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# Luteal phase Vitamin C supplementation on the outcome of in-vitro fertilization

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## Abstract

**Background:** In-vitro fertilization is successful Assisted Reproductive Technology for infertility became one of the best modalities for achieving the dream of having children in face of many obstacles in fertility, many works have been done to enhance the outcome of IVF especially the live birth rate and all methods tried to support the luteal phase with many medications.

**Objective:** To evaluate the effect vitamin C supplementation at dose of 1000mg per day on the day of oocyte retrieval on the outcome of the In-vitro fertilization.

**Methodology:** A randomized open-label study was carried out on 200 women undergoing In-vitro fertilization for infertility treatment conducted at AlShorouk IVF clinic Benha city egypt. They were randomly divided in two groups; one group received 1000 mg/day oral Vitamin C supplementation 1000mg divided into two capsules by day and night on the day of oocyte retrieval along with the standard treatment protocol of In-vitro fertilization and other group received standard treatment long protocol for the In-vitro fertilization only. Pregnancy test done after 14 days and pregnant patient followed by regular visits till reaching term and any morbidity recorded.

**Results:** Significant outcome regarding reaching full term pregnancy occurred in the vitamin c group with number of full term pregnancies 65 out of 100 (65%) compared to only 45 out of 100 in the control group (45%) with p value 0.004 also the rate of early miscarriage is higher in the control group 20% and only 8% in the vitamin c study group with p value 0.01.

**Conclusion:** Vitamin C supplementation has significantly improved the outcome of In-vitro fertilization techniques with reduced incidence of spotting and miscarriages along with improved term pregnancy.

However a multimodal approach of analgesia/anaesthesia for TUGOR is recommended to further improve on clients' satisfaction and acceptance.

**Keywords:** In-vitro fertilization, Vitamin C, Infertility, full term pregnancy.

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## **Introduction**

Vitamin C functions as a cofactor in many enzymatic reactions that mediate a variety of essential biological functions, including collagen synthesis.

Vitamin C deficiency leads to impaired collagen synthesis, contributing to the more severe symptoms of scurvy. Another biochemical role of vitamin C is to act as an antioxidant (a reducing agent) by donating electrons to various enzymatic and non-enzymatic reactions.<sup>[10]</sup>

Dietary sources of vitamin C are guava, lime, lemon, green leafy vegetables, Milk, and Animal products like liver and fish.

Ascorbic acid is necessary for the post translational hydroxylation of proline and lysine residues to form hydroxyproline & hydroxy lysine that make collagen strong by cross linking collagen fibers which constitutes most of connective tissue and intercellular cement substances of capillaries

Vitamin c also involved in Hydroxylation of tryptophan to 5-hydroxy tryptophan with formation of serotonin; serotonin is the key hormone that stabilizes our mood, feelings of well-being, and happiness.

Vit C reduces ferric iron to ferrous state, which is preferentially absorbed from intestine and consequently raising Hemoglobin level and correct anemia. Also vitamin c involved in Folic acid metabolism and Helps the enzyme folate reductase to reduce folic acid to tetrahydrofolic acid thus helps in maturation of RBC.

Unexplained infertility affects 15% of couples in the United States; Its pathophysiology remains unclear; Evidence suggests that oxidative stress (OS) and low antioxidant status may be associated with infertility of both known and idiopathic origin (1).

Lower total antioxidant status (TAS) is observed in serum of women with polycystic ovarian syndrome (a known risk factor for female infertility) and the peritoneal fluid of

women with idiopathic infertility compared with fertile control women (2,3).

Ascorbic acid is essential for maintenance and synthesis of collagen during tissue development and at sites of tissue damage, and also for the maintenance of the slow collagen turnover which occurs in mature tissues.

Vitamin c acts as an electron donor, it is an essential co-factor for the enzymes that hydroxylate proline and lysine residues during the post-translational processing of pro-collagen. Collagen synthesis is required for follicle growth, for repair of the ovulated follicle and for corpus luteum development.

Ascorbate will also be needed for secretion of collagen and proteoglycans into follicular fluid. To gauge the requirement during follicle growth, the follicular basement membrane and theca can be considered as the surface of a growing sphere whose quantity will increase as the square of follicular radius.

The radius of the graffian follicle may double on a daily basis so the local demand for collagen synthesis, and for ascorbate, will be intense. (4).

These concepts have yet to be investigated directly, but shown relevance in an early study of infertility in scorbutic guinea pigs The antioxidant properties of ascorbic acid are known to protect tissues from reactive oxygen species such as  $O_2^-$ ,  $OH^-$ ,  $H_2O_2$ ,  $O_2$ ,  $OCI^-$ ,  $NO$ , and metal-oxygen complexes (5,6).

The ovary has long been recognized as a site of ascorbic acid accumulation and turnover, with the highest concentrations in the theca interna, granulosa, and luteal compartments. (6)

LH blocks the uptake of ascorbic acid by gonadotropin primed ovaries. A change in the retention and excretion of ascorbic acid occurs at mid-cycle in women, associated with LH secretion and temperature rise, and has been proposed as a definitive marker of ovulation [7].

There appears to be a biphasic change such

that excretion increases in the late follicular phase, declines immediately prior to ovulation, and increases again immediately after the rise in body temperature.; these changes was assumed due to changes in the uptake of ascorbic acid by the periovulatory ovary.

It has been suggested that changes in retention before ovulation facilitate luteal steroidogenesis, and this explains its cycle-protective effects.

Recent studies with luteinizing granulosa cells show that ascorbate is stimulatory to progesterone and oxytocin secretion, consistent with its known roles in hormone biosynthesis, and synergizes with neurotransmitters in stimulating hormone secretion. the concentration of ascorbic acid in the corpus luteum appears to be greatly in excess of that required to facilitate hormone production [8].

In the present study, we explored a factor of high Ascorbic Acid intake by the female partner undergoing IVF and its relation to various parameters and outcome of pregnancy.

## **Methodology**

A randomized open-label study spread over 1 year from the period of January 2020 to January 2021 was carried out in the specialty Alshorouk IVF clinic located in Benha city Egypt. All the patients were explained clearly about the purpose and nature of the study and those who are willing to given written informed consent were enrolled for the study.

## **Participant Selection**

**Sample size:** 200 hundred patients undergoing IVF in that period with one undred as cases with vitamin c supplementation and one hundred as controls and not given vita

**Inclusion criteria:** Patients who were <40years of age, had >2 years of infertility and required infertility treatment by IVF-ET and came to hospital for the infertility treatment were included in this study.

**Exclusion criteria:**

endocrine diseases such as diabetes mellitus, hypothalamic pituitary dysfunction, or thyroid dysfunction; autoimmune disease, cardiovascular disease, and liver and kidney dysfunction; treatment with oral contraceptives and gonadotropin-releasing hormone agonists within 3-months

## **Study Duration**

These 200 patients were enrolled from January 2020 to January 2021.

## **Study Procedure in Detail**

Written informed consent was obtained before including them in the study. All the patients fulfilling the inclusion-exclusion criteria were interviewed for the first time on the day of enrolment, and their case sheets were reviewed to gather necessary information.

History and examination were carried out. All the demographic, disease related parameters, clinical examinations, details of IVF technique and antenatal visits, spotting/bleeding occurred or not and outcome of pregnancy was recorded.

## **Group Allocation**

All patients were randomly assigned into groups A and B using random number table. Group A patients received 1000 mg/ day of oral Vitamin C. The vitamin C supplementation was started on the day of follicle aspiration and continued for entire duration of gestational period. Group B patients did not the vitamin C supplementation as oral tablets.

## **Fertilization Assessment, Embryo Assessment and Pregnancy Evaluation**

All oocytes were inseminated or injected with sperm using the standard intracytoplasmic sperm injection (ICSI) technique. Fertilization was confirmed by the presence of two pronuclei and extrusion of the second polar body.

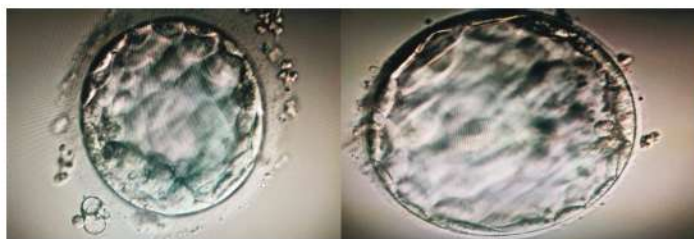
A preferable embryo was defined as one that had reached the four-cell stage on day 2, reached the seven-cell stage on day 3, and had less than 20% of its volume filled with fragments and a preferable blastocyst was defined as being in a full blastocyst stage. A pregnancy test was performed two weeks at day 12-14 post transfer. Pregnancy was confirmed when fetal heart activity was detected



M2 oocyte



Embryos at different stages



Blastocyst embryo



Good quality 16 cells embryo

### **Patients Follow up**

Routine check-up of patients was done with respect to blood pressure, heart rate, weight, complete gestational assessment throughout pregnancy till delivery period.

Warning sign and symptoms are given in a written format for reminding and confirmation that they understand like vaginal spotting, bleeding, and frequent abdominal pain referred to the back. If any of the warning symptoms were encountered we got an instant feedback by telephony and an emergency visit arranged to assess and treat.

### **Statistical Analysis**

Outcome assessment pertaining to the bleeding/spotting events, need of hospitalization

during pregnancy, duration of term pregnancy, any cases of preterm delivery and miscarriages recorded and then by using Fischer's exact test we compared the results of patients prescribed with Vitamin C against the control group of patients. P value less than 0.05 was considered significant.

### **Result**

Out of 200 patients, 100 patients were randomized in the vitamin C group and remaining 100 patients were randomized in the control group and analyzed. There were no significant differences between the two groups at baseline in relation to age, parity and BMI (Table 1).

**Table 1:** Epidemiological data

variable	Vitamin c group	Control group	P value
age	32(20-40)	31(20-40)	>0.05
BMI(kg/m2)	26 (18-29)	25(18-28)	>0.05
Duration of infertility	4.5	5	>0.05

**Table 2:** Efficacy related parameters in both study groups.

variable	Vitamin c (cases)group	Control group	P value
Spotting	15	25	0.07
miscarriage	8	20	0.01
Preterm pregnancy	27	35	0.2
Term pregnancy	65	45	0.004

significant difference in result of Term pregnancy was achieved in Vitamin C group, 65 out of 100 women (65%) compared to 45 out of 100 (45%) in the control group with p value 0.004.

A Total of 8 women out of 100 (8%) in vitamin C group compared to 20 from 100 in the control group who experienced miscarriage (p Value 0.01) which is statistically significant.

Also, there was no statistically significant difference found in the results of spotting between two groups.

## **Discussion**

In this cohort of women enrolled in a randomized controlled study to evaluate the role of antioxidant for unexplained infertility outcome, we found evidence that increased intake of certain antioxidants as ascorbic acid is associated with higher levels of term pregnancies compared to preterm pregnancy and miscarriages.

It is hypothesized that female antioxidant intake and oxidative stress may influence the timing and maintenance of a viable pregnancy.

Many preliminary studies have also emphasized the importance of Ascorbic acid in luteal formation and regression, but no examination

of dietary supplementation during luteal phase has been reported.

Studies have revealed that relatively high bioavailability of vitamin C inside the Graafian follicle and the results obtained from clinical trial suggest a very important role of the vitamin C in follicular genesis, follicular maturation, ovulation and term pregnancy. The efficacy of supplemental use of vitamin C above a level that can be supplied by means of diet alone has been evidently playing beneficial role in reduced spotting and miscarriages [9-11].

Low level of Ascorbic acid disturbs the Follicular Fluid (FF) microenvironment which adversely influences IVF outcome parameters such as oocyte quality, fertilization rate, and high-grade embryos.

Ascorbic acid restores the balance between oxidation and antioxidant action and associated with the maturation of oocytes as shown by the positive correlation between appropriate Reactive oxygen species levels in free fluid and the term pregnancy [12,13].

Low plasma ascorbic acid leads to elevated ROS levels which appear to be responsible for oxidative stress injury, leading to denaturation of oocyte DNA and cytoskeletal damage, an increase of embryonic debris, and abnormal embryonic development. (18-20)

Supplemental Vitamin C maintains balance of the ROS level and antioxidant capacity in the free fluid follicular environment proves to be essential for the acquisition of high-quality oocytes and embryos following IVF treatment [14,15].

Ascorbic acid performs a major biological role; it is required for the biosynthesis of collagen, for the biosynthesis of steroid and peptide hormones, and to prevent or reduce the oxidation of biomolecules.

Ascorbic acid concentrations at the time of oocyte recovery in women undergoing IVF procedures revealing a strong correlation between follicular fluid and serum concentrations of ascorbic acid to facilitate rapid follicular expansion during the approach to ovulation and/or post-ovulatory steroidogenesis [16-17].

In present study supplementation of Ascorbic acid provided important clinical signs in the group of women treated.

A high percentage of term pregnancies were achieved in group A with vitamin c supplementation 65% compared to only 45% in the control group B which received no vitamin c and this could be interpreted as more related to the continuous follow up and regular multivitamin supplementation.

Significantly lower incidence of spotting was observed in women undergoing IVFET with vitamin C supplementation compared to control group. It is suggested that Vitamin C supplementation help in reducing incidence of spotting and bleeding incidences which helps psychologically also to the mothers in early phase of embryo transfer by reducing anxiety and hospitalization.

The present investigation has investigated role of vitamin C supplementation post embryo transfer and its influence on various clinical parameters like term pregnancy, and miscarriages.

## **Conclusion**

Oxidative stress has been identified as major factor adversely affecting outcome of IVF. Vitamin C has been identified as one of the nutrients which help in reducing oxidative stress. Supplementation of large dose of Vitamin C post embryo transfer orally, has shown statistically significant improvement in the outcome of IVF techniques with reduced incidence of spotting and bleeding, reduced hospitalization and miscarriages along with improved term pregnancy.

**Conflict of interest:** The author has no competing interests to declare.

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