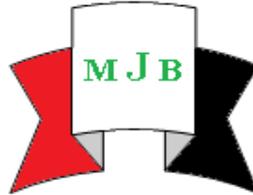# Vitamin E and C Status In The Sera and Cervical Mucus Secretion of Infertile Female With Unexplained Infertility

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Abstract

Back ground : Infertility is one of the most important and under appreciated reproductive health problems in developing countries.

The causes of infertility can be found in about 90% of cases, while about 10% of patients don't know why they can not conceive .The causes of this case (unexplained infertility) seems to be heterogeneous, with suggested potential causes ranging from disturbances in endocrinological, immunological , genetic and reproductive physiological factors.

One of the main causes of unexplained infertility is the reactive oxygen species (ROS). Therefore this study is aimed to estimate and calculate the vitamin E and C level. To carry out this aim, the vitamin E and C level had been estimated in both sera and cervical mucus secretions among healthy fertile women (control) and patients with unexplained infertility.

Patients and method: The study groups were attended to Babylon Maternity and Pediatric Hospital and privet clinics. All studied patients are suffering from primary or secondary unexplained infertility types and are diagnosed by gynecologist. The study is carried out on (30) apparently healthy fertile women as a control group, their mean age (30.133±8.011years) and (60) infertile women as patient group , their mean age (29.866 ±7.195 years).

Results: Vitamin C levels decrease insignificantly(p>0.05) in serum and cervical mucus secretion(9.944 ± 1.549mg/l and14.233 ±3.458mg/l) in patient group comparing with(10.383±3.655mg/l and(16.447± 4.042mg/l) in control group.

Vitamin E levels decrease significantly (p<0.05) in serum and cervical mucus secretion(5.272 ± 1.228mg/l and 4.644 ± 1.343 mg/l) in patient groups comparing with (7.337±3.535 mg/l and 7.023± 0.754 mg/l) in control groups.

Conclusion: The present study shows that the vitamins level plays an important role in human fertility. Vitamin E levels is significantly decreased in sera and cervical mucus secretion in patients with unexplained infertility.

الخلاصة

يعتبر العقم من أهم المشاكل الصحية في الدول النامية.و يمكن تحديد أسباب العقم في ٩٠% من الحالات بينما يبقى السبب في ١٠ % من الحالات غير معروف.وهذا العقم غير المفسر ربما تكمن أسبابه في الاضطرابات الفسلجية :هرمونية أو مناعية أو وراثية.

ويبدو في الوقت الحاضر إن أنواع الأوكسجين الفعالة واحدة من الأسباب الرئيسية للعقم الغير مفسر .لذلك صممت هذه الدراسة لتحديد وقياس أنواع الأوكسجين الفعالة والتي تسمى بالجذور الحرة وكذلك قياس مضادات الأكسدة في مصل الدم و الإفرازات المخاطية لعنق الرحم لمعرفة مدى علاقتها بالعقم غير المفسر.

المرضى وطرق العمل : شملت الدراسة (٦٠) امرأة مصابة بالعقم غير المفسر متوسط أعمارهن (٢٩.٨٦٦±٧.١٩٥)عاما واللاتي شخصن سرريا و مختبريا . تم فحص النساء من قبل أخصائية نسائية في مستشفى بابل للولادة والأطفال وفي العيادات الخاصة في بابل

و للفترة من أيلول ٢٠٠٨ ولغاية أيار ٢٠٠٩. كما تضمنت الدراسة (٣٠) عينة قياسية من النساء المنجبات متوسط أعمارهن (٣٠.١٣٣±٨.٠١١)عاما ، حيث تم دراسة نسبة فيتامين أي (E) و نسبة فيتامين سي(C) في مصل الدم وفي الإفرازات المخاطية لعنق الرحم و لكلا المجموعتين.

النتائج : بينت النتائج إن نسبة فيتامين إي قد انخفض انخفاض معنوي في مصل الدم و الإفرازات المخاطية لعنق الرحم 1.228 (5.272و4.644 ± 1.343 ملغم /لتر) للنساء العقيمات عند مقارنتهم بالنساء المنجبات(٧.٣٣٧ ±٣.٥٣٥ و ٠.٧٥٤ ± ٧.٠٢٣ ملغم /لتر.

إما نسبة فيتامين سي فقد وجد انخفاض غير معنوي في مصل الدم و الإفرازات المخاطية لعنق الرحم(9.944±1.549 و 3.458 14.233± ملغم /لتر) للنساء العقيمات عند مقارنتهم بالنساء المنجبات(١٠.٣٨٣±٣.٦٥٥ و ١٦.٤٤٧ ± ٤.٠٤٢ ملغم /لتر).

الاستنتاج: ان نسبة فيتامين اي له علاقة وثيقة بالعقم وان نسبته منخفضة في حالات العقم غير المفسر .

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Introduction

Infertility is defined as 'the inability to conceive following 12 months of unprotected sexual intercourse, before an investigation is undertaken unless medical history and physical findings dictate earlier evaluation and treatment (1).

Unexplained infertility is a diagnosis of exclusion, when the standard investigation of both the female and male partner has ruled out other infertility diagnoses (2).A couple is considered to have unexplained infertility if the woman ovulated and had a normal and hysterosalpingogram, and the man a normal semen analysis .Critical factors to be considered in evaluating and managing unexplained infertility are the duration of infertility and female age (3).

Vitamin C is a water soluble vitamin found in many fruit and vegetable(4).

It is required for optimal functions of number of enzymes ;deficiency cause scurvy and poor wound repair. It is also considered a chain breaking antioxidant that stops the propagation of the peroxidative process.

Ascorbic acid has three main biological functions, each dependent on its role as a reducing agent:

- It is required for biosynthesis of collagen.
- For biosynthesis of steroids and peptide hormones (5) and

- Protects the body from the harmful effects of free radicals and pollutants (6).

Vitamin E is fat - soluble vitamin**.** The primary role of vitamin E within the body is to function as an antioxidant (7).Within cells and organelles (e.g. mitochondria) vitamin E is the first line of defense against lipid peroxidation. Moreover, it plays a very important function in lending red blood cells flexibility as they make their way through the arterial network and helps prolong the life of erythrocytes, immune function, and has positive effects in the fertility (8).

Aims of The Study

The aims of the present work is to explore some vitamins level in unexplained infertility among women and relationship between these vitamins and some factors (age, body mass index ,smoking ,infertility duration and type of infertility).

Materials and Methods

Patients and control groups:

Control group: This group consists of thirty apparently healthy fertile women, with a mean age (30.133±8.011years), and range from 18 to 44 years were investigated to serve as a controlling group. None of them had clinical or laboratory evidence of diseases that would affect the parameters to be measured.

Patients group: Couples resident in Babylon with unexplained infertility of

more than 12 months duration were identified from the Fertility Clinic database in Babylon maternity hospital and private clinic. The patients were seen between August 2008 and May 2009.

The following criteria have been used to establish the diagnosis of unexplained infertility: mid-luteal serum progesterone concentration >20 nmol/l, bilateral tubal patency demonstrated by laparoscopy or hysterosalpingogram and normal semen parameters (WHO,1999).

Sixty women had been studied, with a mean of age (29.866 ±7.195 years), and from 18 to 44 years ; 26 with primary unexplained infertility and 34 have secondary unexplained infertility. Those having male factor of infertility or female factors or any other an associated condition which could alter the level of free radicals like, hypertension, diabetes mellitus, heart disease, malignancy, and antioxidant therapy, had been excluded from the study. Each subject was involved to detailed clinical history and physical examination. The infertile group had undergone baseline investigations of infertility.

Method

Collection of blood and serum preparation

About 5 ml of venous blood for specific test of markers was collected by vein puncture using 5ml disposable syringes. Some blood drops are put on slide for blood group typing(9) . The obtained sera was put then in another disposable tubes and labeled. The samples were transferred to the biochemical laboratory for analysis of vitamin C and vitamin E.

Collection of cervical secretion

About 0.5 ml of cervical secretion was taken by syringe from high cervix using cusco speculum, labeled and storage at − 20 C◦.

For biochemical tests, the mucus must be liquefied by mucolytic agent of N-acetyl L- cycteine at concentration 0.2 mg/ml which prepared by weight 0.2 mg of N-acetyl L- cycteine and complete to one milliliter with DW (10,11).

Biochemical tests

Determination of total vitamin C in sera and cervical secretions: vitamin C was determined according to 2,4-dinitrophenylhydrazine (DNPH) methods (12)

Determination of Vitamin E in sera and cervical secretions: The α-α dipynidyl was added to an aliquot of the upper layer to estimate the principal interfering substance, B-carotene, at 460 nm. At this time, the ferric chloride $(FeCl_3)$ reagent was added to the system to produce the colour which was measured at 510 nm (13).

Statistical Analysis

SPSS program was used in this study.All values were expressed as mean ± standard deviation (SD). Independent t-test was used to estimate differences between groups. The differences were considered significant when the probability (P) was less than 0.05(P< 0.05)(14).

The Results

Measurement of vitamin C activity

Vitamin C levels in sera and cervical secretions of infertile women with unexplained infertility show insignificant decrease (p>0.05) when compared with fertile control as shown in Table (1):
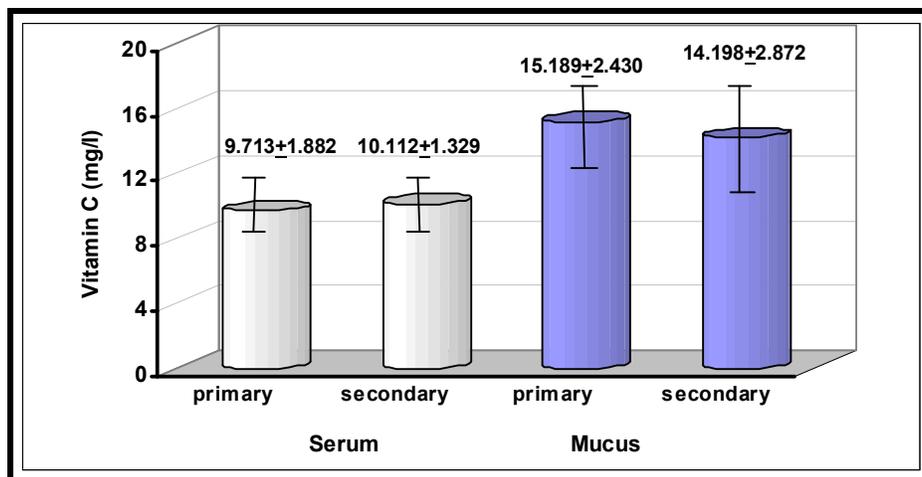
Table (1) Vitamin C levels(mg/l) in sera and cervical secretions of infertile women with unexplained infertility and Control.

Sample	Control Mean ±SD mg/l	Patients Mean ±SD mg/l	P value
Serum Vitamin C	10.383 ± 3.655	**1.549 9.944 ±**	**P>0.05**
C.mucus Vitamin C	**16.447 ± 4.042**	14.233 ± 3.458	**P>0.05**

Vitamin C levels and types of infertility

Vitamin C levels in sera and cervical secretions of infertile women with unexplained infertility show insignificant differences($p>0.05$) between primary and secondary infertility as shown in Figure (1):
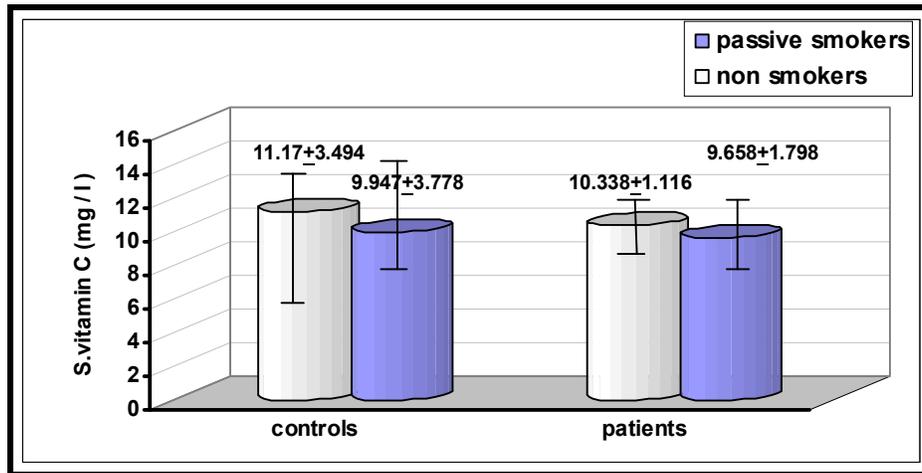

Figure (1) Vitamin C levels (mean ±S.D mg/l) in sera and cervical secretions in primary and secondary infertile women with unexplained infertility($p>0.05$).
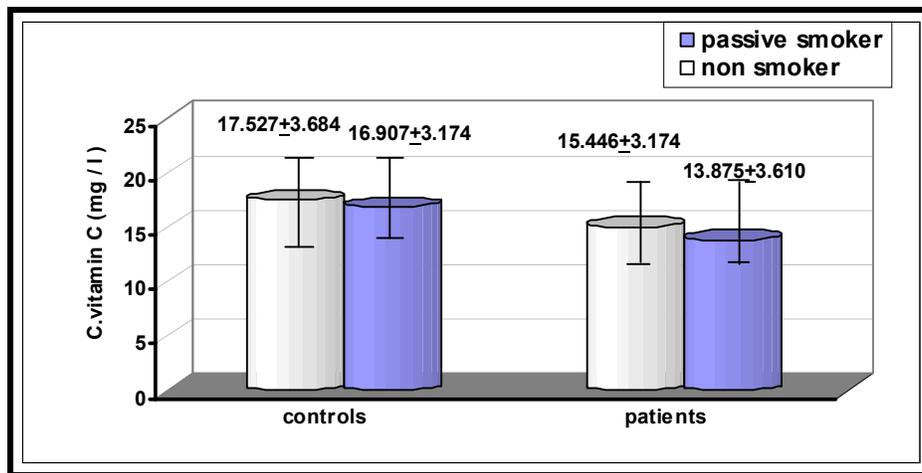
Vitamin C levels and smoking

Vitamin C levels in sera and cervical secretions show insignificant differences($p> 0.05$) between passive and non smoker in infertile women with unexplained infertility and fertile control as shown in Figure (2) and (3):

Figure (2) The serum vitamin C levels (mean ±S.D mg/l)in passive and non smoker in infertile women with unexplained infertility and fertile control($p>0.05$) .
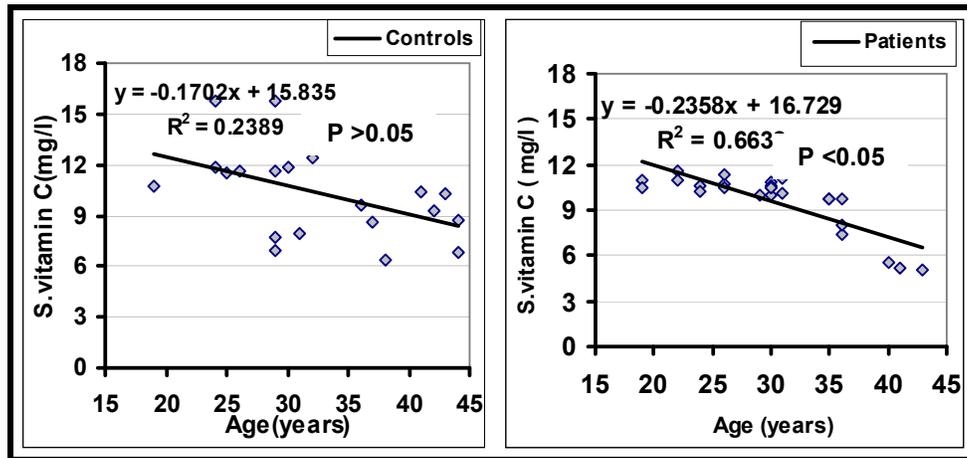

Figure (3) Vitamin C level in cervical secretions (mean ±S.D mg/l)in passive and non smoker in infertile women with unexplained infertility and fertile control($p>0.05$) .
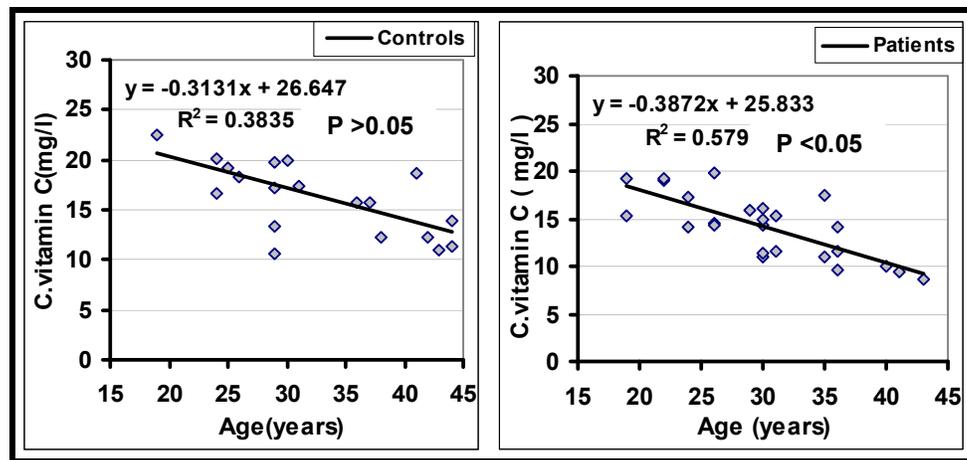
The Relationship between age and vitamin C

Vitamin C levels in sera and cervical secretions of infertile women with unexplained infertility show a significant negative correlation with age increment when compared with fertile controls ($p<0.05$) as shown in Figure (4) and (5):

Figure (4) Relationship between age (years) and serum vitamin C levels (mean ±S.D mg/l) in infertile women with unexplained infertility and fertile control.
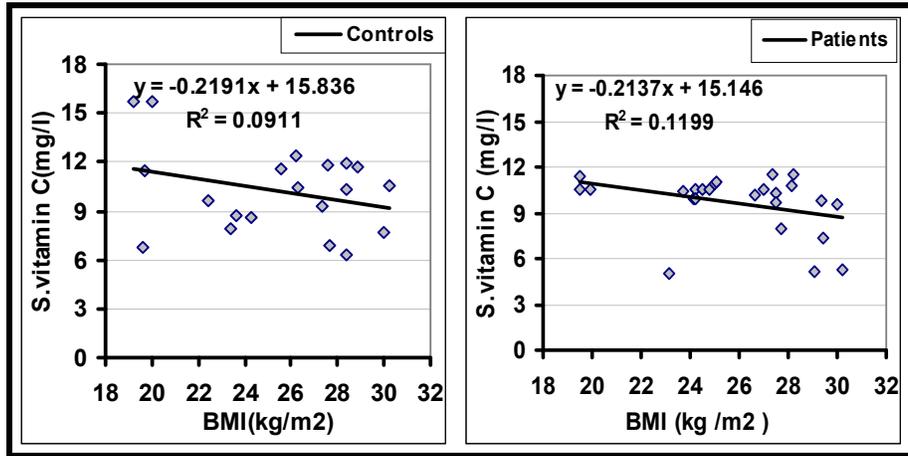

Figure (5) Relationship between age (years) and vitamin C levels in cervical secretions (mean ±S.D mg/l) in infertile women with unexplained infertility and fertile control.
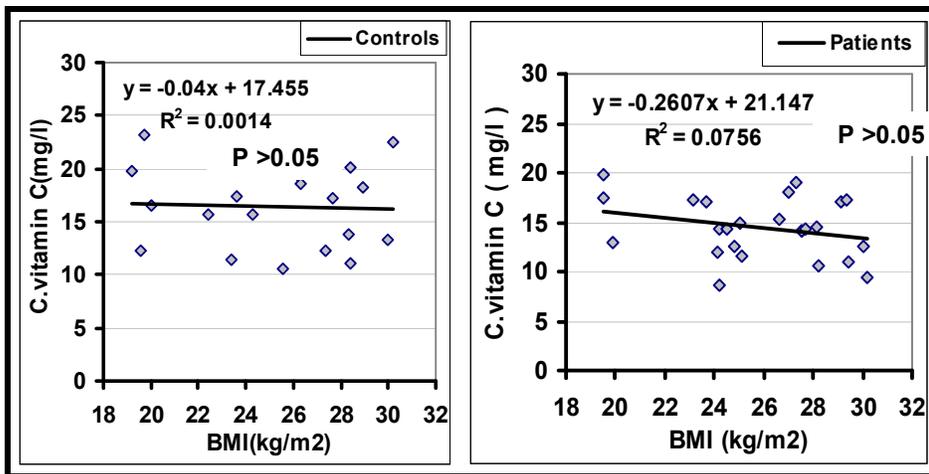
The Relationship between body mass index and vitamin C

Vitamin C levels in sera and cervical secretions of infertile women with unexplained infertility show insignificant negative correlation($p > 0.05$) with BMI increment when compared with fertile control as shown in Figure (6) and (7):

Figure (6) Relationship between BMI (kg/m^2) and serum vitamin C levels (mean ±S.D mg/l) in infertile women with unexplained infertility and fertile control.
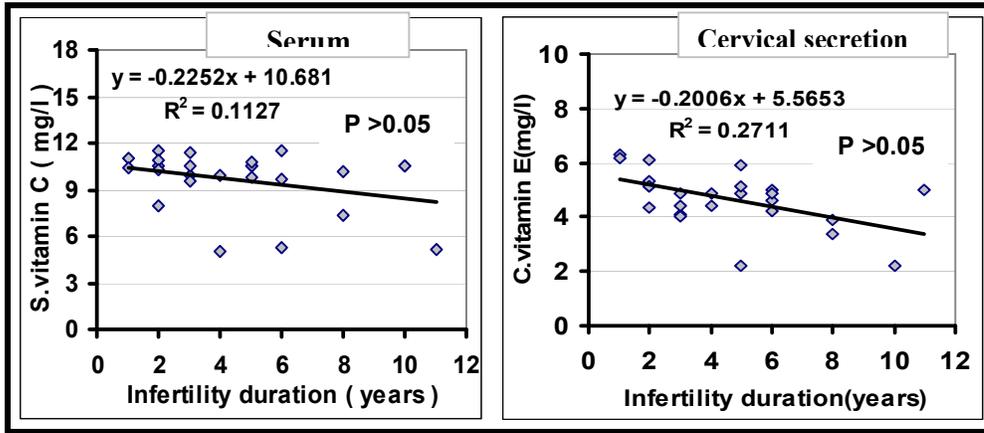

Figure (7) The correlation between BMI (kg/m^2) and vitamin C levels in cervical secretions (mean ±S.D mg/l) in infertile women with unexplained infertility and fertile control.

The Relationship between duration of infertility and vitamin C

Vitamin C levels in sera and cervical secretions of infertile women with unexplained infertility show insignificant negative correlation ($p > 0.05$) with duration of infertilityas shown in Figure(4.38):

Figure (8) Relationship between duration of infertility in years and vitamin C levels (mean ±S.D mg/l) in sera and cervical secretions of in infertile women with unexplained infertility.

Measurement of vitamin E levels

Vitamin E levels in sera and cervical secretions of infertile women with unexplained infertility show a significant decrease ($p<0.05$ and $p< 0.001$) respectively when compared with fertile control as shown in Table (2):
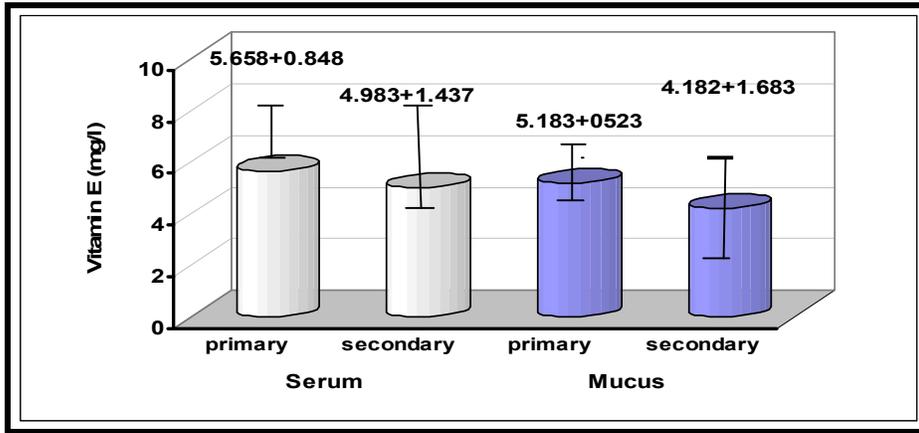
Table (2) Vitamin E levels in sera and cervical secretions (mg/l) of infertile women with unexplained infertility and fertile control.

Sample	Control Mean ±SD mg/l	Patients Mean ±SD mg/l	P value
Serum Vitamin E	7.337 ± 3.535	1.228 5.272 ±	$p<0.05$
C.mucus Vitamin E	7.023 ± 0.754	4.644 ± 1.343	$p< 0.001$

Vitamin E levels and types of infertility

Vitamin E levels in sera and cervical secretions of infertile women with unexplained infertility show insignificant differences($p>0.05$) between primary and secondary infertility as shown in Figure(9):
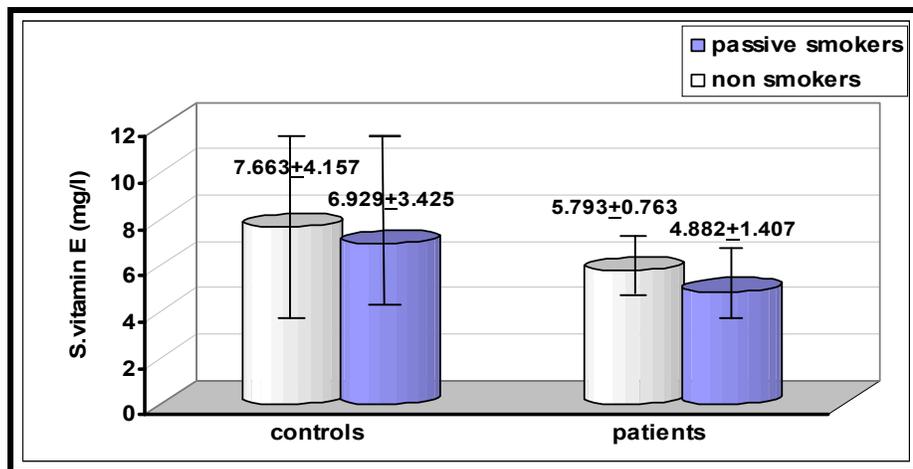
Figure (9) The vitamin E levels in sera and cervical secretions (mean ±S.D mg/l) in primary and secondary infertile women with unexplained fertility(p>0.05).

Vitamin E levels and smoking
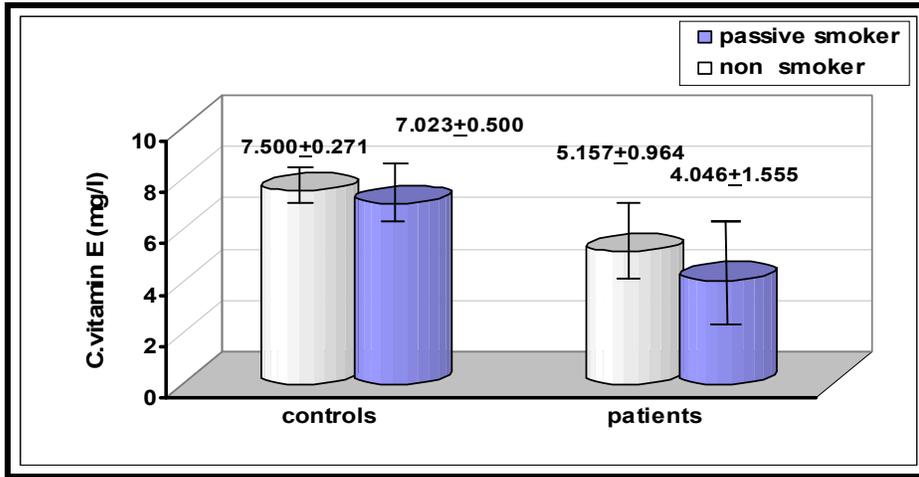
Vitamin E levels in sera and cervical secretions show insignificant differences between passive and not smoker in infertile women with unexplained infertility and fertile control (p> 0.05) as shown in Figure(10) and (11):

Figure (10) The serum vitamin E levels (mean ±S.D mg/l) in passive and non smoker of infertile women with unexplained infertility and fertile control(p>0.05).

Figure (11) The vitamin E level in cervical secretions (mean ±S.D mg/l) in passive and non smoker of infertile women with unexplained infertility and fertile control(p>0.05) .

The Relationship between age and vitamin E

Vitamin E levels in sera and cervical secretions of infertile women with unexplained infertility show insignificant negative correlation (p>0.05) with age increment when compared with fertile controls as shown in Figure (12) and (13):
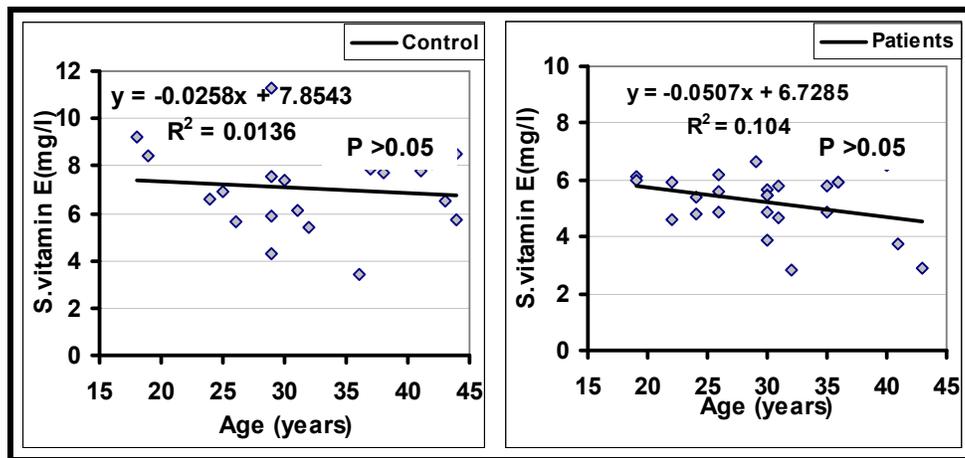
Figure (12) Relationship between age (years) and serum vitamin E levels (mean ±S.D mg/l) in infertile women with unexplained infertility and fertile control.

Figure (13) Relationship between age (years) and vitamin E levels in cervical secretions (mean ±S.D mg/l) in infertile women with unexplained infertility and fertile control.
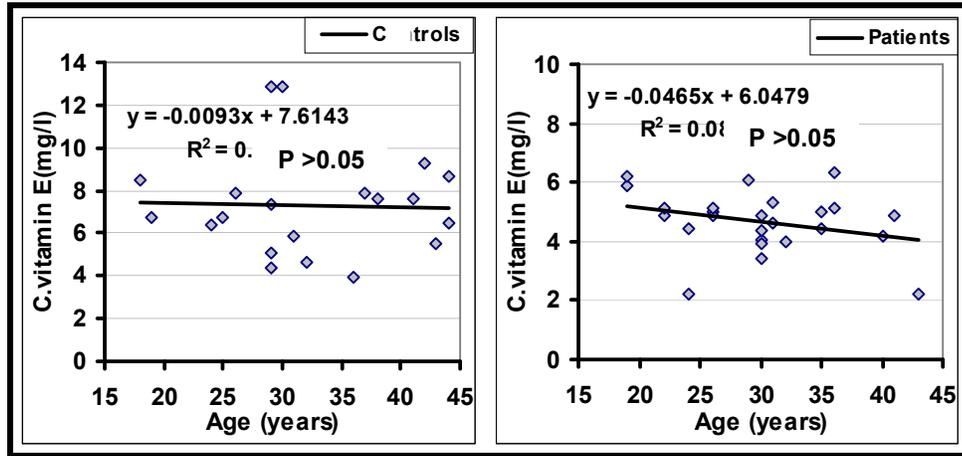
The Relationship between body mass index and vitamin E

Vitamin E levels in sera and cervical secretions of infertile women with unexplained infertility show insignificant decrease (p<0.05) with body mass index increment when compared with fertile controls as shown in Figure (14) and (15):

Figure (14) Relationship between BMI (kg/m^2) and serum vitamin E levels (mean ±S.D mg/l) in infertile women with unexplained infertility and fertile control.
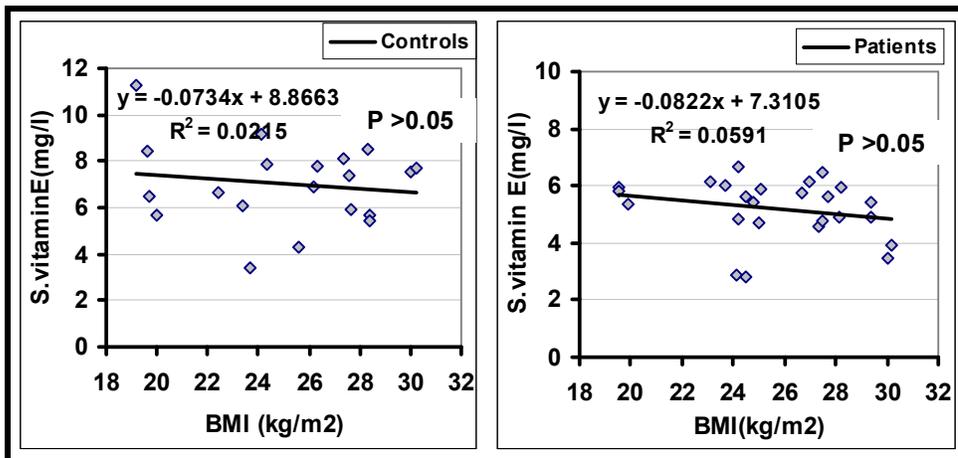
Figure (15) The correlation between BMI (kg/m^2) and vitamin E levels in cervical secretions (mean ±S.D mg/l) in infertile women with unexplained infertility and fertile control.

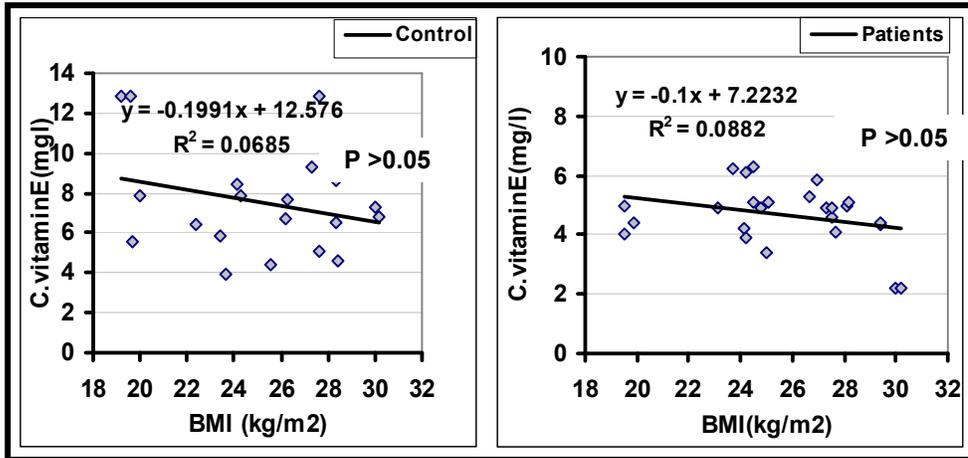**The Relationship between duration of infertility and vitamin E**

Vitamin E levels in sera and cervical secretions of infertile women with unexplained infertility show insignificant negative correlation (p> 0.05) with duration of infertility as shown in Figure(16):
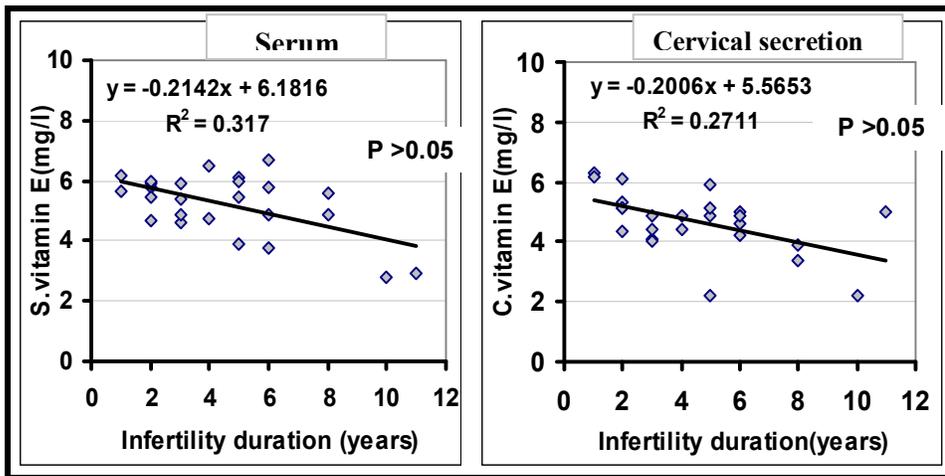
Figure (16) Relationship between duration of infertility (years) and vitamin E levels in sera and cervical secretions (mean ±S.D mg/l) in infertile women with unexplained infertility .

Discussion
Measurement of vitamin C activity
In female reproductive tract, vitamin C is located mainly in the ovary.

Vitamin C levels in sera and cervical secretions of infertile women with unexplained infertility show insignificant decrease (p>0.05) when compared with fertile controls as shown in Table (1)

Many studies indicate that the addition of ascorbic acid does prevent oocyte membrane damage and increases basement membrane turnover (15), leading to increased follicle integrity and survival. Other researchers propose that certain concentrations of alpha-tocopherol or ascorbic acid facilitate meiotic maturation of cumulus free oocytes and can protect cumulus cell DNA damage and apoptosis (16).

Supplementation inhibits follicular apoptosis and causes premature resumption of meiosis (1).

Vitamin C levels and types of infertility
Vitamin C levels in serum and cervical secretion of infertile women with unexplained infertility show insignificant differences between primary and secondary infertility (p>0.05) as shown in Figure (1).Therefore, this result may be explained by the fact of dietary vitamin C intake in which each type of female infertility are near equal.

Vitamin C levels and smoking
Vitamin C levels in sera and cervical secretions of infertile women with unexplained infertility show insignificant differences between passive and not passive smoker in infertile women with unexplained infertility and fertile controls (p> 0.05) as shown in Figure (2) and (3).

Ayaori ,et al. (2000) report that plasma ascorbic acid is inversely associated with smoking(17).Vitamin C helps to combat the pollution surrounding us. Moreover; it has been shown to reduce the DNA damage (18).No dietary difference between passive smoker and not smoker might be the cause behind such a result..

The Relationship between age and vitamin C
A significant negative correlation between vitamin C levels in sera, cervical secretions of infertile patients and the age increment when compared with fertile controls (p<0.05, r = -0.814, p<0.05, r = -0.761) as shown in Figure (4) and (5). Tarín ,et al.(1998) find that supplementation of vitamin C reduces the risk of ovulating aneuploid and diploid oocytes in aged female mice. Furthermore, dietary supplementation of mice with the antioxidants vitamins C and E, resulted in improvements in both the quantity and quality of oocytes when compared to unsupplemented older mice (19). This reduction of vitamin C with age may be due to increase OS with age increment which leads consequently to depletion of antioxidants(19).

The Relationship between body mass index and vitamin C
Vitamin C levels in serum and cervical secretion of infertile women with unexplained infertility show insignificant negative correlation with BMI increment when compared with fertile controls (p>0.05, r = -0.346; p>0.05, r = -0.274)respectively as shown in Figure (6) and (7).This result may be regarded to increase of polyunsaturated lipid , increase lipid peroxidation which consequently leads to decrease antioxidants levels.

The Relationship between duration of infertility and vitamin C
Vitamin C levels in sera and cervical secretions of infertile women

with unexplained infertility show insignificant negative correlation with duration of infertility (p>0.05, r = -0.335; p>0.05, r = -0.217) respectively as shown in Figure(8) and this result is in agreement with a result obtained by Crha ,et al.(2003) who report higher pregnancy rate with vitamin C supplementation when compared to the a controling group(20).

Measurement of vitamin E levels

Vitamin E is a major chain breaking antioxidant in membranes, located mainly in the ovary specially in follicular fluid.

Vitamin E levels in sera and cervical secretions of infertile women with unexplained infertility show a significant decrease (p<0.05 ;p< 0.001) respectively when compared with fertile controls as shown in Table (2). This result matches with the results got by Makinde and Adedeji(1994) and Mehendale, et al.(2009) who state that plasma vitamin E level is greater in fertile women than in infertile women(21&22).

Savita, et al.(2009) suggest that the increased OS are associated with the decrease of antioxidants and associated with infertility(23).

Vitamin E directly neutralizes superoxide anion, hydrogen peroxide, and hydroxyl radical ;so increase these types of free radical may lead to depletion of vitamin E. Also vitamin E increases number of embryos developing to the expanded blastocysts and increases viability of embryos exposed to heat shock. So any change in its concentration may have a role in infertility(1).

Vitamin E may increase oocyte quality. In a human trial, infertile couples given vitamin E show a significant increase in fertility (24).

Vitamin E levels and types of infertility

Vitamin E levels in sera and cervical secretions of infertile women with unexplained infertility show insignificant differences between primary and secondary infertility(p>0.05)as shown in Figure(9). This result may be due to the lack of vitamin E in diet as well as to other factors such as environmental factors which nearly affect both types of female infertility equally .

Vitamin E levels and smoking

Vitamin E levels in serum and cervical secretion of infertile women with unexplained infertility show insignificant differences between passive and not passive smoker in infertile women with unexplained infertility and fertile controls (p> 0.05) as shown in Figure (10) and (11) . Tiboni, et al. (2004)(25) state that no smoking-related differences in follicular fluid or plasma concentrations of vitamin E between fertile and infertile women as well as to Dietrich ,et al. (2003) research who find that no significant differences in plasma concentrations of alpha-tocopherol between fertile and infertile smoker. This result may be due to the same level of vitamin E in diet in both passive smoker and non smoker(26).

The Relationship between age and vitamin E

Vitamin E levels in serum of infertile women with unexplained infertility show a insignificant negative correlation (p>0.05, r = -0.332; p>0.05, r = -0.292)respectively with age increment when compared with fertile controls as shown in Figure (12) and (13).This result approves with the results obtained by Yeh, et al.(2005) who say that decrease in vitamin E and other antioxidant defense with age(27).

In this respect, Makinde and Adedeji (1994) show that vitamin E values are increased with age up to the 25-29 year age group(21). Other evidences researching by Carbone , et al. (2003) show that the aged ovaries have elevated vitamin E content. Thus, a shift toward a higher concentration of vitamin E (and low serum vitamin E level) may occur to help protect the

aging ovary during luteolysis and compensate for the decline in the luteal cell ability to quench ROS, as evidenced by lower glutathione reductase enzyme. Other researchers, such as Ruder,*et al.*(2008) (1)find that vitamin E supplementation reduces the risk of ovulating aneuploid and diploid oocytes in aged female mice and increases survival of explanted rat conceptuses in vitro. In general, when supplemental vitamins E is given to older mice, the age-associated reduction in ovulation is partially prevented (29).

The Relationship between body mass index and vitamin E

Vitamin E levels in serum and cervical secretion of infertile women with unexplained infertility show insignificant decrease ($p>0.05$, $r = -0.243$; $p>0.05$, $r = -0.296$)respectively with body mass index when compared with fertile controls as shown in Figure (14) and (15). Increase BMI may lead to increase unsaturated lipid which lead to higher ROS levels production . Higher ROS levels production would lead to reduce levels of antioxidants such as vitamin E that would ultimately reduce ROS-scavenging ability and prevent the neutralization of toxic ROS effects (30) .

The Relation between duration of infertility and vitamin E

Vitamin E levels in serum and cervical secretion of infertile women with unexplained infertility show insignificant negative correlation with duration of infertility ($p>0.05$, $r = -0.563$; $p>0.05$,

$r = -0.520$) respectively as shown in Figure(16).

This result may be due to the fact that the vitamin E level may be affected by the level of free radical production and level of vitamin E intake during time of infertility.Antioxidant levels in patients with unexplained infertility are significantly lower than those of healthy controls (31).Ruder, *et al.*, (2009) find that the use of

multivitamins, impacts the generation of ROS and may play a beneficial role in female fertility(1).

Conclusions

The present study shows that the vitamins level plays an important role in human fertility:
- Vitamin E levels is significantly decreased in sera and cervical mucus secretion in patients with unexplained infertility.
- There is an inverse significant relationship between vitamin C level and age increment in patients with unexplained infertility.

Recommendations
- Studying the role of oxidants in multiple sites such as ovary, peritoneal cavity and uterus separately.
- Study the role of oxidants in other types of infertility.
- Strategies to overcome OS in-vitro conditions and balancing between in vivo and in vitro environments can be utilized in assisted reproductive technique , to successfully treat infertility.

References

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