# Role of Ascorbic Acid in Male Fertility and its Relation with Free Testosterone

**Radhika Kothari[1], Satish Waghmare[2], A.R.Choudhari[3]**


## ABSTRACT

**Introduction:** Ascorbic acid is present at approximately ten fold higher concentration in seminal plasma as compared to blood and is mostly secreted from seminal vesicles. The present study aimed to assess the influence of seminal plasma ascorbic acid on seminogram characteristics and to assess the effect of endogenous testosterone on the seminal levels of ascorbic acid.

**Material and Methods:** Routine seminogram parameters were analysed from the semen samples obtained from150 male partners of infertile couples of age 21-50 who attended the Reproductive Biology Unit of the department of Physiology. Subjects were classified into two main groups. Group A-the subjects with normal ejaculates and group B-the subjects with abnormal ejaculates. These two groups were further subdivided into the following groups: i) Asthenoteratozoospermics (n=43); ii) Oligoasthenoteratozoospermics (n=24); and iii) Azoospermics (n=21). The seminal plasma ascorbic acid was measured spectrophotometrically and sample for serum free testosterone was sent to Thyrocare laboratory.

**Results:** We found that the seminal plasma ascorbic acid was significantly lower in the abnormal ejaculates than in the normal ejaculates. It was observed that there is statistically significant positive correlation between the seminal plasma ascorbic acid and levels of free testosterone in serum ($p<0.05$, $r=0.279$). Statistically significant correlation was also found between seminal plasma ascorbic acid and all parameters of seminogram like sperm concentration, sperm motility and sperm morphology ($p<0.05$, $r=0.77$, 0.85 and 0.83 respectively).

**Conclusion:** The significant causative factor in impairing sperm functions may be the low levels of seminal plasma and its dependence on endogenous free testosterone is observed from a positive correlation between the two.


**Keywords:** Seminal Plasma, Male Infertility, Ascorbic Acid, Serum Free Testosterone

## INTRODUCTION

A major water soluble antioxidant in biological fluids and an essential micronutrient required for normal metabolic functions of the body is Ascorbic acid or Vitamin C. Ascorbic acid has the most striking property that it acts as a reducing agent (electron donor). Ascorbic acid acts as an electron donor that reacts with superoxide, hydroxyl and peroxide radicals to form dehydroascorbic acid. Donation of electron by ascorbic acid gives rise to dehydroascorbate which must be recycled back to ascorbic acid in healthy condition.[1] It also recycles Vitamin E. In comparison to plasma, many tissues need to conserve or sequester the reduced form of ascorbic acid at high levels to protect themselves from oxidative stress.[2]

Sperm cytoplasmic volume is very low and its cytoplasm contains only low concentrations of free radical scavenging enzymes. In contrast, seminal plasma is well endowed with an array of antioxidant defence mechanism to protect spermatozoa against oxidants. It protects sperms against DNA damage induced by $H_2O_2$ radical and also reduces nitrite.[3] Song et al reported that patients with low levels of seminal ascorbic acid had increased sperm DNA damage.[4]

The present study aimed to assess the influence of seminal plasma ascorbic acid on seminogram characteristics and to assess the effect of endogenous testosterone on the seminal levels of ascorbic acid.

## MATERIAL AND METHODS

Detailed history of present and past illness as well as medical and surgical intervention was taken and semen samples were obtained from 150 male partners of infertile couples. External genitalia examination was done at surgery OPD. The study was done during the period from December 2012 to June 2014 in the Reproductive Biology Unit (Infertility Clinic) in the Department of Physiology. Patients were referred from department of Gynaecology and Surgery.

**Inclusion Criteria**: Primary or secondary infertile men aged 21-50 years who have not conceived after one year of regular, unprotected intercourse.

**Exclusion Criteria**: Subjects suffering from febrile illness or history of any treatment which may suppress the spermatogenesis were excluded from the study. Those subjects having diseases which may interfere with male infertility like hydrocele, hernia, undescended testes, varicocele, or any surgical history of genitourinary tract, were also excluded from the study. Permission of ethics committee was obtained from the institute and written consent was taken from each subject. After three days of abstinence, semen samples of the subjects were delivered on fourth day. Samples of semen were collected by masturbation. Each sample was tested after complete liquefaction at room temperature.

**Parameters studied**

**1) Routine Semen Analysis**

SQA II C-P (Sperm Quality Analyser) {Medical Electronic System Ltd. Israel} was used for
analysis of sperm concentration (millions/ml), sperm motility and sperm morphology.

[1]Tutor, Mahatma Gandhi Institute of Medical Sciences, Sevagram. Dist. Wardha, [2]Assistant Professor, Dr Vasantrao Pawar Medical College, Nashik, [3]Professor and Head, Department of Physiology, Mahatma Gandhi Institute of Medical Sciences, Sewagram. Dist. Wardha, Maharashtra, India

**Corresponding author:** Dr Satish Waghmare, Assistant Professor, Dr Vasantrao Pawar Medical College, Nashik, India

**How to cite this article:** Radhika Kothari, Satish Waghmare, A.R.Choudhari. Role of ascorbic acid in male fertility and its relation with free testosterone. International Journal of Contemporary Medical Research 2017;4(6):1232-1234.

Subjects were grouped into four categories according to WHO guideline[5,6] with following criteria:

i) Normozoospermics: Those subjects who have sperm concentration of 15 millions/ml or more; sperm motility (progressive + non progressive) being 40 % or more; normal sperm morphology in 30% cells or more.
ii) Oligoasthenoteratozoospermics: Those subjects who have sperm concentration less than 15 millions/ml; sperm motility (progressive + nonprogressive) below 40%; normal sperm morphology in less than 30% of sperms.
iii) Asthenoteratozoospermics: Those subjects who have sperm concentration of 15 millions/ml or more; sperm motility (progressive + nonprogressive) below 40%; normal sperm morphology in less than 30% of sperms.
iv) Azoospermics: Those subjects in whom there is total absence of spermatozoa in semen (even after centrifugation).

**2) Estimation of serum free testosterone**

Blood samples of the subjects were collected in the hospital. The blood samples were sent to Thyrocare laboratory for measurement of serum free testosterone by Radioimmunoassay technique and the report was collected after three days.

**3) Estimation of Ascorbic acid[7]:**

Principle: Ascorbic acid in seminal plasma is oxidized by cupric($Cu^{+2}$) to form dehydro ascorbic acid which reacts with acidic 2,4-dinitrophenyl-hydrazine to form a red bis-hydrazone which was measured at 520 nm spectrophotometrically.

## STATISTICAL ANALYSIS

Present study used descriptive and inferential statistics applying Z-test, One-way ANOVA and Pearson's Correlation Coefficient (SPSS version 17.0) and Graph Pad Prism (version 5.0).The p value less than 0.05 was considered as level of significance.

## RESULTS

The mean Ascorbic acid levels (mg/dl) of seminal plasma was found to be highest in Normozoospermics (18.21 ± 3.52; Range 10.00-27.50), followed by Asthenoteratozoospermics (12.86 ± 3.55; Range 7.75-22.00), Oligoasthenoteratozoospermics (8.07 ± 3.30; Range 4.00-19.75) and Azoospermics (4.05 ± 3.36 Range 1.00-17.50). Statistically significant variation was found in mean Ascorbic acid levels of four groups (F=108.65, p=0.000).

Seminal ascorbic acid level was found to have positive correlation with seminogram parameters like sperm concentration, motility, normal morphology (r=0.77, 0.85, 0.83 respectively, p<0.05) [Table 1, Figure 1-3].

Normozoospermics were found to have highest mean serum free testosterone level (pg/ml). It was found that there is statistically significant variation in mean free testosterone levels of four groups.

It was also found that there is significant positive correlation between serum free testosterone (pg/ml) and seminal plasma Ascorbic acid levels(mg/dl) (r=0.279, p<0.05) [Table 2, Figure 4].

## DISCUSSION

The very basic test to assess a man's fertility is semen analysis. Apart from sperm concentration, motility and morphology, in this study, we have investigated seminal concentration of Ascorbic acid. We have also tried to evaluate how Ascorbic acid

| Parameters | Mean | Std. Deviation | N | Correlation 'r' | p-value |
|---|---|---|---|---|---|
| Ascorbic Acid Level | 13.07 | 6.19 | 150 | - | - |
| Concentration | 48.35 | 44.61 | 150 | 0.77 | 0.000, S,p<0.05 |
| Motility | 33.75 | 22.02 | 150 | 0.85 | 0.000, S,p<0.05 |
| Morphology | 24.02 | 14.15 | 150 | 0.83 | 0.000, S,p<0.05 |
| Pearson's Correlation Coefficient | | | | | |

**Table-1:** Correlation of Ascorbic Acid Level (mg/dl) with sperm concentration(millions/ml), total % motility and normal morphology.

| Parameters | Mean | Std. Deviation | N | Correlation 'r' | p-value |
|---|---|---|---|---|---|
| Free Testosterone Level | 20.34 | 8.81 | 108 | - | - |
| Ascorbic Acid Level | 13.07 | 6.19 | 150 | 0.279 | 0.003,S,p<0.05 |
| Pearson's Correlation Coefficient | | | | | |

**Table-2:** Correlation of serum free testosterone Level (pg/ml) with seminal plasma Ascorbic Acid (mg/dl)

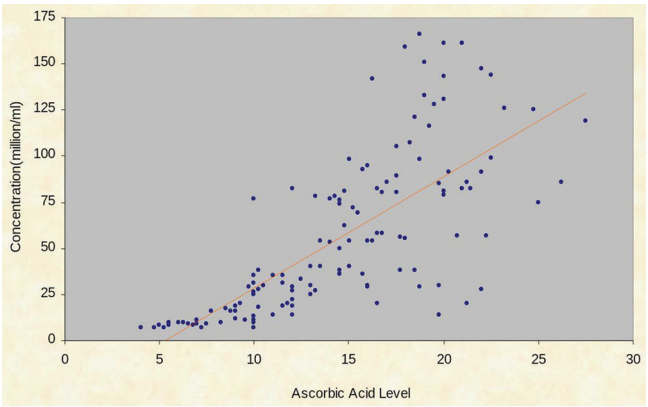


**Figure-1:** Correlation of Ascorbic Acid Level (mg/dl) with sperm concentration (millions/ml)

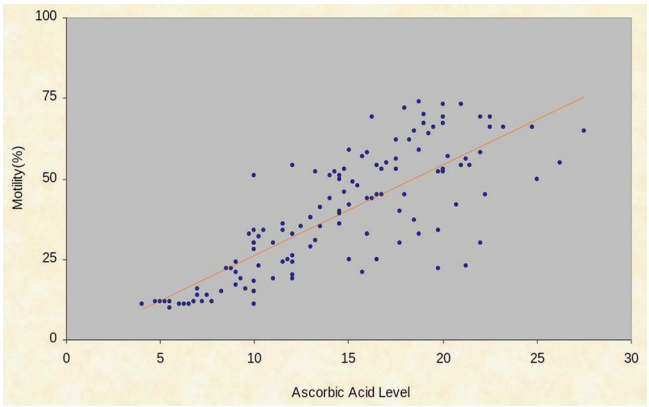


**Figure-2:** Correlation of Ascorbic Acid Level (mg/dl) with sperm % total motility

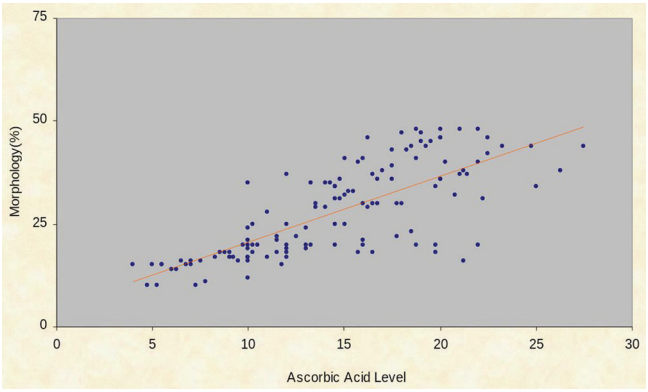


**Figure-3:** Correlation of Ascorbic Acid Level (mg/dl) with sperm normal morphology (%)

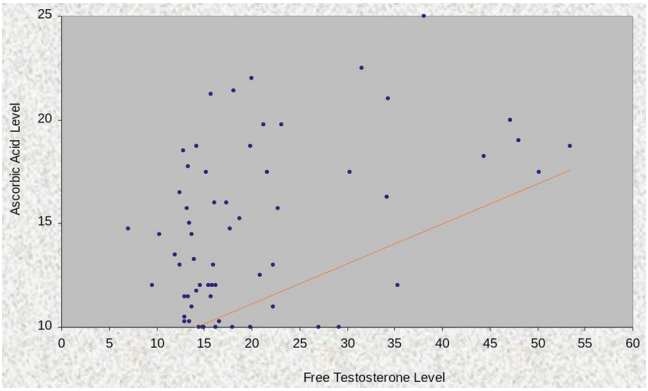


**Figure-4:** Correlation of serum free testosterone Level (pg/dl) with seminal plasma Ascorbic Acid Level (mg/dl)

influence sperm concentration, motility and morphology and whether endogenous testosterone affects the seminal levels of Ascorbic acid.

Ascorbic acid in seminal plasma is mainly secreted from seminal vesicles. It acts as one of the principle antioxidants in seminal plasma and scavenges harmful reactive oxygen species.[4]

In the present study we found the highest level of Ascorbic acid (in mg/dl) in the seminal plasma of Normozoospermics (Mean 18.21±3.52), followed by Asthenoteratozoospermics (Mean 12.86 ± 3.55), Oligoasthenoteratozoospermics (Mean 8.07± 3.30) and Azoospermics (Mean 4.05 ±3.36).

These findings were similar to that observed by Lewis S E et al, 1997,[8] Mohammad Nouri et al, 2008[9] and Piyali Das et al, 2009.[10]

Our study contradicts the findings of Videla E et al, 1981[11], who could not find significant differences among various groups.

We also found a positive correlation between seminal Ascorbic acid and sperm concentration, motility and normal morphology which specifically supports the findings of Piyali Das et al, 2009[10] and Shrikant Shete et al, 2012.[12]

Thus it seems that ascorbic acid is important for semen quality.

The present study also found highest serum free testosterone (in pg/dl) in normozoospermics (Mean 30.98±13.75) followed by azoospermics (Mean 21.55 ± 4.57) Oligoasthenoteratozoospermics (Mean 19.81 ± 4.55) and Asthenoteratozoospermics (Mean 15.10 ±2.81).

Statistically significant positive correlation was found between seminal plasma ascorbic acid with serum free testosterone. Despite of extensive literature search we could not find any direct study elaborating the correlation between serum free testosterone and ascorbic acid. Thus it seems that proper androgenic stimulus is required for ascorbic acid secretion in seminal plasma.

## CONCLUSION

The present study concluded that seminal plasma ascorbic acid may have considerable role in improving male fertility potential. Adequate seminal plasma concentration of ascorbic acid is required for normal sperm function.

Acknowledgments: We are thankful to the staff of the Department of Physiology, Mahatma Gandhi Institute of Medical Sciences, Sewagram, India, for helping us in conducting this study smoothly.

## REFERENCES

1. Halliwell B, Cutteridge JMC,editors. Free radicals in Biology and Medicine, 2nd edn. Oxford university press; 1989. p. 123-419.
2. Bode AM. Metabolism of vitamin C in health and disease. Adv Pharmacol. 1997;38:21-47.
3. Agarwal A, Prabakaran SA. Mechanism, measurement, and prevention of oxidative stress in male reproductive physiology. Ind J Exp Biol. 2005;43:963-74.
4. Song GJ, Norkus EP, Lewis V. Relationship between seminal ascorbic acid and sperm DNA integrity in infertile men. Int J androl. 2006;29:569-75.
5. Cooper TG, editor. WHO laboratory manual for the Examination and processing of human semen, 5th edn. Switzerland: WHO Press; 2010. pp.1-157.
6. Rowe PJ, Comhaire FH, Hargreave TB, Mellows HJ. WHO manual for the standardized investigation and diagnosis of the infertile couple. Cambridge: Cambridge University Press; 1993. pp. 1-34.
7. Mc Cormick DB, Green HL. Methods for the determination of ascorbic acid. In: N W Teitz, editor. Text Book of Clinical Chemistry,2st edn. WB Saunders Company. 1994; 1313–1314.
8. Lewis SE, Sterling ES, Young IS, Thompson W. Comparison of individual antioxidants of sperm and seminal plasma in fertile and infertile men. Fertil Steril. 1997;67:142-9.
9. Mohammad Nouri, Aliyeh Ghasemzadeh, Laya Farzadi, Vahideh Shahnazi, Marefat Ghaffari Novin. Vitamins C, E and lipid peroxidation levels in sperm and seminal plasma of asthenoteratozoospermic and normozoospermic men. Iranian Journal of Reproductive Medicine. 2008;6:1-5.
10. Das P, Choudhari AR, Dhawan A, Singh R. Role of Ascorbic acid in Human Seminal plasma against the oxidative damage to the sperms. Indian J of Clinical Biochemistry. 2009;24:312-15.
11. Videla E, Blanco AM, Galli ME, Fernández-Collazo E. Human seminal biochemistry: fructose, ascorbic acid, citric acid, acid phosphatase and their relationship with sperm count. Andrologia. 1981;13:212–4.
12. Shete SA, Hulke SM, Thakare A, Patil P, Kaore. Antioxidant level in the Seminal Plasma of Human subjects with different Fertility Potential. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2012;3:653-9.

**Source of Support:** Nil; **Conflict of Interest:** None

**Submitted:** 17-05-2017; **Accepted:** 06-06-2017; **Published:** 27-06-2017