

Evaluation The Role Of Vitamin C In The IUI-Treatment Of Female Infertility.

تقييم دور فيتامين ج في علاج عقم النساء بطريقة حقن السائل المنوي داخل الرحم

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الخلاصة:

الخلفية:تقدر نسبة الأزواج الذين يعانون من العقم ما بين 7%-28% ويتأثر ذلك كثيرا بعمر الزوجة و على اية حال فان اضطرابات التبويض مسؤولة عن خمس حالات عقم النساء الكلي. في حالة الجسم الصحي تكون انواع الاوكسجين التفاعلية في حالة توازن مع مضادات الأوكسدةفإذا ما تأثر هذا التوازن لصالح الاولي حدث ما يسمى بالشد التاكسدي. وجدت معلمات الشد التاكسدي هذه داخل السوائل الحويصلية للنساء اللواتي يعانين من تأخر الإنجاب الخاضعات للتقنيات المساعدة للإنجاب ووجد ان قلة الاوكسجين داخل الحويصلة نتج عنه تأخر في نمو البويضةيدخل فيتامين ج في صناعة الألياف الرابطة و الهرمونات الاسيروبيدية والامينية كما و يحمي هذا الفيتامين انسجة الجسم من التحطم التاكسدي.إن وفرة هذا الفيتامين في المبايض تتناغم مع ادواره المعروفة في صناعة الهورمونات و تنسيق عمل المحفزات العصبية التي تسيطر بدورها على إفراز الهورمون.

الهدف من الدراسة:تقييم دور فيتامين ج في علاج عقم النساء بطريقة حقن السائل المنوي داخل الرحم و اخذ النتائج مقاسه بحساب العدد الكلي للحويصلات ،حجم الحويصلة الواحدة ،سمك بطانة الرحم و حصول الحمل

المنهجية: دراسة عشوائية مقارنة مسيطرة للفترة من اذار 2010 الى تشرين الاول 2010

الطريقة:تمت الدراسة بأخذ مجموعتين من النساء في مركز الخصوبة التابع لمدينة الصدر الطبية في مدينة النجف و مراقبتهما لدراسة تأثير اضافة فيتامين ج على احد المجموعتين، اضيف فيتامين ج(500-1000 ملغ) الى علاج خمسين امرأة عولجت بأدوية تحريض الاباضة مع حقن السائل المنوي داخل الرحم ولم يتم اضافة الفيتامين الى علاج الخمسين امرأة الاخرىالذين عولجن في ما عدا ذلك بنفس الطريقة ثم تمت مراقبة نتائج العلاج بالفحص المتكرر بالأموح فوق الصوتية لحساب عدد و قطر البويضات، ثخن البطانة الرحمية و تحليل الحمل لتشخيص حصول الحمل او عدمه بعد العلاج. حللت البيانات وصفا باستخدام المعدل و الانحراف المعياري والقيم الصغرى و العظمى.

النتائج عدد و قياس قطر البويض و ثخن البطانة الرحمية وتشخيص الحمل كان اكثر مما هو عليه في حال اضافة فيتامين ج للعلاج. الاستنتاج: قد يكون لإضافة فيتامين ج دور ايجابي على قابلية الانجاب لدى النساء من حيث زيادة عدد و قياس قطر البويضات ،زيادة ثخن البطانة الرحمية و حصول الحمل.

التوصيات

1. قد نحتاج لإجراء الدراسة على عدد اكبر لدعم النتائج التي تم التوصل إليها في هذه الدراسة
2. قد نحتاج الى قياس مستوى فيتامين ج عند النساء اللواتي تقرر ادخالهن في برامج تحريض الاباضة و تزويد من وجد النقص لديها بالفيتامين المذكور

Abstract

Back ground:the prevalence of female infertility ranges from 7% to 28% depending on the age of the woman, however ovulatory disorder can be seen in fifths of infertile women. In a healthy body, ROS (Reactive Oxygen Species) and antioxidants remain in balance, when the balance is disrupted towards an overabundance of ROS; Oxidative stress (OS) occurs. Oxidative stress markers have been localized in follicular fluid of subfertile women undergoing assisted reproduction, Low intrafollicular oxygenation has been associated with decreased oocyte developmental potential. Vitamin C (ascorbic acid) is required for the synthesis of collagen, for the synthesis of steroid and peptide hormones, and it protects tissues from oxidative damage. There is an abundance of ascorbic acid in the ovary, which is consistent with its known roles in hormone synthesis and synergism with neurotransmitters in stimulating hormone secretion.

Objective: The aim of our study is to evaluate the role of Vitamin C in the treatment of female infertility, with outcomes measured by total number of follicles, follicular size, endometrial thickness, and pregnancy outcome.

Method: Randomized, controlled, group- comparative study by taking two groups and observe them to study the effect of adding vitamin C on infertility treatment of half of them.50 women on induction of ovulation prepared for (IUI) with no vitamin C in their treatment and (50 women) prepared for (IUI) with vitamin C of 500-1000mg daily in their treatment and follow up them by serial ultrasound measurement for number of ovarianfollicles , follicular diameter and endometrial thickness and pregnancy outcome.

Result: Follicular diameters, follicular numbers, endometrial thickness at day 10 of cycle, and pregnancy rate in group receiving vitamin C were all higher than group that who are not taking vitamin C.

Conclusion: Vitamin C has a role in female reproduction as it can improve the number, diameter of follicles, endometrial thickness and thereby pregnancy outcome.

Key words: vitamin C, infertility, intrauterine insemination.

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INTRODUCTION:

Infertility is a disease defined as "the inability to conceive following 12 or more months of unprotected sex and investigation is not undertaken unless the medical history and physical finding dictate earlier evaluation and treatment⁽¹⁾. The prevalence of female infertility ranges from 7% to 28% depending on the age of the woman. Oxidative Stress has a role in etiopathogenesis of anovulatory, endometriosis, tubal factor infertility, and unexplained infertility⁽²⁾, as the follicular fluid microenvironment has a crucial role in determining the quality of the oocyte, low intrafollicular oxygenation has been associated with decreased oocyte developmental potential as reflected by increasing frequency of oocyte cytoplasmic defect, impaired cleavage and abnormal chromosomal segregation in oocytes from poorly vascularized follicles⁽³⁾. In a healthy body, ROS (Reactive Oxygen Species) and antioxidants remain in balance. When the balance is disrupted towards an overabundance of ROS, Oxidative stress (OS) occur. OS influences the entire reproductive lifespan of woman and even thereafter (i.e. menopause) OS results from an imbalance between prooxidants (free radical species) and the body's scavenging ability (antioxidants), ROS affect multiple physiological processes from oocyte maturation to fertilization, embryo development and pregnancy. Free radical species are highly reactive. They become stable by acquiring electrons from nucleic acids, Lipids, proteins, carbohydrates or any nearby molecule causing a cascade of chain reactions resulting in cellular damage and disease⁽¹⁻⁴⁾. There are two major types of free radical species: reactive oxygen species (ROS) and reactive nitrogen species (NOS). Three major types of ROS superoxide (O_2^-), hydrogen peroxide H_2O_2 , hydroxyl (OH). ROS have been implicated in more than 100 diseases^(4,5). They have a physiological and

pathological role in female reproductive tract. Such as oocyte maturation, ovarian steroid-ogenesis, corpus luteal function and Luteolysis^(6,7). Antioxidants are commonly added to food products like vegetable oils and prepared foods to prevent or delay their deterioration from the action of air. Under normal conditions, scavenging molecules known as antioxidants convert ROS to H_2O to prevent overproduction of ROS. There are two types of antioxidants in human body: enzymatic antioxidant (natural antioxidant), and non-enzymatic antioxidants^(4,5). They are known as synthetic antioxidants or dietary supplements. In the body, complex antioxidants system is influenced by dietary intake of antioxidants vitamins and minerals such as Vitamin C, Vitamin E, Selenium, Zinc, Taurine, Hypotaurine, glutathione, Beta Carotene^(6,7,8). Ascorbic acid is a dietary requirement for primates, and a few other mammals, which lack the necessary hepatic enzymes for its synthesis, this vitamin has been associated with conditions such as ageing, common cold and cancer, and the recommended daily requirement has been the subject of many debates. It has three biological actions of particular relevance to reproductive tissues: it is required for the synthesis of collagen, for the synthesis of steroid and peptide hormones. There is an abundance of ascorbic acid in the ovary, which is consistent with its known roles in hormone synthesis and synergism with neurotransmitters in stimulating hormone secretion⁽⁹⁾. The follicular-luteal cycle also requires high rates of tissue remodeling and collagen synthesis for follicle growth, for repair of the ovulated follicle, and the development of the corpus luteum. Ascorbic acid acts as a co-factor in the amidation of some proteins and has been implicated in the regulation of oxytocin secretion by ovaries⁽¹⁰⁾. The role of ascorbic acid in promoting collagen biosynthesis has been studied extensively⁽¹¹⁾. During follicular growth,

ovulation and formation of corpora lutea, basement membranes and the extracellular matrix are undergoing constant remodeling and, therefore, have high requirements for collagen. Early studies implicated ascorbic acid in the regulation of the Graafian follicular basement membrane; lack of ascorbic acid causes degeneration of follicle membranes and high doses inhibit collagenolytic activity in mature follicles⁽¹²⁾. Although there is much information on the role of ascorbic acid during formation of corpora lutea, little is known about its role during follicular growth and development⁽¹³⁾, for that this study concentrates on the role of vitamin C on follicular development, growth, endometrial thickness and pregnancy rate.

OBJECTIVES:

To evaluate the role of Vitamin C in the treatment of female infertility, with outcomes measured by total number of follicles, follicular size, endometrial thickness, and pregnancy outcome.

PATIENTS AND METHODS: A prospective, randomized, controlled, group- comparative study done at the infertility center of AL- Sadder Teaching Hospital in AL-Najaf city between March 2010 and October 2010. This study done on numbers of infertile couples attending the center who had inclusion criteria of bilateral tubal patency, normal male side (normal semen), who were decided to be treated by IUI. The number was 112 couples only 100 of them entered our study and divided into 2 groups:

1st Group (50) without adding of Vit.C to their treatment. 2nd Group (50) with supplementation of Vit.C to their treatment of dose (500 – 1000 mg) daily from day two of cycle onwards. After full history and thorough clinical examination including cycle day 2 or 3 transvaginal exam using a 7.5 MHz vaginal probe of Siemens' device, all women had investigations at second day of cycle like hormonal assay, hysterosalpinography,

and seminal fluid analysis. They underwent a standard treatment of ovarian stimulation in the form of either oral clomiphene citrate (100- 150 mg) daily or injectable treatment e.g.: Gonal F (75 IU) once daily till maturation of the follicles. Follow up of the 2 groups done by transvaginal ultrasound from day ten of cycle to evaluate follicular development by measuring the number of follicles and their diameters and endometrial thickness (ET), the latter is measured by longitudinal scanning of the uterus on the frozen image using electronic calipers placed at the level of the junction of the endometrium-myometrium interface at the level of the fundus. H.C.G (5000 I.N. – 10.000 I.U.), was given when the mean diameter of the leading Follicle is 18-20 to achieve final follicular maturation and rupture 36hr prior to the intrauterine insemination (IUI). If the number of mature follicles exceeds 4 or the total number of follicles over 12mm in diameter exceeds 8, H.C.G withheld and the couple advised to abstain from intercourse. Freshly donated semen by husband is produced by masturbation. Suitability of samples donated for IUI were judged, then treated with a swim up technique, culture media used were Ferticult and Earl's media a volume of 1 ml of preparation was taken for insemination using 20 cm IUI catheter (Zavos IUI catheter ZDI, INC, Germany) by the embryologist. Procedure of IUI: In dorsal position, Cervix is exposed with bivalve speculum prepared semen is injected gently, women all were instructed to lie supine for 30 minutes, luteal phase support by oral and vaginal high dose progesterone for total 14 days, women were followed up to prove pregnancy or not by measuring BhCG level and or by transvaginal ultrasound. Analysis and comparison between the two groups done to evaluate the impact of Vit.C on the results.

Statistical Analysis: Data were computed and analyzed using Statistical Package for

Social Sciences (SPSS) Ver.17. Statistical analysis such as descriptive (Mean, standard deviation, minimum and maximum values) were used according to the respective objectives of the study. The appropriate statistical test including, t-test and chi Square (χ^2) test were used to

compare the results . Differences were considered to be statistically significant if the p-values were ≤ 0.05 at 5% and ≤ 0.01 at 1%.

RESULTS:

112 Couples enrolled in our study , from them only 100 continued with us, the other 12, cancelled because they developed no or >5 follicles. 50 women not received Vitamin C, called them Group 1, and other 50 women received Vitamin C, called them group 2.

Table(1) Demographic results of studied groups

Parameters	Mean	Range
Age(years) G1	29.58±5.75	19 - 40
G2	30.44±7.32	16 - 46
Weight (Kg) G1	74.32±11.26	37 – 105
G2	77.10±9.22	60 - 100
Duration of Infertility (years)		
G1	9.62±4.32	3 – 20
G2	7.38±5.88	1- 26
	G1	G2
Type of infertility		
Primary	41(%)	39(%)
Secondary	9(%)	11(%)

Table (1) showed the demographic characteristics of our women under study. The mean age of group 1 was (29.58) and for group 2 was (30.44). In group 1 the mean weight was (74.32) and for group 2 was (77.10). The mean duration of infertility of group 1 was (9.62) and for group 2 was (7.38).

Table(2) Comparison of follicle diameter in millimeters (Day 10) between the two groups.

Groups	Diameter of follicles in millimeters at (Day 10)		P. Value
	Mean	Std. Deviation	
G1	16.37	2.045	0.036*
G2	18.95	2.464	

(t-test);* $P \leq 0.05$.

Table (2) shows comparison between follicular diameter at CD10 in group 2 was 18.95 in comparison to group 1 was 16.37, so there was significant increment in follicular diameters with Vit.C treatment.

Table(3) Comparison of follicle numbers in the studied groups

follicle No.	Total No.	G1	G2	Sig
1	29(29%)	16(32%)	11(22%)	0.021*
2	32(32%)	18(36%)	14(28%)	
3	23(23%)	12(24%)	13(26%)	
4	16(16%)	04(08%)	12(24%)	
Total No.	100	50	50	

Chi square (χ^2 -test);* significant at $P \leq 0.05$

Table (3) shows comparison of follicular number in the studied groups. The women having one mature follicles in G1 was 16 (32%) and in G2 was 11 (22%).In G1 the women having two mature follicles was 18(36%) while in G2 was 14(28%), in G1 the women having 3 mature follicles was 12(24%) while in G2 was 13(26%) also in G1 the women having 4 mature follicles was 4(8%) while in G2 was 12(24%). These results found to be statically significant ($P \leq 0.05$).

Table(4) Comparison of endometrial thickness in millimeters between the two groups.

Groups	Endometrial thickness in millimeters		P. Value
	Mean	Std. Deviation	
G1	6.88	3.575	0.033*
G2	8.95	3.041	

(t-test);* significant at $P \leq 0.05$.

Table (4) shows that group 2 had higher endometrial thickness (8.95 mm) than group 1 (6.88 mm), which is statistically significant (P value ≤ 0.05).

Table(5); Pregnancy outcome of the two groups

Outcome	G1	G2	Sig.
Pregnant	5(10%)	7(14%)	0.537 NS
Non-pregnant	45(90%)	43(86%)	

The group that treated with vit. C shows higher pregnancy outcome (14 %) in comparison with the group that who were not receiving vit. C (10 %) but this result is not reaching statistical significance.

DISCUSSION:

Most studies have concentrated on the well-known effects of ascorbic acid on steroid genesis. It has been suggested that the high ascorbic acid content measured within ovarian tissue is in excess of that required solely for steroid genesis⁽¹¹⁾⁽¹⁴⁾,

however, the ovaries produce increasing concentrations of progesterone in response to LH. Studies on luteinizing granulose cells have shown that ascorbic acid stimulates production of progesterone⁽¹⁵⁾ and that increasing progesterone

concentrations block the uptake of ascorbic acid⁽¹⁶⁾. Therefore, the action of LH may indirectly control the fluctuations in ascorbic acid concentration observed throughout the ovarian cycle. As the ovaries are sites of intensive tissue remodeling, ascorbic acid is probably required as a co-factor in collagen production^(11, 16). Much attention has been paid to the ability of ascorbic acid to act as an antioxidant⁽¹⁷⁾; as both tissue remodeling and steroidogenesis are processes that produce reactive oxygen species, it is likely that ascorbic acid serves this function within the ovaries. The results of our study, as shown in Table (2 and 3), indicate that the multi-functional activities of ascorbic acid contribute to follicular development, both size and number of follicles were higher in group treated with Vit C., this agreed with Rose et al.(1999)⁽¹⁸⁾, who showed in their study that addition of selenium and ascorbic acid increased the percentage of follicles able to maintain their spherical morphology when cultured in vitro. This can be explained according to Luck et al, and Igarashi et al as follows, The ovaries, and other endocrine tissues, accumulate large amounts of ascorbic acid. Within the ovaries, ascorbic acid accumulates in the granulosa, thecal and luteal cells and it has long been associated with fertility (Luck et al., 1995)⁽¹⁹⁾. Ascorbic acid has been used to enhance the effect of clomiphene on induction of ovulation in women (Igarashi, 1977)⁽¹⁴⁾.

About effect of Vit. C. on endometrial thickness to compare our result cited in Table (4), which show significantly thicker endometrium in treated group in whom vitamin C was added to their treatment this met the results obtained by Sami Al-Katib et al 2009⁽²⁰⁾ whose study showed also a significant increase in endometrial thickness in response to vitamin C supplementation, he attributed this to one or more of the following causes protection against oxidative stress and apoptosis,

thus maintain it thick, via the positive effect of vitamin C on ovarian hormones, or due to enhanced collagen synthesis in the luteal extracellular matrix.

Our study showed that the pregnancy rate was higher in supplemented group with Vit C. Table (5), but not reaching significant level. This result was in agreement with Crcha, et al (2003)⁽²¹⁾ who found that the pregnancy rate was higher in the supplemented group than in the control group (34.2) versus (23.7%), but the differences was not statistically significant, while Agarwal, 2005⁽²²⁾ reported that the pregnancy rate was significantly higher in treatment group with Vit. C. compared with the control (25% versus 11%).

CONCLUSION:

Supplementation of Vit. C. in the diet of infertile women may lead to increase the number and diameters of follicles, On the other hand, Vit.C. may increase endometrial thickness thus positively affecting implantation and thus overall pregnancy rate.

RECOMMENDATIONS:

1. Further studies on larger groups of treated women may be needed to support our results
2. serum level of vitamin C needs to be evaluated in woman who would be decided to be treated by ovulation induction for IUI, and vitamin c need to be supplemented at least for those who found to have low serum vitamin C level

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