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Research Article

Effect of Post Embryo Transfer Vitamin C Supplementation on the Outcome of In-Vitro Fertilization: A Randomized Controlled Study

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ABSTRACT

Background: In-vitro fertilization is successful Assisted Reproductive Technology for infertility but it has challenges like recurrent implantation failure, miscarriages and premature birth. Supplementation of different micronutrients during In-vitro fertilization has emerged as upcoming modality to improve outcome.

Objective: To evaluate the effect of post embryo transfer supplementation with vitamin C on the outcome of the In-vitro fertilization.

Methodology: A randomized open-label study was carried out on 120 women undergoing In-vitro fertilization for infertility treatment. They were randomly divided in two groups; one group received 1000 mg/day oral Vitamin C supplementation post embryo transfer along with the standard treatment protocol of In-vitro fertilization and other group received standard treatment protocol for the In-vitro fertilization only. Both the groups were followed up till its outcome and compared statistically in terms of spotting, bleeding, hospitalization, term delivery and child birth weight.

Results: Increased intake of ascorbic acid significantly reduced spotting (81.67% in Vitamin C Group vs. 63.33% in Control Group; p=0.0245) and reduced need of hospitalization (p=0.032). Intake of Vitamin C significantly reduced the incidence of low child birth weight (46.67% in Vitamin C Group vs. 76.67% in Control Group; p=0.0007). Significant improvement in duration of pregnancy was achieved with intake of Vitamin C with Preterm pregnancy 20%, term pregnancy 65% and lower incidence of miscarriages 15% (p=0.0819). It was observed that the technique of In-vitro fertilization does not have significant impact on outcome of pregnancy in both the groups (p>0.05).

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.

Keywords: In-vitro fertilization, Vitamin C, Infertility, Embryo transfer, Outcome of embryo-transfer

INTRODUCTION

Role of dietary antioxidant intake in Infertility and conception has been always an emerging interest for medical fraternity. A growing body of 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. Acting 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,

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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. Since the radius may double on a daily basis, the local demand for collagen synthesis, and for ascorbate, will be intense, particularly in species (human, cow) with relatively large mature follicles. These concepts have yet to be investigated directly, but shown relevance in an early study of infertility in scorbutic guinea pigs [4]. The antioxidant properties of ascorbic acid are known to protect tissues from reactive oxygen species such as 0_2^-, OH^-, H_20_2, 0_2, OCl^-, 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. The ability of LH to block the uptake of ascorbic acid by gonadotropin-primed rat ovaries provided the basis of bioassay. 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. The immediate cause of 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 that this relationship also explains its cycle-protective effects. More 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. Notwithstanding these effects, the concentration of ascorbic acid in the corpus luteum appears to be greatly in excess of that required to facilitate hormone production [8].

The preponderance of lay literature suggesting that antioxidant levels influence female fertility is based on limited scientific research that primarily investigates specific types of infertility, such as ovulatory infertility or the relationship between glucose control and fertility, although one recent investigation suggests that women's adherence to national dietary recommendations increases the chance of ongoing pregnancy in couples undergoing IVF/intracytoplasmic sperm injection treatment. 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 was carried out in the specialty IVF department of a private hospital in Western India. The study protocol was approved by the Human Research Ethics Committee prior to commencement of study. 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: Considering incidence of spotting after IVF as 10% with vitamin C and other micronutrient supplementation as compare to 30-40% in those who don't take any supplements as per literature [11], the required sample size for this randomized study is calculated as 60 participants in each group with alpha error 0.05 and power of study 80%. Therefore, total 120 patients who came for infertility treatment and underwent IVF-ET were recruited from the Department of In-vitro fertilization, Zydus Hospital in the year 2018-19.

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

Exclusion criteria: (1)Patients with infertility complicated by endocrine diseases such as diabetes mellitus, polycystic ovary syndrome, hypothalamic pituitary dysfunction, or thyroid dysfunction; (2) a history of autoimmune disease, cardiovascular disease, and liver and kidney dysfunction; (3) treatment with oral contraceptives and gonadotropin-releasing hormone agonists within 3-months; and (4) a history of alcohol and drug abuse were excluded from the study.

Study Duration

These 120 patients were enrolled from January 2018 – January 2019. The first examination started in January 2018, and, considering follow-up visits, the last patient was enrolled in January 2019 so that the 37-week follow-up could be completed in October 2019.

Study Procedure in Detail

All the patients participating in the study were given a clear explanation about the purpose and nature of the study in the language they understood. Written informed consent was obtained before including them in the study. All the patients fulfilling the inclusion-exclusion criteria attending the IVF department were interviewed for the first time on the day of enrolment, and their case sheets were reviewed to gather necessary information. Detailed history and examination were carried out by the treating Gynaecologist. 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 throughout the duration of the treatment at multiple follow up visits as per the standard protocol guidelines of the department.

Randomization and Group Allocation

All patients were randomly assigned into groups A and B using random number table. Group A patients received all standard treatment as per the IVF treatment guidelines along with 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 received all standard treatment as per the IVF treatment guidelines but not the vitamin C supplementation as oral tablets. Randomization was obtained by a computer-generated, block design sequence to receive vitamin C or not in a 1:1 ratio.

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. Fertilized oocytes were cultured in groups of a maximum of 4 oocytes until day 5. 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 and did not include an inner cell mass or trophectoderm with very few cells. A pregnancy test was performed two weeks after the transfer of embryo. Pregnancy was confirmed when fetal heart activity was detected on transvaginal ultrasound four weeks after embryo transfer.

Patients Follow up and Evaluation

Routine check-up of patients was done with respect to blood pressure, heart rate, weight, complete gestational assessment throughout pregnancy till delivery period. Patients were followed up weekly in first trimester, every 15 days in second trimester and again weekly in third trimester of pregnancy. If patient had any symptom or complaint then she was given consultation additionally and appropriate measures for treatment, if required. All patients were provided with specific nutritional chart and their Body Mass Index (kg/m^2) (BMI) was determined during the entire duration of pregnancy. Necessary diet supplementation and medications as per the routine antenatal evaluation were provided to all patients depending upon the need during pregnancy. Patients were monitored throughout for development of any adverse effects.

STATISTICAL ANALYSIS

Per-protocol based analysis was performed for all patients and GraphPad InStat softwareV2.05 was used for all statistical calculations. Data were represented as actual frequencies, percentages, mean, standard deviation, median and their interquartile range as appropriate. Chi square test was used to analyze non-parametric data and unpaired t-test was used for comparison between the groups for parametric data. Outcome assessment pertaining to the bleeding/spotting events, need of hospitalization during pregnancy, duration of term pregnancy, any cases of preterm delivery and miscarriages along with evaluation of child birth weight were preformed using Fischer's exact test to compare 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 120 patients, 60 patients were randomized in the vitamin C group and remaining 60 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). Average weight gain during pregnancy was of 12 kg in the vitamin C group of women and 13 kg in the control group, this difference was found statistically not significant (Table 1). From baseline to delivery there was a no significant increase in systolic blood pressure both in the vitamin C and control groups (Table 1).

Pregnancy results of embryo transferred on different days in vitamin C group and control group indicates no significant difference. All the women undergone cryopreservation technique for IVF in both the groups (Table 2). During pregnancy, 18 out of 60 (30%) in the vitamin C group and 25 out of 60 (41.67%) in the control group were hospitalized once during the study and 10 out of 60 (16.67%) in the vitamin C group and 17 out of 60 (28.33%) in the control group were hospitalized more than once during the study. This difference was considered statistically significant.

Significant difference in result of Term pregnancy was achieved in Vitamin C group, 39 out of 60 women (65%) compared to 27 out of 60 (45%) in the control group with p value 0.0819. Even significant difference was observed with respect to preterm delivery where 12 out of 60 females (20%) in vitamin C group exhibited preterm delivery which was significantly less compared to 17 out of 60 females (28.33%) in control group. A Total of 9 women out of 60 (15%) in vitamin C group compared to 16 (26.67) in the control group who experienced miscarriage (p Value 0.0819) which is statistically significant. Also, there was a statistically significant difference found in the results of spotting between two groups. Total 49 women (81.67%) out of 60 did not have spotting in vitamin C group compared to 38 women (63.33%) out of 60 in control group indicating statistically significant difference with p value 0.0245 (Table 3).

Table 1: Demographic and clinical characteristics of patients undergoing IVF in both study groups.

Patient Characteristics	Vitamin C group	Control group	P value
Age (years) (Mean, range)	31 (19-40)	32 (18-39)	>0.05
BMI (kg/m^2) (Mean, range)	24 (18-27)	23 (19-27)	>0.05
Increase in Weight During Pregnancy (IWP) kg (Mean, range)	12 (6-22)	13 (5.7-20.12)	>0.05
Blood pressure (Mean mmHg)	126/80	134/78	>0.05
Duration of Infertility in years (Mean, range)	6.1 (3.5-8.2)	6.3 (3-8)	>0.05
All antenatal visits and instruction followed	60	59	>0.05

BMI: Body Mass Index

Table 2: IVF technique related parameters in both study groups

Sr. No.	Parameter	Vitamin C group	Control group	P Value
1	Number of Embryo Transfer			
	1	8 (13.33%)	10 (16.67%)	0.9258 (NS)
	2	44 (73.33%)	45 (75%)	
	3	8 (13.33%)	5 (8.33%)	
2	Day of Embryo Transfer			
	D2	3 (5%)	8 (13.33%)	0.4254 (NS)
	D3	30 (50%)	26 (43.33%)	
	D5	24 (40%)	22 (36.67%)	
	D6	3 (5%)	4 (6.67%)	

Table 3: Efficacy related parameters in both study groups.

Characteristics	Vitamin C group	Control group	P value
Hospitalization Required			
0	32 (53.33%)	18 (30%)	0.032 (Significant)
1	18 (30%)	25 (41.67%)	
More than 1	10 (16.67)	17 (28.33%)	
Spotting			
Observed	11 (18.33%)	22 (36.67%)	0.0245 (Significant)
Not Observed	49 (81.67%)	38 (63.33%)	
Pregnancy			
Preterm Pregnancy	12 (20%)	17 (28.33%)	0.0219 (Significant)
Term Pregnancy	39 (65%)	27 (45%)	
Miscarriage	9 (15%)	16 (26.67%)	
Child Birth Weight			
Low Birth Weight	28 (46.67%)	46 (76.67%)	0.0007 (significant)
CBW kg (Mean, range)	3.31 (2.68-4.14)	2.89 (2.49-3.87)	>0.05

CBW= Child Birth Weight

Child low birth weight was found in 28 women in the vitamin C group and in 46 women among the control group, this difference was considered significant (Results are summarized in Table 3). Seemingly, the average child birth weight at delivery was 3.31 in the vitamin C group and 2.89 in Control group of women and the difference found statistically not significant (Table 3). Comparing the groups in terms of overall number of hospitalizations during pregnancy, this different trend was found significant (Table 3). All the patients tolerated vitamin C well with no adverse drug reactions with Vitamin C.

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 significantly reduced spotting, less frequent hospitalization with evidentiary higher levels of term pregnancies compared to preterm pregnancy and miscarriages with significant reduction in low child birth weight but the relationship varied. 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. Higher plasma concentration of Ascorbic acid restores the balance between oxidation and antioxidant action in FF is associated with the maturation of oocytes as shown by the positive correlation between appropriate Reactive oxygen species levels in FF 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 oocyte DNA and cytoskeleton damage, an increase of embryonic debris, and abnormal embryonic development. Supplemental Vitamin C maintains balance of the ROS level and antioxidant capacity in the FF environment proves to be essential for the acquisition of high-quality oocytes and embryos following IVF treatment [14,15].

Besides the effect of Vit C on oxidative stress, it has also been clinically indicated that Vitamin C supplementation via oral or intravenous administration led to a significant reduction in the volume and weight of the endometriotic cysts and number of natural killer cells and inhibited endometriotic implantation in a dose dependent manner [16-18]. Clinical investigation reveals that patients with severe EMs who received Vitamin C (1000 mg/ day) and Vitamin E (800 IU/day) for 8 weeks before IVF-ET treatment showed significantly suppressed levels of myeloperoxidase in Follicular fluid. Also, it was observed that Vitamin C and Vitamin E supplementation for 6 months led to a decrease in the plasma and peritoneal fluid concentrations of Malondialdehyde and lipid hydroperoxides in patients with EMs. In this study, supplementation of Vitamin C was given after post embryo transfer and continued throughout the pregnancy; which also confirms the effect of the medication time (in terms of phase of IVF cycle) on reduction in oxidative stress levels of Follicular fluid [19,20]. Ascorbic acid performs a major biological role in relevance to reproduction, dependent on its role as a reducing agent: 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. It is frequently involved in mixed-function oxidation, resulting in the incorporation of oxygen from molecular oxygen into a substrate. 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 [21,22].

In present study supplementation of Ascorbic acid provided important clinical signs in the group of women treated. Frequency and extent hospitalization during IVF-ET in women with supplemental high level of Ascorbic acid was found significantly lower compared to women in control groups. A high percentage

of term pregnancies were achieved in both groups and this could be interpreted as more related to the continuous follow up and regular multivitamin supplementation provided to all women than a real picture of the cohort's pregnancy outcome. In the case of children born with a body weight lower than 2500 g, the significant difference found among groups in favour of the vitamin C women is intriguing as it can be interpreted as more and better energy supply to child during gestation. The clinical observation of the characteristic trait of women hospitalized led us to perform a correlation test and a linear correlation was found in both groups between hospitalization rate and the Body Mass Index, which reflects women's body fitness during child bearing as confirmed by study of Hans U et al [23]. Significantly lower incidence of spotting was observed in women undergoing IVF-ET 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, investigation of low child birth weight, frequency and extent of hospitalization and miscarriages. Few limitations of the study include it was carried out in single centre and small sample. Genetic variation and geographical variations could not be studied here. We suggest considering positive effect of Vitamin C supplementation of IVF outcomes, larger multicentre studies should be carried out focusing on this area.

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.

DECLARATION

Conflict of interest: The authors have no competing interests to declare.

Ethics Approval: The study protocol was reviewed and approved by the Institutional Ethics Committee. The study was carried out following the standards of clinical study as laid down in Schedule Y and new drugs and clinical trial act, 2020.

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