**Original paper**

# Vitamin C Supplementation in Relation to Subfertility and Ovarian Function

Wasan Gh. Aboud[1*]

[1]Department of Obstetrics & Gynecology, College Of Medicine, University Of Kerbala, Kerbala, Iraq

## Abstract

**Background**: subfertility is a common medical problem affecting about 15-20 % of women consulting the gynecological outpatients in the whole world. Vitamin C is an important substance that plays different roles in the body and part of this action is in relation to subfertility.

**Objective**: to evaluate the effect of vitamin C supplementation on the ovarian hormones estrogen and progesterone in subfertile women.

**Methods**: This prospective study was carried out on thirty women attending private gynecological clinics, twenty of them were infertile and the other ten were normal fertile women as control group. They were subjected for the measurement of serum concentrations of both estrogen and progesterone. At the start, The investigations began at the 23rd day of a regular menstrual cycle(B), 5 ml of blood was taken for the measurement of the serum concentrations of both ovarian hormones.

Each woman then took vitamin C tab. 1.5 gm (orally) daily and continued for the next 23rd day (AI), At the same day serum E & P was repeated and the vitamin C supplementation continues to the next 23rd dayof second period, a total period of 58±2 days (AII). So at this day hormonal assessment was repeated and vit C supplementation was stopped.

**Results and Discussion**: The results showed that vitamin C supplementation causes an increased serum progesterone concentration significantly($p<0.05$)after two months supplementation of oral vit C, as compared with the baseline serum level in both fertile and infertile women, while the effect of vitamin C supplementation on serum estrogen is increased significantly after the second months of the vitamin supplementation as compared with the baseline level in fertile women only. In this study, we found a role of vitamin C supplementation to affect the subfertility through increasing the endometrial thickness either directly by its antioxidant effect on endometrial cell or through increasing the level of progesterone.

**Conclusion**: our study shows an effect of vitamin C on the progesterone level and hence this may play a role in infertility treatment as it will increase the receptivity and preparation of the endometrium to the fertilized ovum.

**Key words:** vitamin C, ovarian hormones, subfertility

## Introduction

Vitamin C (L-ascorbic acid) is an essential nutrient for humans(1). Vitamin C is an important antioxidant (electron donor) in the aqueous milieu of the body. This function of ascorbic acid may be important in preventing degenerative diseases, cardiovascular diseases, and some cancers. It enhances non-heme iron absorption, the transfer of iron from transferrin to ferritin(2),about 60 mg/day is required in the diet for normal adult, this is usually increased during fever, infection, and toxic conditions(3).Many

*For Correspondence: wasanobgy@gmail.com

minor components of food, such as minerals and antioxidant vitamins, have been shown to alter biological processes which may reduce the risk of chronic diseases in humans[4].

Ascorbate is required for a range of metabolic reactions in all animals and plants. It is essential for life and powerful organic compound that participates in many biological processes as antioxidant in humans, and is a cofactor in several vital enzymatic reactions[5]. The only way human uptake ascorbic is via the food the main source of vitamin C is fruit and vegetable, and hence plasma vitamin C concentration is a marker of fruit and vegetable intake[6].

Reproductive failure is a significant public health concern. Infertility, defined as the failure to conceive a recognized pregnancy after 12 months of unprotected intercourse, it carries significant personal, social and financial consequences[7]. Although relatively little is known about factors affecting fertility and early pregnancy loss, most of literatures suggest that environmental and lifestyle factors play an important role. There is sufficient evidence to hypothesize that diet, particularly its constituent antioxidants, and oxidative stress may influence the timing and maintenance of a viable pregnancy[8]. Infertility is a benchmark of ascorbate deficiency in the guineapig, a species that, like humans, requires a dietary source ofascorbate[9].

Systemic supplementation with antioxidant may help overcome oxidative stress in the female infertility; therefore, vitamin C may play a role in fertilization[10]. Systemic supplementation with vitamin C is used in women who are infertile, in those with luteal phase defects and in those who have experienced recurrent abortions[11].

The ovary has long been recognized as a site of ascorbic acid accumulation and turnover. The highest concentrations are found in the theca interna, granulosa, and luteal compartments[12,13].

Another study demonstrated that patients undergoing in vitro fertilization-embryo transfer, vitamin C supplementation is given during the period of hormonal stimulation, which resulted in higher follicular fluid concentrations of vitamin C[14].

Another study by Westphal *et al.*, 2004, showed that three months of supplementation results in a trend towards an increase in mean mid-luteal progesterone concentrations[15].

Another randomized controlled trial examined the effects of vitamin C supplementation (750 mg daily) in patient with luteal phase defects. Ascorbic acid supplementation resulted in significantly higher serum progesterone concentrations[16]. Our study now is about the effects of vitamin C supplementation on the ovarian hormones, which in turn may play a role to solve infertility in the women.

## Materials and Method

### Study Population:

This prospective study was carried out on thirty women over 6 months from July-December 2011, the women were collected from private outpatient clinics in Kerbala city, ten of them were fertile as control group and the remaining twenty were infertile of unexplained causes, any woman with a known cause for her infertility was excluded from the study. All of them were in reproductive age of 15-45 years.

The BMI for each woman in the study was calculated according to the following equation:

BMI = Weight (kg) / Height ($m^2$) [17].

and it was ranging as 16-35 kg/$m^2$..

Vitamin C was used as oral tablet (Cetavit tab. 500 mg) manufactured by ALSHAHBA LAB. ALEPO INDUSTRY- SYRIA

**The ovarian hormones estrogen E2& progesterone P, were measured by Hormone measurement kits:**

Using Enzyme Immunoassay for the quantitative determination of both progesterone and estrogen concentration in Human Serum, by using available kits (PROGESTERONE AND ESTRADIOL (E2) ENZYME IMMU-NOASSAY TEST KIT), Catalog Number: BC-1113, BC-1111 respectively with using ELISA machine.

From each woman, history was taken, determination of the 23rd day of the cycle was done, At this day a sample of venous blood (5 ml) was withdrawn from each woman for estimating the serum concentration of both ovarian hormones (estrogen and progesterone) as baseline level**(B).**

After that, each woman started to take the first dose of vitamin C 1.5 gm/day (1tablet of 500 mg, 3 times daily), ingested after meal. This dose was continued for the next 23rdday of the following cycle (first period 29±1 days). Again, the same hormonal assay mentioned above was repeated(**AI**), supplementation with 1.5 gm vit c tab is continued until they reached next 23rd day from the second cycle (second period: 58 day±2) (**AII**), lastly, at this day each woman underwent the same investigations and stopped the oral intake of vitamin C.

## Statistical analysis:

Statistical analysis was performed by using the least significant differences (LSD) and analysis of variance (ANOVA), utilizing (SPSS: ver. 17 for windows). All values were expressed as Mean± SD, P-value of less than 0.05 was considered statistically significant.

## Results

The mean ± SD of age and BMI of women included in our study (fertile and infertile) in which there statistical analysis showing no significant differences (P>0.05) as shows in table (1)

The effects of vitamin C on serum progesterone concentration in fertile and infertile women were noted in table (2) in which it shows significant increase (p<0.05) in AII as compared with B in fertile and infertile women. On the other hand, there isno significant increase (P>0.05) inAI in compared with B in all women**.**

The effect of vitamin C on serum estrogen concentration in fertile and infertile women was noted in table (3). Similarly, results shows significant increase (p<0.05) in AII as compared with B in fertile women only.

Table 1: Anthropometric data (Mean ±SD) for fertile and infertile women:

| Anthropometric data | Fertile N=10 | Infertile N=20 | P-value |
|---|---|---|---|
| Age (years) | 30.6±6.2 | 25.4±6.83 | NS |
| BMI(kg/$m^{2)}$ | 26.93±3.29 | 25.07±2.99 | NS |

NS: no significant differences (P>0.05) in ages andBMI as compared between fertile and infertile women.

Table 2: The Effect of vitamin C on serum progesterone concentration (Mean ±SD) in fertile and infertile women

| fertility status | Serum progesterone concentration (ng/ml) | | |
|---|---|---|---|
| | Before vitamin C supplementations (B) | After vitamin C Supplementations | |
| | | 29 ±1 days (AI) | 58 ± 2days (AII) |
| Fertile | 0.98±1.2 | 1.75±1.37 | 2.42±1.42* |
| Infertile | 1.07±0.35 | 1.98±0.65 | 2.55±0.83* |

***:** significant increase **(P<0.05)** as compared between AII & B
**B:** Serum progesterone concentration before vitamin C supplement.
**AI:** Serum progesterone concentration after first period (29 ± 1day) with vitamin C supplementation.
**AII:** Serum progesterone concentration after second period (58± 2 day) with vitamin C supplementation.

Table 3: The Effect of vitamin C on serum estrogen concentration (Mean ± SD) in fertile and infertile women

| fertility status | serum estrogen concentrations (pg/ml) | | |
|---|---|---|---|
| | Before vitamin C supplementations (B) | After vitamin C Supplementations | |
| | | 29 ±1 days (AI) | 58 ± 2days (AII) |
| Fertile | 44.9±14.3 | 51.0±15.87 | 62.6±15.16* |
| Infertile | 44.1±17.54 | 51.3±14.86 | 56.4±17.04 |

***:** significant increase **(P<0.05)** as compared between AII&B
**B:** Serum estrogen concentration before vitamin C supplement.
**AI:** Serum estrogen concentration after first period (29 ± 1day) with vitamin C supplementation.
**AII:** Serum estrogen concentration after second period (58± 2 day) with vitamin C supplementation.

## Discussion

In our study we found that vitamin C has an effect on sex hormones (progesterone and estrogen) as shown in the results of tables (2,3) in which there is significant increased in serum progesterone after AII as compared with B in both fertile and infertile women, also serum estrogen was significantly increased after AII as compared with B in fertile, women and this may help to increase the endometrial thickness during luteal phase, this results agree with the explanation of both **McKinley and Olouchlinin his study in 2006,**on responsibility of both (progesterone and estrogen) in increasing the endometrial thickness during luteal phase of menstrual cycle[18].

The free radicals play a significant role in physiological processes within the ovary. Many studies have demonstrated involvement of reactive oxygen species (ROS) in the follicular-fluid environment, folliculogenesis, and steroidogenesis [19-21].

There is an ongoing debate on the role of antioxidant supplementation in both male and female infertility[22]. Oxidative stress has been shown to affect the midluteal corpus luteum and steroidogenic capacity both in vitro and in vivo. Oxidative stress and inflammatory process have roles in the pathophysiology of polycystic ovarian disease and drugs such as Rosiglitazone may be effective by decreasing the levels of oxidative stress[23,24].

There is an explanation by **Karanthet al**. in 2001,whosuggested that the

antioxidants properties of vitamin C stimulate the release of gonadotrophins from adenohypophysis[25]. Also vitamin C supplementation potentiate the antioxidant properties of estrogen which was investigated in human female reproductive organs by **Lui*et al***[26], while **Murdoch** was investigated antioxidant properties of estrogen in pig luteal and follicular tissue exposed to hydrogen peroxide in vitro, in which high doses of estrogen (≥40 pg/ml) protected against apoptosis, suggesting that ovarian estrogen ($E_2$) function as ROS scavenger during pregnancy mediated luteal rescue and folliculogenesis [27].

Vitamin C supplementation has a role in increasing S. progesteronein A2 and this agree with the results of previous study by **Henmi*et al*** in 2003, that showed the effects of vitamin C supplementation 750 mg daily in patient with luteal phase defects due to progesterone deficiency[16].

The effect of vitamin C on increasing serum progesterone concentration may be caused by the role of vitamin C as antioxidant to improve the tissue of corpus luteum (CL)and lead to improve the secretion of progesterone hormone[8]. Another studies found that CL concentrated with vitamin C about 100 times the level in blood plasma of this vitamin[28], this give us indication about the importance of vitamin C in improving the function of this gland in secretion of sex hormone. Also Ascorbic acid deficiency characteristically produces ovarian atrophy and extensive follicular atresia and causes premature resumption of meiosis[29].And More recently, ascorbic acid and other antioxidants were shown to inhibit follicular apoptosis in cultured rat follicles[30].

Follicular phase events are known to affect subsequent progesterone production in the luteal phase. Ascorbic acid supplementation enhances the ovulation inducing effects of clomipheneby an apparently local ovarian effect [31].

The ovary has long been recognized as a site of ascorbic acid accumulation and turnover. The highest concentrations are found in the theca interna, granulosa, and luteal compartments[32,33]. The concentration of ascorbic acid is reported to be much higher in human follicular fluid than in blood serum. This suggests active transport of ascorbic acid against the concentration gradient[34,35], and that a scorbic acid may play a role as an antioxidant vitamin during folliculo-genesis[36].

In **Henmi** study, ascorbic acid supplementation significantly increased serum Progesterone levels in patients with luteal phase defect. The clinical pregnancy rate was significantly higher in the ascorbic acid supplementation group than the control group. Thus, ascorbic acid supplementation is an effective treatment for some patients with luteal phase defect. Ascorbic acid supplementation significantly increased serum E2 levels[16].Which was not shown in our infertile women but only the fertile group, this may mandate the need to include more women with infertility to show vitamin C effect on E2.

Endo in 1993, previously reported that hydrogen peroxide reduced production of both progesterone and estradiol by human cultured granulosa lutein cells[37]. Henmi et al believed that ascorbic acid supplementation improved steroid oogenesis in his study, although it is not known whether this occurred via antioxidant effects of vitC [16].

## Conclusion

in conclusion vitamin C has important role in the increasing serum

progesterone and estrogen which plays a important role in increasing the endometrial thickness to prepare the uterus for the implantation, also this vitamin can causes increase the progesterone hormone which of benefit to support the early pregnancy.

## Recommendations

1. There is a benefit from taking vitamin C supplementation in as it can result in increasing the endometrial thickness which may have a role in subfertility treatment
2.Vitaminc is found in different types of fruits and vegetables so it is easily supplemented.
3.Furtherstudy is needed for the role of other antioxidants in increasing fertility through their effects on the pituitary hormones endometrial thickness and cervical receptivity.

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