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ORIGINAL ARTICLE

Effect of Ubiquinol on Serum Reproductive Hormones of Amenorrhic Patients

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Abstract In neuroendocrine system the increase in oxidative status is produced by a glucocorticoid—dependent and transcriptional increase in pro-oxidative drive, with concurrent inhibition of the antioxidant defense system, ultimately leading to increased neuronal cell death. Functional hypothalamic disturbances and neuroendocirne aberrations have both short and long term consequences for reproductive health. Understandably, an impaired or diminished hypothalamic–pituitary–ovarian axis leads to anovulation and hypoestrogenism. Anovulation is directly linked to the neurohormonal and hormonal background of Functional Hypothalamic Amenorrhea. Impairment of pulsatile Gonadotropin Releasing Hormone secretion causes the impairment of pulsatile Lutenizing Hormone (LH) and Follicle Stimulating Hormone (FSH) secretion. The importance of oxidative stress in various pituitary disorders suggesting a possible clinical usefulness of antioxidant molecules like the lipophilic antioxidant Ubiquinol. Coenzyme Q10 or Ubiquinol is an essential part of the cell energy-producing system of mitochondria. However, it is also a powerful lipophilic antioxidant, protecting lipoproteins and cell membranes from autooxidation. Due to these unique actions Ubiquinol is used in clinical practice as an antioxidants for neurodegenerative diseases. So to identify the role of Ubiquinol on reproductive hormones FSH and LH, we have included 50 infertile patients of age group of 20–40, which are mostly amenorrhic. Out of 50 only 30 patients were in continuous follow up after supplementing them with 150 mg of Ubiquinol every day for 4 months. The hormonal levels were estimated by Enzyme Linked Immuno Sorbent Assay technique at follicular phase. The result suggests that FSH concentration is increased up to three times (from 3.10 ± 2.70 to 10.09 ± 6.93) but remains within the normal limit ($P < 0.05$). LH values were found doubled ($P < 0.05$) than its normal range (from 14.83 ± 10.48 to 27.85 ± 22.30). The Prolactin values were decreased while Progesterone values were high but not in the significant range ($P > 0.05$). The supplementation of 150 mg of Ubiquinol may reduce the oxidative stress in neuroendocrine system which further improves the function of diminished HPA axis. Hence increased level of FSH and LH may be due to reduced oxidative stress by Ubiquinol.

Keywords Ubiquinol · Functional Hypothalamic Amenorrhea (FHA) · Hypothalamic–Pituitary–Ovarian axis (HPA axis) · Follicular phase · Lipophilic antioxidant

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Introduction

Infertility can be defined as a lack of pregnancy after 1 year of regular unprotected intercourse. Approximately 15–20 % of couples of reproductive age are infertile, which can be attributed equally to both male and female factors [1]. Amenorrhea is one of the major causes of female

infertility. Functional Hypothalamic Amenorrhea (FHA) is one of the most common causes of secondary amenorrhea, which is mostly due to stress and exercise-related. Functional hypothalamic disturbances and neuroendocirne aberrations have both short and long term consequences for reproductive health. An impaired or diminished hypothalamic–pituitary–ovarian axis leads to anovulation and hypoestrogenism. Lack of estradiol cyclical changes and progesterone concentrations leads to abolished endometrial cyclicity and typically endometrium continues to persist in early proliferative phase. Anovulation is directly linked to the neurohormonal and hormonal background of FHA. Impairment of pulsatile Gonadotropin Releasing Hormone (GnRH) secretion causes the impairment of pulsatile Lutenizing Hormone (LH) and Follicle Stimulating Hormone (FSH) secretion. The basic approach to this distinction is an assessment of the gonadotropins and identifying hypo-gonadotrophic hypogonadism. If such a diagnosis has been made, the key diagnostic tool is a GnRH stimulation test, which in the case of FHA shows a positive response of the gonadotropins to exogenous GnRh [2].

Organisms survive by maintaininga dynamic equilibrium with their environment [3]. Oxidative stress, a condition defined as unbalancing between production of free radicals and antioxidant defenses, is an important pathogenetic mechanism in different diseases [4]. The glucocorticoids released from the adrenal gland in response to stress—induced activation of the hypothalamic–pituitary–adrenal (HPA) axis induce activity in the cellular reduction–oxidation (redox) system. It also induces neuronal oxidative stress directly through enhanced mitochondrial respiration and oxidative phosphorylation. The increase in oxidative status is produced by a glucocorticoid—dependent and transcriptional increase in pro-oxidative drive, with concurrent inhibition of the antioxidant defense system, ultimately leading to increased neuronal cell death. Although oxidative stress and elevated glucocorticoids are both observed in a number of chronic pathologies, the delineation between physiological function and pathological insult is complex and remains unclear. This may have important implications for neurodegenerative conditions involving redox-sensitive regions. These observations highlight that maintenance of a balanced redox state is critical for normal cellular homeostatic function and relies heavily on hormonal cues from the neuroendocrine stress system [5].

Reactive Oxygen Species (ROS) appears to have physiological role oocyte maturation, fertilization, luteal regression, and endometrial shedding of female reproductive system [6]. Estimation of ROS levels in follicular fluid may be used as marker for predicting the success of in vitro fertilization (IVF) [7]. Oxidative stress can affect the female fertility potential in number of ways such as ovulation, fertilization, embryo development and implantation. The sources of ROS in Graffian follicle may be macrophages, neutrophils and granulose cells. Follicular fluid contains high levels of antioxidants, which protect oocytes from oxidative damage. Elevated levels of oxidants can damage the ovum after its release from the ovary, the zygote/embryo and most importantly, spermatozoa, which is very sensitive to oxidative stress [8, 9].

Low antioxidants and high reactive oxygen species (ROS) are observed with age, and also in sera and peritoneal fluid of women with idiopathic infertility, and elevated follicular fluid homocysteine was observed in women with endometriosis [9–16]. Oocyte ROS exposure associated with intracellular signaling which induces angiogenic response, resumption of meiosis I at puberty, decreased fertilization and blastocyst development, decline in ovarian function, decreased steroidogenesis [17–21] and inhibit thecal interstitial cell proliferation in corpus luteal function [22].

Recent research on the role of reactive oxygen species (ROS) in human infertility has received a great deal of interest from the scientists and medical practitioners, which lead into the development of newer therapeutic approaches to treat infertility with antioxidants [1]. Co-enzyme Q10 (Co-Q10), also known as ubiquinone for its presence in all body cells and as an essential part of the cell energy—producing system. However, it is also a powerful lipophilic antioxidant protecting lipoproteins and cell membranes from its autooxidation. Ubiquinol inhibits lipid peroxidation by preventing the production of lipid peroxyl radicals (LOO). [$L^* + CoQH_2 \rightarrow LH + CoQ^{*-}$, $LO^* + CoQH_2 \rightarrow LOOH + CoQ^{*-}$]. $CoQH_2$ reduces the initial perferryl radical and singlet oxygen [$CoQH_2 + \rightarrow Fe^{+++} - O_2{}^{*-} \rightarrow CoQ^{*-} \rightarrow + Fe^{++++}H_2O$]. It also

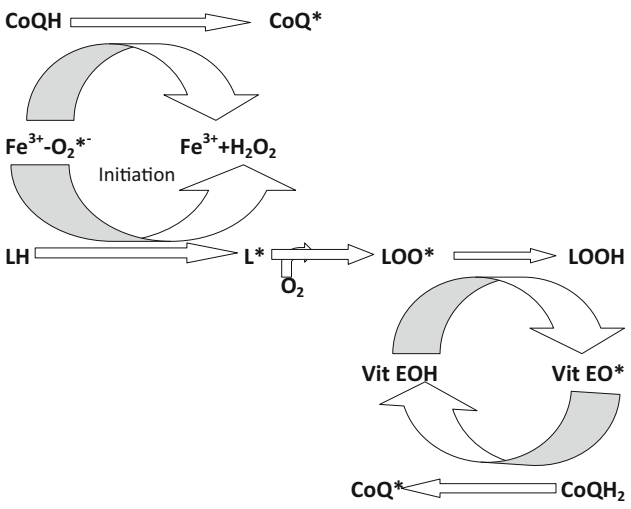

Fig. 1 Schematic diagram of antioxidant activity of Ubiqinol

regenerates other antioxidants such as vitamin E. [Vit $E - O^* + CoQH_2 \rightarrow VitE - OH + CoQ^{*-}$] (Fig. 1). Due to these properties, of Ubiquinol which is *r*educed form of CoQ10 is an active form and commonly used in clinical practice in chronic heart failure, infertility, and neurodegenerative diseases, etc.,. Ubiquinol level can be affected by different mechanism; its low levels in plasma, in fact, can be due to accelerated metabolism and/or consumption, such as in hyperthyroidism and acromegaly, or a reduced synthesis, such as in hypoadrenalism and hypogonadism. The CoQ10 oral supplementation as a support to the specific endocrine therapy, has already been demonstrated in many cases [23]. The importance of oxidative stress in various pituitary disorders, suggesting a possible clinical usefulness of antioxidant molecules like the lipophilic antioxidant Ubiquinol [4].

Due to the above mentioned antioxidant property of Ubiquinol and its significance, we have decided to use Ubiquinol supplementation in amenorrhic patients as one of the cause of the amenorrhea is oxidative stress. Our main objective was to provide the antioxidant therapy for the prevention of oxidative damage which causes diminished hypothalamic–pituitary–ovarian axis leads to anovulation and hypoestrogenism. The stress induced Function Hypothalamic Amenorrhea (FHA) is increasing due to stressful social life style of the womens. In nowadays the need to treat infertility is gaining importance as the frequency of FHA patients reporting to the hospital was found increasing. Therefore we have undertaken the study i.e., "supplementation of Ubiquinol to the amenorrhic patients" considering it may be useful to reduce the oxidative stress and may induce the formation of FSH and LH hormones which are essential for the formation and maturation of follicles as well as development of oocytes.

Materials and Methods

The 50 subjects were taken of the age group 20–40 with known cases of amenorrhea related to profound impairment of reproductive functions including anovulation and infertility. Out of 50 only 30 patients were in continuous follow up after supplementing with 150 mg of Ubiquinol per day for continuous 4 months. The parameters studied are FSH, LH Prolactin and Progesterone. A total number of 30 healthy and normal individuals with no previous history of any medical problems, with in the age group of 20–40 were taken as controls for the study without Ubiquinol supplementation.

Patients with known previous history of endometriosis, polycystic ovarian syndrome, abdominal tuberculosis, diabetes, hypothyroidism, hyperthyroidism and patients with known mental disturbances were excluded from the study.

5 ml of blood sample was collected and the hormonal level was estimated at the first day of the follicular phase and after 2 and 4 months successively by Enzyme Linked Immuno Sorbent Assay (ELISA) method using Biorad instrument.

The Hormonal level is measured by ELISA technique using Thermo scientific instrument. The testosterone assay procedure is as follows.

Bring all the kit components and the test serum to room temperature (20–25 °C) prior to the start of the assay. One set of Standards should be run with each batch of test serum. Secure the desired number of coated wells in the holder. Record the position of the standards and the test serum on the EIA date recording sheet provided. Unused strips should be resealed in the foil bag containing the desiccant, using the resealing Zip-lock before being replaced at 2–8 °C. Dispense 10 µl of standards, test serum and controls into the appropriate wells. Dispense 100 µl of Hormone HRP conjugate reagent into each well. Dispense 50 µl of rabbit anti-hormone reagent to each well and mix for 30 s. Place in a wet box with some moist paper. Incubate at 37 °C for 90 min. At the end of the incubation period, discard the contents of the wells by flicking plate contents into a Biohazard container. Then strike the wells sharply against absorbent paper.

Hand Washing: Fill the wells with a minimum of 300 µl of distilled water per well. Flick plate contents into a Biohazard container, then strike the wells sharply against absorbent paper or paper towel to remove all residual water droplets.

Machine Washing: Ensure that 300 µl of distilled water is dispensed per well and that an appropriate disinfectant is added to the water collection bottle. Wash the empty wells five times. After washing remove excess fluid by striking the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.

Dispense 100 µl of substrate solution into each well and gently mix for 5 s. Incubate in the dark at room temperature for 20 min (20–25 °C). Stop the reaction by adding 100 µl of stop solution to each well, gently mix for 30 s. Read the absorbance at their respective wavelengths generally at 420 nm with microtitre well reader within 10 min [24].

Results and Discussion

The result suggests that FSH concentration is increased approximately three times (Fig. 2; Table 1) from 3.10 ± 2.70 to 10.09 ± 6.93 but remains within the

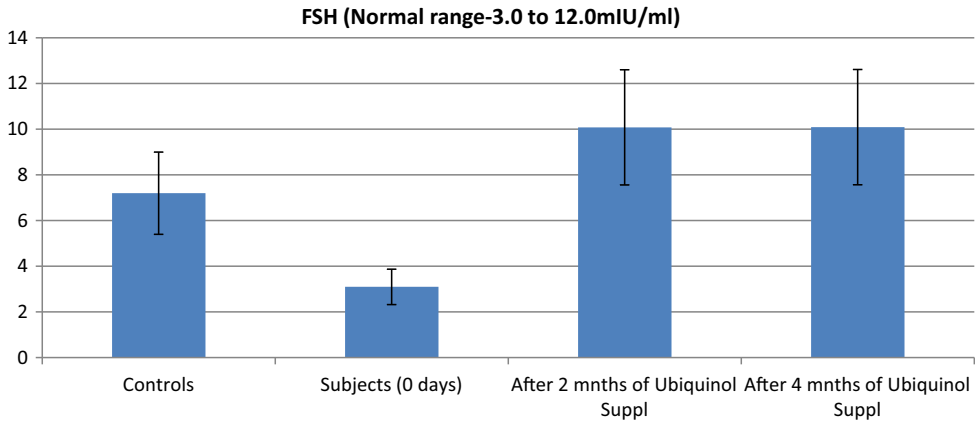

Fig. 2 Figure showing the statistically significant levels of serum FSH after Ubiquinol supplementation

Table 1 Table showing comparative values of serum reproductive hormones before and after supplementation of 150 mg of Ubiquinol per day

Hormone (normal range in follicular phase)	Controls (mean ± SD) (n = 30)	Subjects (0 days) (mean ± SD) (n = 30)	After 2 months of Q10 suppl (mean ± SD) (n = 30)	After 4 months of Q 10 suppl (Mean ± SD) (n = 30)	*P* value
FSH (3.0–12.0 mIU/ml)	7.2 ± 1.61	3.10 ± 2.70	10.08 ± 6.93*	10.09 ± 6.95*	*
LH (0.5–10.5 mIU/ml)	5.3 ± 1.45	14.83 ± 10.48	27.85 ± 22.30*	28.02 ± 21.18*	*
Prolactin (1.2–19.5 ng/ml)	9.6 ± 1.61	34.71 ± 27.88	32.26 ± 19.88	31.03 ± 19.53	ns
Progesterone (0.15–0.70 ng/ml)	0.45 ± 0.11	1.72 ± 1.30	2.40 ± 1.45	2.40 ± 1.43	ns

ns not significant; *n* number of patients

* Significant from 0 days

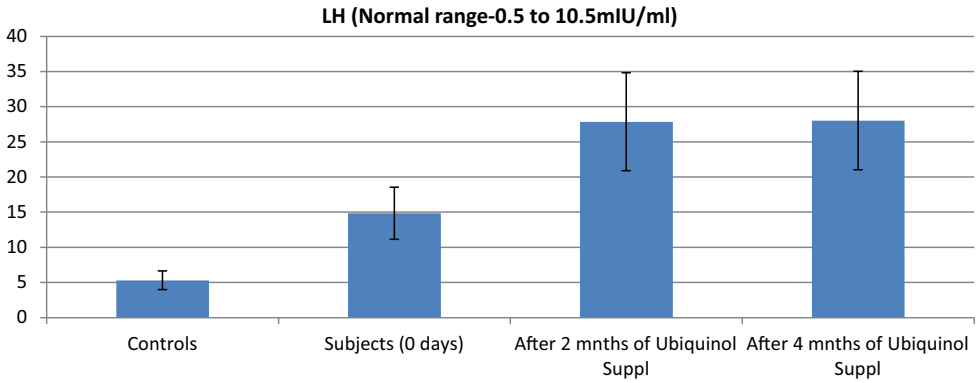

Fig. 3 Figure showing the statistically significant levels of serum LH after Ubiquinol supplementation

normal limit ($P < 0.05$) which is significant. LH values were found doubled than its normal range (Fig. 3; Table 1) from 14.83 ± 10.48 to 27.85 ± 22.30 ($P < 0.05$) which is also significant. The Prolactin values were decreased (Fig. 4; Table 1) from 34.71 ± 27.88 to 32.26 ± 19.88 ($P > 0.05$) which is not significant while Progesterone values were approximately 40 % high (Fig. 5; Table 1) from 1.72 ± 1.30 to 2.40 ± 1.45 ($P > 0.05$) but not in the significant range.

Decreased antioxidant capacity during aging is associated with decreased body weight and sex hormones in mouse model [25]. Due to lipophilic antioxidant nature of CoQ10 the various studies has been done on endocrine system. The CoQ10 levels were significantly lower in isolated hypoadrenalism than in patients with adrenal hyperplasia and multiple pituitary deficiencies. The study indicates that secretion of adrenal hormones is in some way related to CoQ10 levels, both in augmented and reduced conditions. However in secondary hypoadrenalism, some other pituitary dependent axes can be affected. It has been also observed that thyroid hormones play an important role in modulating CoQ10 levels and metabolism. The CoQ10 level was significantly observed low in hypogonadism than in normal individuals. The testosterone treatment induced a

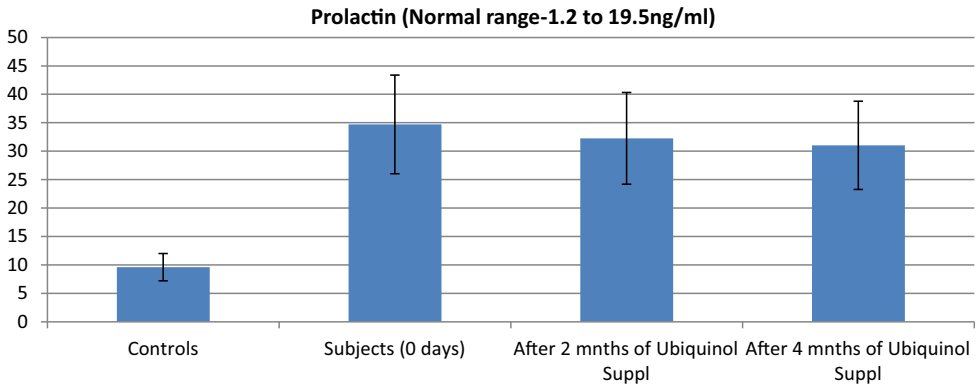

Fig. 4 Figure showing the statistically no significant levels of serum Prolactin after Ubiquinol supplementation

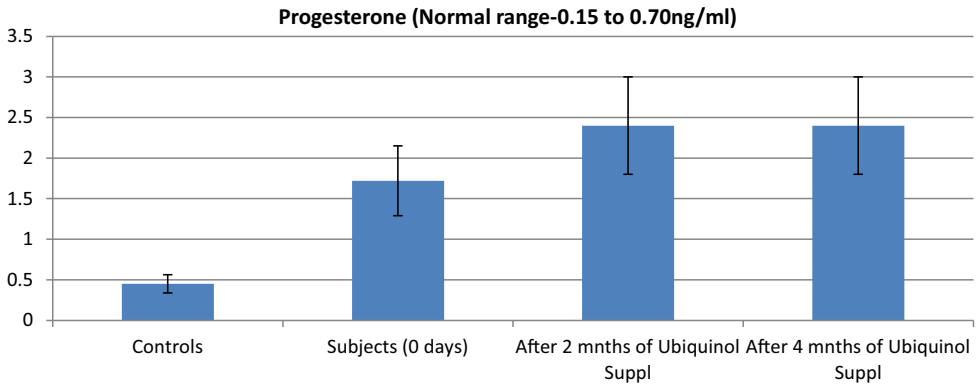

Fig. 5 Figure showing the statistically no significant levels of serum Progesterone after Ubiquinol supplementation

significant change both in CoQ10 level and in Total Antioxidant Capacity. The CoQ10 and total antioxidant capacity values were significantly correlated, suggesting an interrelationship between different antioxidants. The study also indicates that the low CoQ10 values in acromegalic patients harboring a GH-secreting pituitary adenoma. This is probably growth hormone influences CoQ10 consumption due to accelerated metabolism [23].

The report shows that enhancement of ability to conceive was remarkably increased with higher antioxidants in follicular fluid with successful IVF [26]. The antioxidants promote embryonic germ cell proliferation, cytoplasmic maturation of meiosis II oocytes, induces thecal interstitial cell proliferation, corpus luteal collagen formation, dominant follicle selection, and aid in meiotic spindle formation in mature meiosis II oocytes [27–32]. Antioxidant supplements vitamin C and E, improved the embryo development in aging mouse model and Nitric Oxide (NO) may aid in antiplatelet action during implantation [33, 34]. These significant findings suggests that antioxidant therapy may be useful in treating female infertility.

When young rats were subjected to oxidative stress in the form of hyperoxia, hyperoxides increased markedly in the HPA axis and after treatment with vitamin E, inhibited such increased in lipid peroxides in each organ. Furthermore, glucocorticosteroid receptors in pyramidal cells in the cornus ammonis I region of the hippocampus in young rats were markedly decreased by oxidative stress. Similar phenomena were also observed in normal aged rats and young rats fed vitamin E-deficient diet kept in a normal atmosphere. Vitamin E supplementation prevented the decrease in glucocorticosteriod receptors in the hippocampus and the increase in corticosterone secretion caused by hyperoxia [35]. All these parameters are giving positive response towards antioxidant therapy.

In our study the result suggests that the FSH by its action on the ovarian follicle causes: development and proliferation of granulosa cells, formation of estrogen, development of FSH and LH receptors on granulose cells. LH causes ovulation, production of progesterone from lutein cells, conversion of granulose cells into lutein cells, maintenance of corpus luteum (CL). In hypopituitarism, deficiencies of luteinizing hormone and follicle stimulating hormone can produce amenorrhea and infertility in women, and impotence and infertility in men. Further, elevated prolactin levels suppress FSH and LH. In fact, high levels (hyperprolactinemia) can be considered nature's contraceptive. The high concentration of progesterone suppresses the LH and thus ovulation is prevented. Progesterone is thus used in oral contraceptive pill to produce anovulation. In the non

pregnant women, LH causes ovulation and maintenance of corpus luteum and in turn secretes progesterone. But continued presence of progesterone causes extinction of LH, which degenerates corpus luteum and stoppage of progesterone secretion. In this study the secretion of progesterone has not found significantly, which may not having degenerative effect on corpus luteum. Thus the cyclicity of progesterone is explained. The increased secretion of FSH and LH after the supplementation of 150 mg of Ubiquinol per day in reduced form might have also reduce the oxidative stress by its lipophilic antioxidant activity which might have normalizes the function of hypothalamic–pituitary–ovarian axis leads to normal secretion of FSH and LH.

Conclusion

The study concludes that, the supplementation of 150 mg of Ubiquinol may reduce the oxidative stress in neuroendocrine system which further improves the function of diminished hypothalamic–pituitary–ovarian axis and leads to increased secretion of FSH and LH. Thus increase in concentration of FSH and LH may be due to reduced oxidative stress by Ubiquinol. Further studies are required with more number of subjects for getting much more statistically significant data.

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Compliance with Ethical Standards

Conflict of interest A. S. Thakur, G. P. Littaru, I. Funahashi, U. S. Painkara, N. S. Dange, P. Chauhan declares that have no conflict of interest.

Ethical approval All procedures performed in this study were in accordance with ethical standards of the institution and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study has been conducted on approval by the college ethical committee.

Informed consent Informed consent was obtained from all the individual participants included in the study.

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