Effects of Vitamin C on the Endometrial Thickness and Ovarian Hormones of Progesterone and Estrogen in Married and Unmarried Women

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ABSTRACT

Vitamin C supplementation has effect on the endometrial thickness and ovarian hormones in married and unmarried women during luteal phase. The objective of this study was to determine the effect of vitamin C on the endomerium thickness and ovarian hormones of progesterone and estrogen in married and unmarried women. This study was carried out on thirty women selected randomly from private clinics and relatives in a period of eight months started from December 2008 to July 2009. Ten of them were unmarried, another twenty were married: ten of them fertile and the remaining ten were infertile. They were subjected to ultrasonic examination for estimations of their endometrial thickness, and measurements of their serum concentrations of both estrogen and progesterone that are associated with vitamin C supplementations in a period that continues for about (58 ±2 days). The results of this study show the effect of vitamin C supplementation on the endometrial thickness which is increased significantly during first and second periods (A1 and A2) as compared with the period before vitamin C supplementation of control group (C) in married and unmarried women. It also shows the effect of vitamin C supplementation on serum progesterone concentration which is increased significantly in A2 as compared with C in fertile and infertile women only and the effect of vitamin C supplementation on serum estrogen that is increased significantly in A1 and A2 as compared with C in both fertile and unmarried women. In conclusion, the results show that there was significant increase on the endometrial thickness in both A1 and A2 as compared with C for both fertile and infertile women.

Keywords: Vitamin C, Endometrium, Progesterone, Estrogen, Married, Unmarried Women

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INTRODUCTION

Vitamin C (L-ascorbic acid) is an essential nutrient for humans and other mammalian species (Eteng *et al*., 2006). Ascorbate is required for a range of essential metabolic reactions it is made internally by almost all organisms. Ascorbate is an antioxidant, which might protect the body from some oxidative stresses, and is considered a cofactor in several vital enzymatic reactions (Padayatty *et al.,* 2003). The main source of vitamin C is found in the fruits and vegetables, therefore the plasma vitamin C concentration is a marker of fruits and vegetables intake. Previous studies showed that the occurrence of cardiovascular disease and cancer is inversely related to

vitamin C intake and plasma vitamin C concentrations (Block *et al.,* 2001). Hence, the protective effects were attributed to the fruits and vegetables intake specifically those containing vitamin C, which might contribute to this protection (Padayatty *et al*., 2003).

Female reproductive failure is a significant public health concern. Although, relatively little is known about factors affecting fertility and early pregnancy loss, most of literatures suggested that the environmental and lifestyle factors played important roles of fertility. There is sufficient evidence to hypothesize that diet particularly its constituent antioxidants and oxidative stress might influence the timing and maintenance of a viable pregnancy (Ruder *et al*., 2008). Systemic supplementation with antioxidant may help overcome oxidative stress in the female infertility; therefore, vitamin C might play a role in fertilization. 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 (Agarwal *et al.,* 2005). Vitamin C has multiple functions including antioxidant and collagen-stimulating properties. A study performed on anovulatory women for whom clomiphene failed showed that oral supplementation with vitamin C (400 mg per day) increased the ovulation in both with and without clomiphene citrate (Chris and Nieske, 2006; Igarashi, 1977).

The concentration of ascorbic acid showed higher in mature ovarian follicles than in the serum (Paszkowski and Clarke, 1999), this suggests an active uptake of vitamin C by the follicle resulting in the sequestration of vitamin C (Agarwal *et al*., 2005). In patients undergoing *in vitro* fertilization (IVF)-embryo transfer, the vitamin C supplementation is given during the period of hormonal stimulation, which resulted in higher follicular fluid concentrations of vitamin C (Crha *et al*., 2003). Another study showed that three months of supplementation results in a trend towards an increase in mean mid-luteal progesterone concentrations and a significant increase in the number of days with basal body temperatures >37^{O}C. After five months of supplementation, the pregnancy rates were significantly higher in the supplemented group (33 versus 0%, P<0.01) (Westphal *et al.,* 2004).

Another randomized controlled trial examined the effects of vitamin C supplementation (750 mg daily) in patient with luteal phase defects. The serum progesterone concentrations (13.27±0.63 versus 7.51±0.22 ng/ml) and pregnancy rates were increased significantly with ascorbic acid supplementation. Most of literatures collected showed lack the study about the effects of vitamin C on the endometrial thickness. Hence, the objective of this study was to determine the effect of vitamin C on the endomerium thickness and ovarian hormones of progesterone and estrogen in married and unmarried women.

MATERIALS AND METHODS

Study Population

The study was achieved throughout a period extended from December 2008 to July 2009. The total number of participations in study was 30 women that involved 10 unmarried and fertile, which randomly selected from relatives and friends to ensure successful results, while 20 married women were divided into two groups with 10 individuals each named fertile and infertile selected from private clinics of gynecology and obstetrics. All participants were in reproductive age, the age range was 15-45 years in unmarried women, while the age range of fertile and infertile was 18-38 years and 17-40 years respectively. The body mass index (BMI) ranges was 16-35 kg/m^2. The proposal of this study was approved by the ethics committee of medicine college Al-kufa University. The synthetic vitamin C was used as oral tablets (250mg) manufactured by Iraqi Pharmaceutical Industry (IPI) Company.

Demographical Information

The history was obtained from each woman involved in the study that including the name, address, contact number, age, marital status (married or unmarried), fertility state (fertile or infertile) and type of fertility (explained or unexplained infertility). Menstrual history included the duration, regularity, the date of last cycle to determine the 23^{rd} day (mid luteal phase) were recorded. Past medical and surgical, medicines, degree of fruit consumption (DFC) rich in vitamin C as lemon and orange that considered good degree (GD) of daily or every other and poor degree (PD) every three day or more of fruit consumption were recorded.

Vitamin C Supplementation, Ultrasound Examinations and Hormone Measurement

Determination on the 23^{rd} day of the cycle as a mid secretary phase, and before vitamin C supplementation, each woman was sent for abdominal ultrasonic examination to estimate the endometrial thickness. Before half an hour of ultrasound examination, the examined women drank a water to get full bladder to give an acoustic window for proper uterine examination. The ultrasound was achieved using Ultra mark ATL 9 (USA, 1992). The convex probe 3.5 MHz was used for transabdominal approach examination (Daftary and Patki, 2009).

Blood specimens were collected for serum estrogen and progesterone concentrations measurement. However, five ml blood specimens were collected from all participants before vitamin C supplements, which consider as a control group (CG). Also, five ml blood specimens were collected from all participants after vitamin C supplements, where each woman started to

take 2 tablet of 250 mg 3 times/ day of vitamin C that ingested orally after meal. The first dose of vitamin C supplementation was taken on the first day of next period (29±1 day) as a first period after the vitamin C supplements group (A1). On 23rd day of the first cycle, the last same dose and duration of treatment was repeated. On the 23rd day of the second period group (A2), the serums' progesterone and estrogen concentrations as quantitative determined using progesterone and Estradiol (E2) Enzyme Immunoassay Test Kit using ELIZA apparatus.

Height and Weight Measurements

The height of women was measured using tap measurement and the weight was measured using movable balance. Measurement of the height and weight for each woman was archived to calculate their body mass index (BMI) according to the following equation: BMI = Weight (kg) / Height (m^2) (Eknoyan and Garabed, 2008).

Statistical analysis was performed using the least significant differences (LSD) and analysis of variance (ANOVA), utilizing (SPSS: ver. 17 for windows) and (Excel) programs. Student t-test and X -2-chi-square also were used in this study. All values were expressed as Mean± SD, P-value of less than 0.05 was considered statistically significant.

Results

The results of this study showed there was no significant difference in the age and body mass index (BMI) (kg/m^2) between unmarried, fertile and infertile women (Table 1).

Table (1): Anthropometric data for unmarried, fertile and infertile women (n=30)

Anthropometric data (Mean ±SD)	Unmarried N=10	Married		P-value
		Fertile N=10	Infertile N=10	
Age (years)	28.3±10.57	30.6±6.2	25.4±6.83	NS
BMI($kg/m^{2)}$	25.72±6.21	26.93±3.29	25.07±2.99	NS

NS: No significant differences at P<0.05

The effect of vitamin C on the endometrial thickness in fertile, infertile and unmarried women

The ultrasound examination of endometrial thickness showed increase significantly at P<0.05 in group A1, and A2, where the highest thickness was seen in the married infertile (10.1±1.76) and unmarried women (10.44±0.85) compared control group (CG). Meanwhile there was no significant increase at P<0.05 between A1and A2 groups. The highest endometrial thickness showed increase in the unmarried women (8-11.5 mm in A2), fertile (7-13mm A1) and infertile

(6-12.5 mm in A1) in a period extends for two successful regular cycles with vitamin C supplements (Table 2).

Table (2): The Effect of vitamin C on the endometrial thickness of all groups

Marital status	Endometrial thickness range (mm) in association with vitamin C supplementations (Mean ± SD)					
	CG		A1		A2	
Married Fertile	6.87±1.56	5-9	9.7±2.0*	7-13	9.57±1.59*	7.5-12
Married Infertile	5.95±2.07	3-9	9.9±2.44*	6-12.5	10.1±1.76*	7.5-12
Unmarried	6.84±2.15	4-10	9.05±1.89*	5.5-11	10.44±0.85*	8-11.5

* Significant increase differences at (P<0.05)

The effect of vitamin C on serum progesterone (P) (ng/ml) concentration showed significant increase (P<0.05) in group A2 in fertile, infertile and unmarried women compared with CG. The effect of vitamin C on serum estrogen (E) (pg/ml) concentration in fertile, infertile and unmarried women showed significant increase (P<0.05) in A2 compared with CG in both fertile and unmarried women only, while there is no significant difference (P<0.05) in serum estrogen concentration in all group when compared between fertile, infertile and unmarried women (Table 3).

Table (3): The Effect of vitamin C on serum progesterone (P) and estrogen (E) concentrations in fertile, infertile and unmarried women

Marital Status	Serum progesterone and estrogen concentrations (Mean ±SD)					
	CG		A1		A2	
	P	E	P	E	P	E
Married Fertile	1.2±0.98	44.9±14.3	1.75±1.37	51.0±15.87	2.42±1.42*	62.6±15.16*
Married Infertile	1.07±0.35	44.1±17.54	1.98±0.65	51.3±14.86	2.55±0.83*	56.4±17.04
Unmarried	1.18±0.89	33.7±11.75	1.52±0.97	42.2±15.01	2.21±1.31*	48.7±15.17*

*Significant increase (P<0.05)

The results of relation between the degree of fruit consumption those rich in vitamin C and vitamin C supplements showed increases significantly (P<0.05) of endometrial thickness of fertile and infertile women in A1and A2 compared with CG in both GD and PD of fruit consumption. While there was no significant increase (P<0.05) showed in A1 compared with A2 (Table 4) (Figure 1).

The results of this study showed there was significantly increase (p<0.05) of the serum progesterone concentrations in the A1and A2 compared with of fertile and infertile, the highest increasing showed the in A2 group fertile women with PD (Table 5).

Table (4): The relation between the degree of fruit consumption rich in vitamin C and endometrial thickness in association with vitamin C supplementation in fertile (n=10) and infertile (10) women

DFC+ C	Fertility Status	Endometrial thickness (mm) Mean ±SD		
		CG	A1	A2
GD	Fertile	7.92±0.66	9.83±1.94*	10.3±1.68*
	Infertile	8.67±0.58	11.17±1.44*	10.67±0.58*
PD	Fertile	3.53±1.01	9.5±2.38*	8.89±1.38*
	Infertile	4.97±1.04	9.36±2.66*	9.86±2.08*

*Significant increase (P<0.05) compared A1or A2 with CG

Table (5): The relation between the degree of fruit ingestion riches in vitamin C and serum progesterone and estrogen concentrations in association with vitamin C supplementations in fertile and infertile women

DFC+C	Fertility state	Serum progesterone and estrogen concentrations (Mean ±SD)					
		CG		A1		A2	
		P	E	P	E	P	E
GD	Fertile	1.4±1.03	46.3±14.29	1.72±1.23	55.±15.53	2.57±1.46	65.17±15.82
	Infertile	1.13±0.29	61.33±26.8	2.33±0.76*	61.33±23.3	2.8±0.97*	67.66±22.50
PD	Fertile	0.35±0.05	42.5±16.2	1.8±1.78*	47±17.4	2.2±1.54*	58.75±15.48
	Infertile	1.04±0.4	36.71±3.25	1.83±0.6*	47±9.09*	2.31±0.77*	51.57±13.26*

*Significant increase (P<0.05)

Figure (1): The ultrasound examination of endometrial thickness showed increase significantly the highest thickness was seen in the married infertile 1A1 (10.1±1.76) and unmarried women 1A2 (10.44±0.85) compared control group (1C).

DISCUSSION

The effect of vitamin C supplementation on the endometrial thickness and ovarian hormones in married and unmarried women during luteal phase was shown a significant increasing in thickness of the endometrium after first period 29±1days and second period 58±2 days as compared with thickness before vitamin C supplementation for married (fertile and infertile) and unmarried women and this significant increasing may be attributed to vitamin C. Firstly, vitamin C may have indirect effect on the thickness of the endometrium by its effect on ovarian hormones of progesterone and estrogen. There was significant increased in serum progesterone after A2 as compared with CG in both fertile and infertile women, also serum estrogen was significantly increased after A2 compared with B in married (fertile) and unmarried women and this may help to increase the endometrial thickness during luteal phase. The results of this study agreed with the pervious study by McKinley and Olouchlin (2006) on responsibility of both progesterone and estrogen in increasing the endometrial thickness during luteal phase of menstrual cycle, another report by Karanth et al., (2001) suggested that the antioxidants properties of vitamin C stimulate the release of gonadotrophins from adenohypophysis. Luteal phase defects decreased about 53% in the supplemented group while the defects spontaneously improved about 22% in non-supplemented group (Henmi *et al.,* 2003). The maximal thickening of the endometrial thickness that reached during this secretary phase between 23^{rd}-25^{th} day of cycle (Kenneth *et al*., 2001).

Secondly, the role of vitamin C as antioxidant that may help to protect the tissue of the endometrium from oxidative stress (Ruder *et al*., 2008). Also the effect of vitamin C supplementation on the endometrial thickness might be related to antioxidant properties of estrogen which investigated in woman's reproductive organs (Liu *et al*., 2006). Murdoch (1998) was investigated the 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 reactive oxygen species scavenger during pregnancy mediated luteal rescue and fulliculogenesis.

Thirdly, the endometrial thickness increasing might related to the role of vitamin C as cofactor in the synthesis of collagen in the luteal extra cellular matrix (Eteng *et al.,* 2006). The formation of collagen fibers can lead to increase in thickness of the endometrium and this agree with previous study on bovine endometrium in which bovine endometrial stromal (BES) cells were cultured to confluence in medium with L-ascorbic acid phosphate magnesium salt n-hydrate which stimulate collagen synthesis of BES (Yamauchi e*t al*., 2003). In conclusion, the results show that there was

significant increase on the endometrial thickness in both A1 and A2 as compared with C for both fertile and infertile women.

REFERENCES

Agarwal, A; Sajal, G and Rakesh, S (2005). Oxidative stress and its implications in female infertility. Reproductive Bio-Medicine Online; 11. (5): 641-50.

Block, G; Norkus, E; Hudes, M Mandel, S and Helzlsouer, K (2001). Which plasma antioxidants are most related to fruit and vegetable consumption. Am. J. Epidemiol., 154: 1113-8.

Chris D. Meletis and Nieske Zabriskie (2006). Natural Approaches for Treating Polycystic Ovary Syndrome. Alternative and Complementary Therapies, 12(4): 157-164

Crha, I; Hruba, D and Ventruba, P (2003). Ascorbic Acide and infertility treatment. Central European Journal of public health, 11: 63-7.

Daftary NS and Patki A. 2009. Reproductive Endocrinology & Infertility. Endocrine gynecology. BI Publications Pvt Ltd, 54 Janpath, New Delhi 110001, Pp 464.

Eknoyan, L and Garabed, N (2008). The average man indices of obesity. Nephrol. Dial. Transplant., 23: 47-51.

Eteng, MU; Ibeekwe, HA; Amatey, TE; Bassey, BJ; Uboh, FU *et al*., (2006). Effect of Vitamin C on serum lipids and electrolyte profile of albino wistar rats. Nigerian Journal of Physiological Sciences, 21 (1-2): 15-9.

Henmi, H; Endo, T and Kitajima, Y (2003). Effects of Ascorbic Acide supplementation on serum progesterone levels in patients with a luteal phase defect. Fertility and Sterility, 80:459-61.

Igarashi, M (1977). Augmentative effect of ascorbic acid upon induction of human ovulation in clomiphene ineffective anovulatory women. International Journal of Fertility, 22: 168-73.

Karanth, S; Yu, WH; Walczewska, A; Mastronardi, CA and McCann, SM (2001). Ascorbic acid stimulates gonadotrophin release by autocrine action by means of NO. Proc Natl Acad Sci USA. 98: 11783-8.

Kenneth M. Nalaboff, John S. Pellerito, and Eran Ben-Levi (2001). Imaging the Endometrium: Disease and Normal Variants. Radio Graphics, *21, 1409-1424.*

Liu, A; Schisterman, EF and Wu, C (2006). Estrogen and progesterone effects on biomarkers of oxidative stress and antioxidant status during the menstrual cycle. Biometrics, 62: 1190-6.

Mckinley, M and Olouchlin, VD (2006). Human Anatomy. Business unit of the Mc Grow-Hill companies. Pp: 861-3.

Murdoch, WJ (1998). Inhibition by oestradiol of oxidative stress induced apoptosis in pig ovarian tissues. J Repord Fertil. 114: 127-130.

Padayatty, S; Katz, A; Wang Y; Eck, P; Kwon, O *et al.,* (2003). Vitamin C as an antioxidant: evaluation of its role in disease prevention. J. Am. Coll. Nutr., 22(1): 18-35.

Paszkowski, T and Clarke, RN (1999). The Graafian follicle is a site of L-ascorbate accumulation. Journal of Assisted Reproduction and Genetics, 16: 41-5.

Ruder, EH; Hartman, J; Blumberg, J and Goldman, M (2008). Oxidative Stress and antioxidants: exposure and impact on female fertility. Human Reproduction Update Advance Access: 1000-93.

Westphal, LM; Polan, ML and Trant, AS (2004). A nutritional supplement for improving fertility in women: a pilot study. Journal of Reproductive Medicine, 49: 289-93.

Yamauchi N, Yamada O, Takahashi T, Imai K, Sato T, Ito A, Hashizume K. (2003). A three-dimensional cell culture model for bovine endometrial: regeneration of a multicellular spheroid using acrobate. Placenta. 24(2-3): 258-67.