNA expression by Rt–qPCR

Total RNA was extracted using RNA Mini Kit (Geneaid Biotech Ltd, Taiwan). One μg of each RNA sample was converted to cDNA using the Bioline SensiFAST cDNA synthesis kit (according to the manufacturer's protocol) in a 20 μL reaction volume according to the manufacturer's protocol. The cDNA template was amplified using Bioer LineGene 9600 PCR machine with 10 μL reaction volume, as previously reported by Olaniyan *et al*[23]. *β-actin* was used as the housekeeping gene using the same PCR condition. The primer sequence used in this study are androgen receptor forward 3'-ATGCTGGGCCTGTAGCCCCCT-5', reverse 3'-CAGGCAGGTCTTCTGGGGTGGG-5'; β-actin forward 3'-CCTCCGTCGCCGGTCCACACC-5', reverse 3'-TCTTGCTCTGGGCCTCGTCGC-5'.

2.7. Histopathological evaluation

Histopathological evaluation was done by hematoxylin and eosin (H & E) staining. Tissues were cut transversely (0.5 cm thick) and passed through a graded series of alcohol (70%, 90%, and 100%) and xylene for different durations. These were then transferred into molten paraffin wax for 60 min at 65℃ for embedding. After that, the processed tissues were embedded, and serial sections were done using a rotary microtome at six microns (6 μm). The tissues were mounted on albumenized slides and allowed to dry for 2 min. The slides were dewaxed with xylene and transferred through 100% ethanol (2 changes), 70%, 50% alcohol, and water for 5 min. After that, the slides were stained with H & E.

2.8. Immunohistochemical analysis of Bax, Bcl–2, and E–cadherin

Samples fixed in 10% formalin were used for immunohistochemical analysis. Samples were cut into 7 μm sections after dehydration and paraffin embedding. Preparations from the groups were used to analyze Bcl-2, Bax, *E*-cadherin proteins, and negative control (slide with no primary antibody), using Rabbit Bax, Bcl-2, and *E*-cadherin antibodies as primary antibodies. The tissue sections were later incubated with secondary antibody and horse-radish peroxidase-conjugated with streptavidin. After that, chromatogen 3,3'Diaminobenzidine (DAB) was added, which gave rise to a dark-brown color.

2.9. Statistical analysis

Data were analyzed with Graph-Pad prism version 6.0. Data were subjected to a normality test and conformed to a normal distribution; comparison between means was done using one-way analysis of variance (ANOVA) followed by a Tukey *post–hoc* test. Data were presented as mean±standard deviation (mean±SD) and considered statistically significant at $P<0.05$.

2.10. Ethics statement

All animal protocols were ethically approved (2018-07-25; BHU-ACURE/18/0124) and done according to the experimental guidelines of Bingham University Animal Ethics Committee, which was established under the internationally accepted laboratory animal use and care principles by the National Institute of Health 1996.

3. Results

3.1. Vitamin E supplementation reduced ovarian MDA and increased antioxidant levels in DHEA–induced PCOS

MDA level significantly increased in the DHEA-treated group compared to the control group (P<0.05). However, co-administration of vitamin E and DHEA significantly decreased the MDA level compared to the DHEA group (P<0.05) (Table 1).

3.2. Vitamin E supplementation increased antioxidant levels in the ovary of DHEA–induced PCOS

The antioxidant level (SOD, CAT, GST and GSH) significantly reduced in the DHEA group compared to the control group. SOD, CAT, GST and GSH significantly increased in the group co-treated with vitamin E and DHEA compared to the DHEA group (P<0.05) (Table 1).

3.3. Vitamin E supplementation increased progesterone and estrogen serum concentration in DHEA–induced PCOS

DHEA treatment significantly decreased progesterone and estrogen levels compared to the control group (P<0.05). However, co-administration of vitamin E and DHEA significantly increased progesterone and estrogen levels compared to the DHEA group (P<0.05) (Figure 1A).

3.4. Vitamin E supplementation increased ATPase activity (Na^+/K^+, Ca^{2+} and H^+ ATPase) in the ovary of DHEA–induced PCOS

DHEA treatment significantly reduced proton pump enzyme activity (Na^+/K^+, Ca^{2+} and H^+ ATPase) compared to the control group (P<0.05). However, vitamin E and DHEA co-administration significantly increased enzyme activities compared to the DHEA group (P<0.05) (Table 2).

3.5. Vitamin E supplementation reduced TNF–α and VEGF concentration in the ovarian homogenate of DHEA–induced PCOS

TNF-α and VEGF concentrations increased significantly in the DHEA-treated group compared to the control group (P<0.05). However, vitamin E and DHEA co-administration significantly reduced TNF-α and VEGF concentration compared to the DHEA group (P<0.05) (Figure 1B).

Table 1. Effects of vitamin E on antioxidant enzymes and malondialdehyde in DHEA-induced PCOS in rats.

Parameters	Control	DHEA	Vitamin E	Vitamin E+DHEA
SOD, U/mL/mg protein	1.63±0.08	1.38±0.18$^{\#}$	1.52±0.13	1.71±0.13**
CAT, µmole/min/mg protein	106.61±5.68	92.37±2.27$^{\#}$	103.08±7.08	104.66±12.28*
GST, µmole/min/mg protein	0.08±0.03	0.04±0.01$^{\#}$	0.07±0.02	0.08±0.02**
GSH, mg/mL/mg protein	7.50±1.37	4.33±0.69$^{\#}$	6.77±1.21	7.19±0.45**
MDA, mol/mg protein	0.60±0.10	0.95±0.21$^{\#}$	0.55±0.11	0.46±0.05**

Data are expressed as mean±SD; n=7. $^{\#}P$<0.05: compared to the control group; $^{*}P$<0.05, $^{**}P$<0.01: compared to the DHEA treated group. DHEA: dehydroepiandrosterone; PCOS: polycystic ovary syndrome; SOD: superoxide dismutase; CAT: catalase; GST: glutathione-S-transferase; GSH: reduced glutathione: MDA: malondialdehyde.

Table 2. Effect of vitamin E on ATPase activity in DHEA-induced PCOS in rats.

Parameters	Control	DHEA	Vitamin E	Vitamin E+DHEA
Na^+/K^+ ATPase, Piµmol/mg protein/h/10^{-3}	0.49±0.11	0.39±0.03$^{\#}$	0.47±0.06	0.44±0.06*
Ca^{2+} ATPase, Piµmol/mg protein/h/10^{-3}	4.59±0.45	3.84±0.32$^{\#}$	4.61±0.11	4.37±0.48*
H^+ ATPase, Piµmol/mg protein/h/10^{-3}	0.43±0.03	0.40±0.03$^{\#}$	0.43±0.05	0.43±0.03*

Data are expressed as mean±SD; n=7. $^{\#}P$<0.05: compared to the control group; $^{*}P$<0.05: compared to the DHEA treated group.

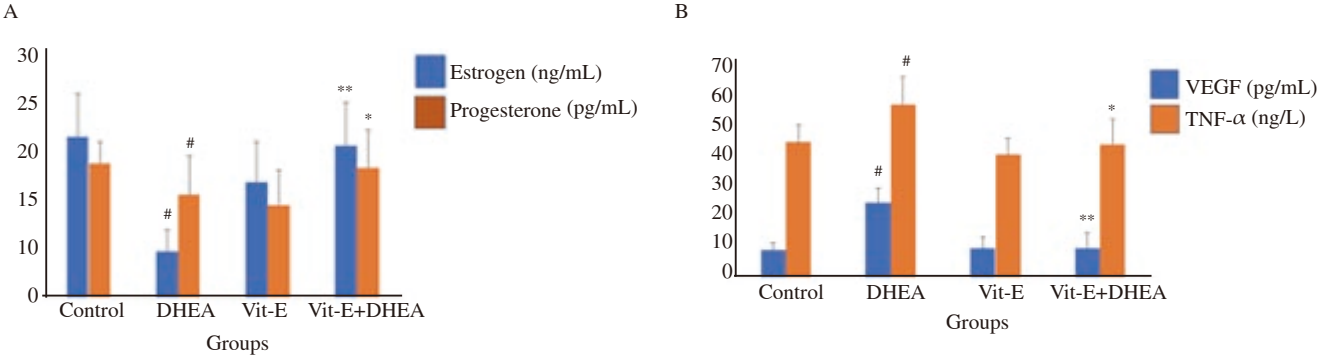

Figure 1. (A) Effects of vitamin E (Vit-E) on estrogen and progesterone concentration in DHEA-induced polycystic ovary (n=7 in each group); (B) Effects of vitamin E (Vit-E) on TNF-α and VEGF concentration in DHEA-induced polycystic ovary (n=7 in each group).$^{\#}P$<0.05: compared to the control group; $^{*}P$<0.05, $^{**}P$<0.01: compared to the DHEA treated group. TNF-α: tumour necrosis factor-alpha; VEGF: vascular endothelial growth factor.

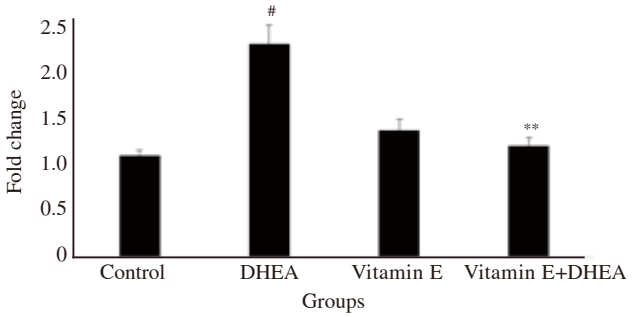

Figure 2. Effects of vitamin E on androgen receptor mRNA fold change ($2^{\wedge\text{-ddct}}$) (n=7 in each group). $^{\#}P$<0.05: compared to the control group; $^{**}P$<0.01: compared to the DHEA treated group.

3.6. Vitamin E supplementation downregulated granulosa cell androgen receptor mRNA expression in the ovary of DHEA–induced PCOS

Androgen receptor mRNA expression levels in the DHEA-treated group significantly increased compared to the control group (P<0.05). However, co-administration of vitamin E and DHEA significantly decreased androgen receptor mRNA expression compared to the DHEA group (P<0.05) (Figure 2).

3.7. Vitamin E supplementation reduced morphological derangements and apoptotic biomarkers in the ovary of DHEA–induced PCOS

3.7.1. Histopathology of the ovaries in rats

In the control group, the ovary showed normal follicular differentiation and a well-arranged epithelium with no cyst. The corpus-luteum and stromal cells were well-developed (Figure 3A). In the DHEA-exposed rats, there were numerous cystic follicles, thick theca cell layer, and high collagen level surrounding the follicle with reduced corpora lutea (Figure 3B). The vitamin E-treated rats showed normal histomorphology and interstitial tissues (Figure 3C). Administration of vitamin E to DHEA-treated rats caused a reduction in follicular cysts, decreased collagen content around the follicle and the theca cell layer appeared thinner (Figure 3D).

3.7.2. Immunohistochemical expression of Bax apoptotic protein

In the control group, the preantral follicles, theca cells, and granulosa cells appeared normal, while Bax was mainly expressed in the granulosa cells of the antral follicles (Figure 4A). In the DHEA-treated rats, there was a greater expression of Bax in the granulosa cells of both the antral and preantral follicles, with numerous cystic and atretic follicles (Figure 4B). In the vitamin E treated rats, the granulosa cells, antral and preantral follicles were normal, with little or no Bax expression (Figure 4C). Administration of vitamin E to DHEA-treated rats caused a reduction in follicular cysts and reduced Bax expression in the antral follicles (Figure 4D).

3.7.3. Immunohistochemical expression of Bcl–2 anti–apoptotic protein in the ovaries

In the control rats, the preantral follicles, theca cells and granulosa cells appeared normal, while Bcl-2 was mainly expressed in the granulosa cells of the antral follicles (Figure 5A). In the DHEA-exposed rats, there was little or no Bcl-2 expression in the antral follicles with numerous cystic follicles (Figure 5B). In the vitamin E treated rats, there was the localization of Bcl-2 in the granulosa cells of the antral follicles, while the antral follicle also appeared normal (Figure 5C). Administration of vitamin E to DHEA-exposed rats caused a reduction in follicular cysts, with mild expression of Bcl-2 in the antral follicles (Figure 5D).

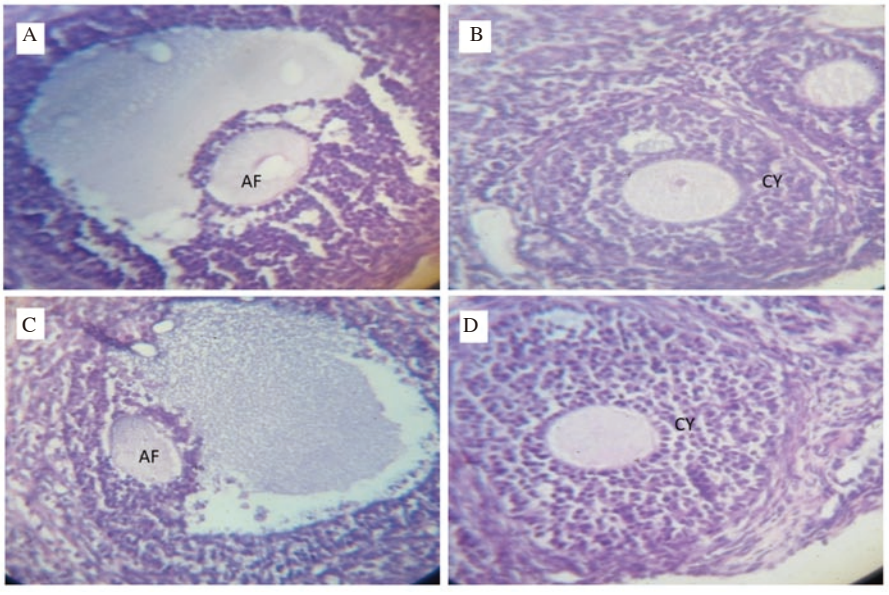

Figure 3. The histoarchitecture of the ovaries after 15 days of administration (H & E staining, ×40 magnification). A: In the control rats, the ovary shows normal follicular differentiation and a well-arranged epithelium with no cyst. The corpus-luteum and stromal cells are well-developed. B: In the DHEA-exposed rats, there are numerous cystic follicles, thick theca cell layer, and high collagen level surrounding the follicle with reduced corpora lutea. C: The vitamin-E-treated rats show normal histomorphology and interstitial tissues. D: Administration of vitamin-E to DHEA-treated rats causes a reduction in follicular cysts, decreases collagen content around the follicle and the theca cell layer appears thinner. AF: antral follicle; CY: cystic follicle.

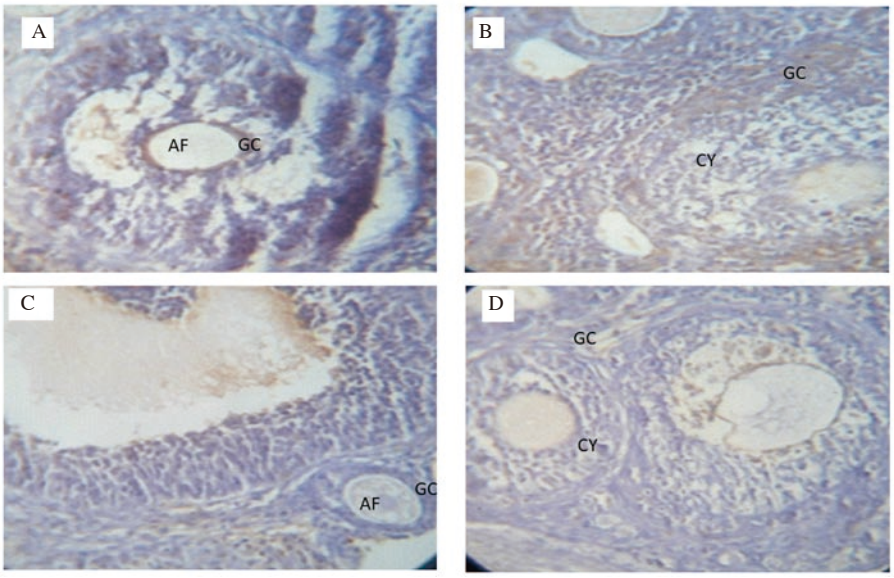

Figure 4. The immunohistochemical expression of Bax apoptotic protein after 15 days of treatment (immunohistochemical staining; ×40 magnification). A: In the control rats, the preantral follicles, theca cells, and granulosa cells appear normal, while Bax is mainly expressed in the granulosa cells of the antral follicles. B: In the DHEA-treated rats, there is a greater expression of Bax in the granulosa cells of both the antral and preantral follicles, with numerous cystic and atretic follicles. C: In the vitamin E treated rats, the granulosa cells, antral and preantral follicles are normal, with little or no Bax expression. D: Administration of vitamin E to DHEA-treated rats causes a reduction in follicular cysts and reduces Bax expression in the antral follicles. AF: antral follicle; GC: granulosa cell; CY: cystic follicle.

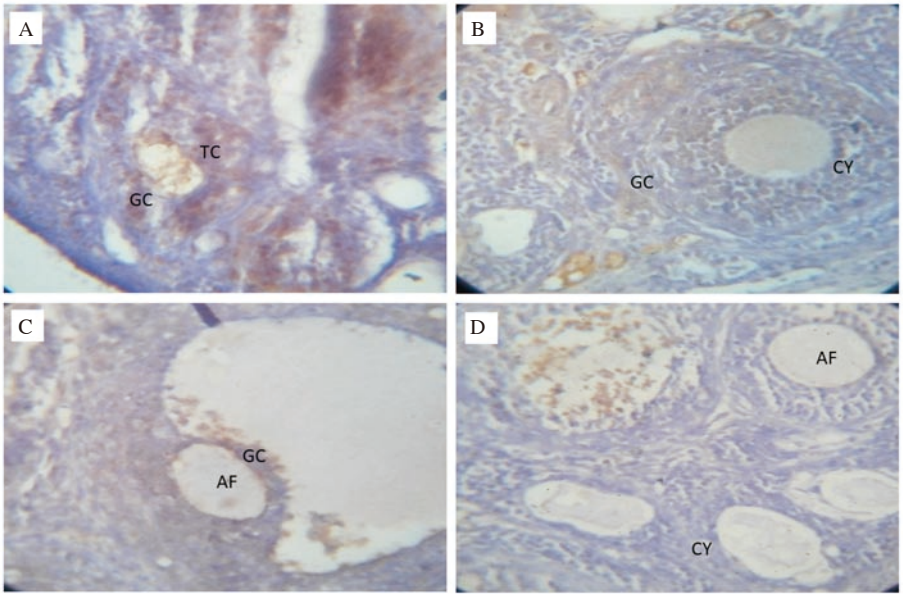

Figure 5. The immunohistochemical expression of Bcl-2 anti-apoptotic protein in the ovaries of rats (immunohistochemical staining; ×40 magnification). A: In the control rats, the preantral follicles, theca cells and granulosa cells appear normal, while Bcl-2 is mainly expressed in the granulosa cells of the antral follicles. B: In the DHEA-exposed rats, there is little or no Bcl-2 expression in the antral follicles with numerous cystic follicles. C: In the vitamin E treated rats, there is the localization of Bcl-2 in the granulosa cells of the antral follicles, while the antral follicle also appears normal. D: Administration of vitamin E to DHEA-exposed rats causes a reduction in follicular cysts, with mild expression of Bcl-2 in the antral follicles. TC: theca cell; GC: granulosa cell; CY: cystic follicle; AF: antral follicle.

3.7.4. Immunohistochemical expression of E–cadherin in the ovaries

In the control rats, *E*-cadherin was adequately expressed and majorly localized in the interstitium of theca cells (Figure 6A). In the DHEA-exposed rats, there were numerous cystic follicles with the localized expression of *E*-cadherin in the stromal area, indicating a decrease in follicular growth (Figure 6B). In the vitamin E treated rats, the antral follicle and granulosa cells appeared normal with adequate *E*-cadherin expression suggesting normal follicular development (Figure 6C). Administration of vitamin E to DHEA-exposed rats caused a reduction in the number of follicular cysts. Also, there was a reduced *E*-cadherin expression in the antral follicle and increased expression in the granulosa cells (Figure 6D).

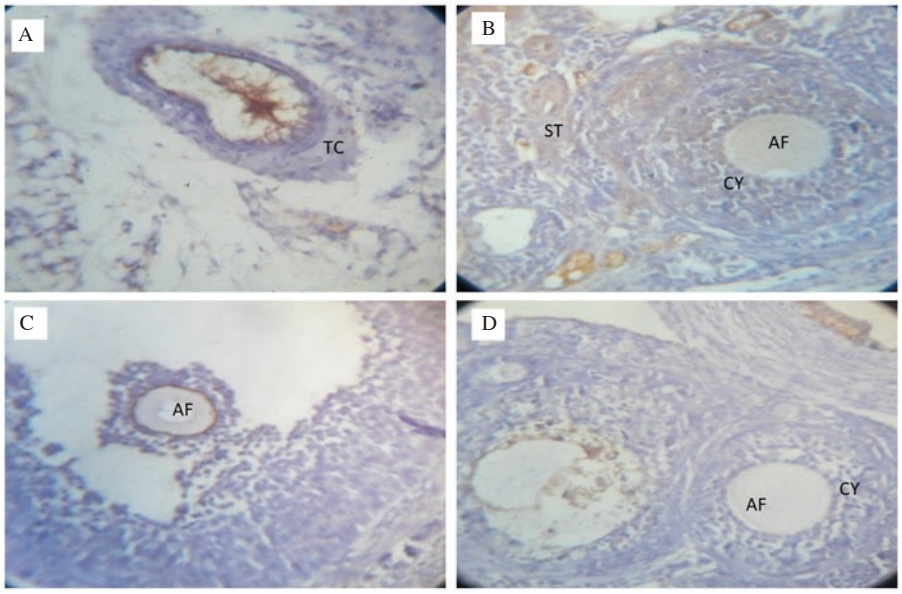

Figure 6. Immunohistochemical expression of *E*-cadherin in the ovaries of rats (immunohistochemical staining; ×40 magnification). A: In the control rats, *E*-cadherin is adequately expressed and majorly localized in the interstitium of theca cells. B: In the DHEA-exposed rats, there are numerous cystic follicles with the localized expression of *E*-cadherin in the stromal area, indicating a decrease in follicular growth. C: In the vitamin E treated rats, the antral follicle and granulosa cells appear normal with adequate *E*-cadherin expression, suggesting normal follicular development. D: Administration of vitamin E to DHEA-exposed rats causes a reduction in the number of follicular cysts. Also, there is a reduced *E*-cadherin expression in the antral follicle and increased expression in the granulosa cells.

4. Discussion

This study evaluated the effects of vitamin E on DHEA-induced polycystic ovary. We demonstrated that exogenous administration of vitamin E inhibited androgen production through anti-inflammatory and antioxidant pathways and the downregulation of androgen receptor genes in the granulosa cells of the ovary. Polycystic ovary is among the major endocrine abnormalities affecting women of reproductive age and is primarily implicated in anovulatory infertility[26].

The pathogenesis of PCOS shows that oxidative stress is a major cause of this syndrome, which can be indicated by the significant alteration between free radical production and detoxification[27]. In this study, DHEA treatment significantly increased MDA levels, resulting from increased free radicals and lipid peroxidation production that compromise cell membrane integrity. However, vitamin E supplementation in DHEA-exposed rats significantly reduced the MDA (a biomarker of lipid peroxidation) level. This result is in accordance with the report by van Dam *et al*[28], where it was shown that vitamin E inhibited lipid peroxidation to protect against endothelial damage. This implies that the efficacy of vitamin E as a reducing agent and chain-breaking antioxidant can reduce free radical production to inhibit lipid peroxidation that compromises cell membrane integrity. Furthermore, the administration of vitamin E improved antioxidant enzyme activity in the DHEA-exposed animals. The improved antioxidant capacity in the vitamin E treated DHEA group implies that this flavonoid may upregulate the antioxidant signaling cascade to enhance antioxidant enzyme activity, thereby reducing the production of reactive oxygen species, and detoxifying the harmful effect of free radicals. This can also be attributed to the inherent potential of vitamin E to improve the concentration of non-enzymatic antioxidants, thereby reducing the detrimental effect of free radicals.

Chronic exposure to estrogen without sufficient progesterone is also a major factor promoting hyperplasia in PCOS[29]. The present study showed that exposure to DHEA significantly decreases progesterone concentration. The reduced progesterone level in the DHEA rats could be due to alterations in gonadotropin-releasing hormone neurons (especially the GABAergic neurons) in the hypothalamus, which cause increased gonadotropin-releasing hormone (GnRH) pulsatile secretion, resulting in low plasma follicle-stimulating hormone. Thus, women with PCOS need high progesterone to reduce GnRH pulsatile secretion[30]. Interestingly, co-administration of vitamin E and DHEA significantly increased progesterone concentration compared to the DHEA-treated group, while synergistic administration of vitamin E plus DHEA significantly increased estrogen concentration compared to the DHEA treatment alone. This is in line with other studies that reported increased progesterone levels during vitamin E supplementation[31,32]. Vitamin E is essential and potent in hormone production in the pituitary-gonadal axis. It increases the size and area of gonadotropic cells, thereby promoting the synthesis and secretion of gonadotropins in the anterior pituitary with a resultant increase in progesterone secretion by the gonads[33].

In this study, supplementation of vitamin E to DHEA-exposed rats improved Na^+/K^+, Ca^{2+}, and H^+ ATPase activities. A similar result has been reported by Ademoğlu *et al*[34], where vitamin E protects ATPase activities in various tissues of hypercholesterolemic rats. These biochemical ATPase enzymes are important biomarkers for health and play a vital role in several biochemical processes in the body. For instance, Na^+/K^+ ATPase is crucial for epithelial polarization needed for tissue development and optimal function[35,36], suggesting that vitamin E may improve ovarian cell development and enhance the adequate movement of nutrients across

the cell membrane. The improved ATPase activity in the vitamin E treated DHEA rats group implies that this flavonoid may exert therapeutic benefit to improve ovarian granulosa cell function, which is compromised by excessive androgen exposure.

Chronic inflammation is another risk factor of PCOS, with reports showing that inflammatory biomarkers such as TNF-α and interleukin-6 are often increased in women with PCOS compared with healthy women[37]. Evidence has shown that oxidative stress and inflammation are interrelated and are usually accompanied by each other[38]. The result obtained from this study showed that DHEA significantly increased VEGF and TNF-α levels, which may be correlated with the increased ovarian VEGF levels reported in PCOS patients, thus providing credence to the DHEA-induced polycystic ovary model used in this study. Interestingly, co-treatment with vitamin E and DHEA significantly decreases inflammatory markers compared to DHEA treatment only. The reduced inflammatory cytokines in the vitamin E treated group could be attributed to the inherent anti-inflammatory activity of this biomolecule to downregulate NF-kβ, a major transcription factor in the inflammatory pathway[39].

The result from this study showed that vitamin E significantly downregulates the mRNA expression of granulosa cell androgen receptor. Similar studies have shown that this flavonoid can inhibit androgen receptor function in prostate cancer cells. This result provides further evidence of the efficacy of vitamin E in modulating the transcription and post-transcription signaling cascade of the androgen receptor gene and inhibiting the synthesis of androgen receptor mRNA, thereby resulting in the downregulation of its expression[40,41].

The histopathological and immunohistochemical analysis of the DHEA-treated ovary showed numerous cystic follicles, high collagen level around the follicle with no corpora lutea, and apoptotic damage characterized by increased Bax-positive area. However, the number of cystic follicles and collagen levels around the follicles reduces, with reduced apoptosis in the granulosa cells as evident by the reduced Bax positive area as well as increased Bcl-2 and *E*-cadherin positive area in the DHEA-exposed group treated with vitamin E. The improved ovarian morphology in the vitamin E treated group suggests that this flavonoid prevents structural derangement in the ovaries *via* its anti-apoptotic property. The reduction of granulosa cell death by vitamin E can be due to this flavonoid's efficacy in neutralizing superoxide and other reactive oxygen species that cause lipid peroxidation and compromise cell membrane integrity. Also, the decreased number of cystic follicles and collagen content by vitamin E implies that this flavonoid mitigated abnormal folliculogenesis induced by DHEA.

This study has some limitations. The study provides some preliminary data to support the potential benefits of vitamin E in the management of PCOS. However, due to the paucity of funds, the detailed mechanism underlying the therapeutic effect of this flavonoid could not be evaluated. Therefore, this has been the major objective of our further study.

In conclusion, the study findings have shown that PCOS is associated with excess oxidative stress and decreased antioxidant reserves. This study demonstrates that vitamin E supplementation could alleviate female reproductive dysfunction *via* its antioxidant and anti-inflammatory properties.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Funding

This study received no extramural funding.

Authors' contributions

Olugbemi T Olaniyan conducted conceptualization, design, methodology, formal analysis, and supervision; Ayobami Dare performed formal analysis, data curation, methodology, writing-review and editing; Charles O. Adetunji carried out writing-review and editing; Gloria E. Okotie was responsible for data curation, formal analysis, and writing-draft; Joseph B. Dare conducted design, methodology and formal analysis; Bosun M. Adigun performed data curation, and writing-original draft; Femi Adebayo was responsible for supervision, writing-original draft and formal analysis.

References

[1] Bas D, Abramovich D, Hernandez F, Tesone M. Altered expression of Bcl-2 and Bax in follicles within dehydroepiandrosterone-induced polycystic ovaries in rats. *Cell Biol Int* 2011; **35**(5): 423-429.

[2] Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, et al. Positions statement: Criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: An Androgen Excess Society Guideline. *J Clin Endocrinol Metab* 2006; **91**(11): 4237-4245.

[3] Elia E, Sander V, Luchetti CG, Solano ME, Di Girolamo G, Gonzalez C, et al. The mechanisms involved in the action of metformin in regulating ovarian function in hyperandrogenized mice. *Mol Hum Reprod* 2006; **12**(8): 475-481.

[4] Lee DK, Kurihara I, Jeong JW, Lydon JP, DeMayo FJ, Tsai MJ, et al. Suppression of ERalpha activity by COUP-TFII is essential for successful implantation and decidualization. *Mol Endocrinol* 2010; **24**(5): 930-940.

[5] Agarwal A, Aponte-Mellado A, Premkumar BJ, Shaman A, Gupta S. The effects of oxidative stress on female reproduction: A review. *Reprod Biol Endocrinol* 2012; **10**(1): 49.

[6] Oborna I, Wojewodka G, De Sanctis JB, Fingerova H, Svobodova M, Brezinova J, et al. Increased lipid peroxidation and abnormal fatty acid profiles in seminal and blood plasma of normozoospermic males from infertile couples. *Hum Reprod* 2010; **25**(2): 308-316.

[7] Çelik LS, Kuyucu Y, Yenilmez ED, Tuli A, Dağlıoğlu K, Mete U. Effects of vitamin D on ovary in DHEA-treated PCOS rat model: A light and electron microscopic study. *Ultrastruct Pathol* 2018; **42**(1): 55-64.

[8] Hsueh AJ, Billig H, Tsafriri A. Ovarian follicle atresia: A hormonally

controlled apoptotic process. *Endocr Rev* 1994; **15**(6): 707-724.

[9] Belgorosky D, Sander VA, Yorio MP, Faletti AG, Motta AB. Hyperandrogenism alters intraovarian parameters during early folliculogenesis in mice. *Reprod Biomed Online* 2010; **20**(6): 797-807.

[10]Hsueh AJ, Kawamura K, Cheng Y, Fauser BC. Intraovarian control of early folliculogenesis. *Endocr Rev* 2015; **36**(1): 1-24.

[11]Aldana L, Tsutsumi V, Craigmill A, Silveira MI, Gonzalez de Mejia E. alpha-Tocopherol modulates liver toxicity of the pyrethroid cypermethrin. *Toxicol Lett* 2001; **125**(1-3): 107-116.

[12]Boscoboinik D, Szewczyk A, Hensey C, Azzi A. Inhibition of cell proliferation by alpha-tocopherol. Role of protein kinase C. *J Biol Chem* 1991; **266**(10): 6188-6194.

[13]Fraser PD, Bramley PM. The biosynthesis and nutritional uses of carotenoids. *Prog Lipid Res* 2004; **43**(3): 228-265.

[14]Ikeda K, Baba T, Morishita M, Honnma H, Endo T, Kiya T, et al. Long-term treatment with dehydroepiandrosterone may lead to follicular atresia through interaction with anti-Mullerian hormone. *J Ovarian Res* 2014; **7**: 46.

[15]Heydari M, Ahmadizadeh M, Ahmadi Angali K. Ameliorative effect of vitamin E on trichloroethylene-induced nephrotoxicity in rats. *J Nephropathol* 2017; **6**(3): 168-173.

[16]Olaniyan OT, Bamidele O, Adetunji CO, Priscilla B, Femi A, Ayobami D, et al. Quercetin modulates granulosa cell mRNA androgen receptor gene expression in dehydroepiandrosterone-induced polycystic ovary in Wistar rats *via* metabolic and hormonal pathways. *J Basic Clin Physiol Pharmacol* 2020; **31**(4). doi: 10.1515/jbcpp-2019-0076.

[17]Beauchamp C, Fridovich I. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Anal Biochem* 1971; **44**(1): 276-287.

[18]Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem* 1972; **247**(10): 3170-3175.

[19]Sinha AK. Colorimetric assay of catalase. *Anal Biochem* 1972; **47**(2): 389-394.

[20]Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *J Biol Chem* 1974; **249**(22): 7130-7139.

[21]Jollow DJ, Mitchell JR, Zampaglione N, Gillette JR. Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. *Pharmacology* 1974; **11**(3): 151-169.

[22]Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193**(1): 265-275.

[23]Olaniyan OT, Femi A, Iliya G, Ayobami D, Godam E, Olugbenga E, et al. Vitamin C suppresses ovarian pathophysiology in experimental polycystic ovarian syndrome. *Pathophysiology* 2019; **26**(3-4): 331-341.

[24]Evans DJ Jr. Membrane adenosine triphosphatase of *Escherichia coli*: Activation by calcium ion and inhibition by monovalent cations. *J Bacteriol* 1969; **100**(2): 914-922.

[25]Bonting SL. Sodium potassium activated adenosine triphosphotase and cation transport. In: Bittdr EE. (ed.) *Membranes and ion transport*. vol 1. London: Wiley Interscience; 1970, p. 257-363.

[26]Glintborg D. Endocrine and metabolic characteristics in polycystic ovary syndrome. *Dan Med J* 2016; **63**(4): B5232.

[27]Pisoschi AM, Pop A. The role of antioxidants in the chemistry of oxidative stress: A review. *Eur J Med Chem* 2015; **97**: 55-74.

[28]van Dam B, van Hinsbergh VW, Stehouwer CD, Versteilen A, Dekker H, Buytenhek R, et al. Vitamin E inhibits lipid peroxidation-induced adhesion molecule expression in endothelial cells and decreases soluble cell adhesion molecules in healthy subjects. *Cardiovasc Res* 2003; **57**(2): 563-571.

[29]Dunaif A, Segal KR, Shelley DR, Green G, Dobrjansky A, Licholai T. Evidence for distinctive and intrinsic defects in insulin action in polycystic ovary syndrome. *Diabetes* 1992; **41**(10): 1257-1266.

[30]Velija-Ašimi Z. Evaluation of endocrine changes in women with the polycystic ovary syndrome during metformin treatment. *Bosn J Basic Med Sci* 2013; **13**(3): 180-185.

[31]Yin L, Ran J, Lian T, Yang C, Li S, Liu Y. Effects of vitamin E supplementation on serum hormones and gene expression of anti-season breeding Xingguo grey geese (*Anser cygnoides*). *Braz J Poult Sci* 2019; **21**(4). doi: 10.1590/1806-9061-2018-0892.

[32]Tefagh G, Payab M, Qorbani M, Sharifi F, Sharifi Y, Ebrahimnegad Shirvani MS, et al. Effect of vitamin E supplementation on cardiometabolic risk factors, inflammatory and oxidative markers and hormonal functions in PCOS (polycystic ovary syndrome): A systematic review and meta-analysis. *Sci Rep* 2022; **12**(1): 5770.

[33]Mumford SL, Browne RW, Schliep KC, Schmelzer J, Plowden TC, Michels KA, et al. Serum antioxidants are associated with serum reproductive hormones and ovulation among healthy women. *J Nutr* 2016; **146**(1): 98-106.

[34]Ademoğlu E, Gökkuşu C, Palandüz S. Vitamin E and ATPases: Protection of ATPase activities by vitamin E supplementation in various tissues of hypercholesterolemic rats. *Int J Vitam Nutr Res* 2000; **70**(1): 3-7.

[35]Rajasekaran SA, Palmer LG, Quan K, Harper JF, William J. Ball J, et al. Na, K-ATPase β-subunit is required for epithelial polarization, suppression of invasion, and cell motility. *Mol Biol Cell* 2001; **12**(2): 279-295.

[36]Nejsum LN, Nelson WJ. Epithelial cell surface polarity: The early steps. *Front Biosci (Landmark Ed)* 2009; **14**(3): 1088-1098.

[37]Gao L, Gu Y, Yin X. High serum tumor necrosis factor-alpha levels in women with polycystic ovary syndrome: A meta-analysis. *PLoS One* 2016; **11**(10): e0164021.

[38]Siti HN, Kamisah Y, Kamsiah J. The role of oxidative stress, antioxidants and vascular inflammation in cardiovascular disease (a review). *Vascul Pharmacol* 2015; **71**: 40-56.

[39]Glauert HP. Vitamin E and NF-kappaB activation: A review. *Vitam Horm* 2007; **76**: 135-153.

[40]Zhang Y, Ni J, Messing EM, Chang E, Yang CR, Yeh S. Vitamin E succinate inhibits the function of androgen receptor and the expression of prostate-specific antigen in prostate cancer cells. *Proc Natl Acad Sci U S A* 2002; **99**(11): 7408-7413.

[41]Lee DK, Chang C. Expression and degradation of androgen receptor: Mechanism and clinical implication. *J Clin Endocrinol Metab* 2003; **88**(9): 4043-4054.

Publisher's Note

The Publisher of *the Journal* remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.