

## RESEARCH ARTICLE

# High Dose Vitamin C Administration Effect in Leydig Cells, Sertoli Cells Number, and Sperm Quality on Male Wistar Rats

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### ABSTRAK

**Pendahuluan:** Sebagian besar pria infertil berhubungan dengan spermatogenesis yang buruk akibat stress oksidatif yang dapat dicegah dengan vitamin C. Namun, pemberian vitamin C yang berlebihan dapat menurunkan kualitas sperma. **Tujuan:** Membuktikan bahwa pemberian vitamin C dosis tinggi dapat menyebabkan penurunan jumlah sel leydig, sel sertoli, dan kualitas sperma pada tikus jantan galur wistar.

**Metode:** Penelitian ini menggunakan metode eksperimental dengan rancangan Post Test Only Controlled Group Design. Sebanyak 24 ekor tikus jantan galur Wistar, dibagi secara acak menjadi 4 kelompok. Kelompok normal (Nor-G), hanya diberi akuades 2 ml/hari; kelompok vitamin C (VC18-G, VC36-G, dan VC72-G) masing-masing mendapatkan vitamin C 18 mg/hari, 36 mg/hari dan 72 mg/hari yang dilarutkan dalam 2 ml akuades. Sperma, jumlah sel Leydig dan sel Sertoli diambil dari epididymis dan testis kanan kiri pada hari ke 21. Analisis sperma menggunakan standar WHO, sedangkan jumlah sel Leydig dan sel Sertoli dengan pengecatan HE.

**Hasil:** Uji Mann Whitney menunjukkan bahwa, konsentrasi spermatozoa pada kelompok VC36-G dan VC72-G lebih rendah secara signifikan dibanding kelompok Nor-G dan VC18-G,  $p < 0.05$ . Uji Post-Hoc LSD menunjukkan, motilitas dan morfologi spermatozoa, serta jumlah sel Leydig dan sel Sertoli, paling rendah secara signifikan pada VC36-G dan VC72-G dibanding Nor-G dan VC18-G,  $p < 0.05$ .

**Kesimpulan:** Pemberian vitamin C dengan dosis 36 mg/hari dan 72 mg/hari dapat menyebabkan penurunan jumlah sel Leydig, sel Sertoli dan kualitas sperma yang dinilai dari konsentrasi, motilitas, dan morfologi spermatozoa pada tikus jantan galur wistar.

**Kata kunci:** vitamin C dosis tinggi, sel leydig, sel sertoli, kualitas sperma.

### ABSTRACT

**Introduction:** Most infertile male are associated with poor spermatogenesis due to oxidative stress, and can be prevented with vitamin C. However, excessive amount of high dose of vitamin C can hindered and lower the sperm quality. **Objective:** To prove that high dose vitamin C is capable to decrease the number of leydig cells, sertoli cells, and sperm quality on male wistar rats.

**Methods:** This research was using experimental method with Post Test Only Controlled Group Design. Of 24 male Wistar rats, divided randomly to 4 groups. Normal groups (Nor -G), only given 2 ml/day distilled water; vitamin C group (VC18-G, VC36-G, and VC72-G) given 18 mg/day, 36 mg/day and 72 mg/day vitamin C respectively, dissolved in 2 ml of distilled water. Sperm, the number of Leydig cells and Sertoli cells were taken from the epididymis and left right testicle on day 21. Sperm analysis using WHO standard, while the number of Leydig cells and Sertoli cells with HE staining.

**Results:** Mann Whitney analysis indicated that the number of sperm in VC36-G and VC72-G are lower compared to that of Nor-G and VC18-G groups,  $p < 0.05$ . Post-Hoc LSD analysis showed that the lowest number of Leydig and Sertoli Cell, and the weakest sperm motility and morphology in VC36-G and VC72-G groups, compared to that of Nor-G and VC18-G groups,  $p < 0.05$ .

**Conclusion:** Vitamin C 36 and 72 mg/day were capable of reducing Leydig and Sertoli cells number, and worsen sperm quality, characterized by decreased in sperm concentration, motility and morphology in Wistar male- rats.

**Key words:** high dose vitamin C, Leydig Cell number, Sertoli Cell number, sperm quality.

### INTRODUCTION

Infertility is defined as a couple unable to conceive after regular intercourse for 12 months without the use of protection (Practice Committee of American Society for Reproductive Medicine, 2013). Based on the data, there are 25% of infertile cases are caused by male factors, while 50% are by male and female (Sharlip et al., 2002). Most infertile men are characterized by poor sperm quality due to impaired spermatogenesis.

About 24% to 42% of male infertility cases are caused by decreased in sperm quality, and about 40% are due to impairment of the testicles including Leydig cells and Sertoli cells (Singh et al., 2013). In addition to spermatogonia cells, Leydig and Sertoli cells are important components in the testicles that have a role in differentiation and maturation of spermatogonic cells into mature spermatozoa through the hormone testosterone and growth factors (Kefer, Agarwal and

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Sabanegh, 2009; Singh et al., 2013). Therefore, any damage to Leydig cells and Sertoli cells could greatly affect male fertility status. One of the factors causing male infertility is oxidative stress, which is characterized by elevated levels of Reactive Oxygen Species (ROS) and affects deterioration of the sperm quality (Makker, Agarwal and Sharma, 2009; Ko, Sabanegh and Agarwal, 2014).

Spermatogenesis is a very active cell-replicating process that is capable of forming 1000 spermatozoa/second. As consequences of very high cell replication, mitochondria from epithelial germ cells in seminiferous tubules, using oxygen in high concentration (Aitken and Roman, 2008; Vijayprasad, Ghongane and Nayak, 2014), so that ROS content as a by-product also increases. In the physiological condition of ROS formed in limited quantities and are useful to help the sperm in acrosome reaction, maturation, hyper activation, capacitation, and sperm-oocyte fusion (Ko, Sabanegh and Agarwal, 2014; Vijayprasad, Ghongane and Nayak, 2014). However, when excessive ROS levels exceeded the antioxidant capacity, it will cause DNA damage, decreased sperm motility, and changes in sperm morphology, followed by a decline in male potential fertility (Ko, Sabanegh and Agarwal, 2014).

To improve the quality of sperm parameters that deteriorate due to oxidative stress, vitamin C is often used as antioxidant, either single administered or in combination with other antioxidants. Administration of vitamin C with physiological dose (10mg/kg BW/day) in animal trials (mice) and in humans can improve the quality of spermatozoa parameters (Acharya et al., 2008; Ebesunun et al., 2009). However, some studies have indicated that high dose vitamin C, which is equivalent to 500 to 1000 mg/day or more in healthy individuals is evident to overturn into pro-oxidants by producing hydroxyl radicals (OH<sup>\*</sup>), so it affecting the deterioration of sperm quality and other cells such as Leydig cells and Sertoli cells in the testicles (Podmore et al., 1998). Cell membranes of spermatozoa, Leydig and Sertoli cells are rich in polyunsaturated fatty acids, making it vulnerable to radical OH<sup>\*</sup> attack, and undergo lipid peroxidation (Vijayprasad, Ghongane and Nayak, 2014).

Study conducted by Chakraborty et al. stated that vitamin C is able to transform from the antioxidant form under physiological conditions, become prooxidant under pathological conditions due to the Fenton reaction [ $\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{OH}^*$ ] (Chakraborty et al., 2014; Mojic et al., 2014). Administration of 500 mg/day vitamin C in healthy individuals have a double effects, which are both antioxidants and prooxidant. Antioxidants were performed by ascorbic acid on

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guanine, while pro oxidants are administered to adenine. Study reported by Podmore et.al showed that vitamin C supplementation with a dose of 500 mg/day for 7 weeks in healthy individuals was proved to decrease 8-oxoguanosine levels, but increase 8-oxoadenin levels of peripheral blood lymphocyte. Increased levels of 8-oxoadenin showed a DNA mutation of lymphocytes, which is a marker of DNA damage due to endogenous oxygen radical attack (Podmore et al., 1998). In addition, administration of only vitamin C also has the potential to produce ascorbic free radicals as pro oxidants. Furthermore, various studies reported that combination of vitamin C, vitamin E and reduced glutathione, resulting conversion in ascorbic radicals into ascorbic acid back by vitamin E, while vitamin E was converted to tocopheryl radicals. Tocopheryl radicals by reduced glutathione will be converted to tocopherol and oxidized glutathione, which are subsequently converted back into reduced glutathione by glutathione reductase (van Meeteren et al., 2005).

Thus, the administration of high dose vitamin C could negatively affect spermatogenesis, Leydig, and Sertoli cells due to vitamin C transformation from antioxidants form into pro oxidant. The purpose of this study was to prove that high dose vitamin C higher than 1000 mg/day could interfere spermatogenesis, marked by worsening sperm quality e.g. concentration, motility and morphology of spermatozoa, as well as decreasing the number of Leydig and Sertoli cells.

### **METHODS**

This research used experimental method with Post Test Only Controlled Group Design. Research subjects were 24 Wistar rats, age 12 weeks, weighing 200 grams, divided randomly into 4 groups each consisting of 6 rats. Normal group (Nor-G), only given distilled water of 2 ml/day; the vitamin C groups (VC18-G, VC36-G, and VC72-G) were each getting 18 mg/day of vitamin C, 36 mg/day, and 72 mg/day dissolved in 2 ml of distilled water. On day 21, sperm retrieval from both epididymis duct were performed to count sperm concentration, motility, and morphology. Furthermore, testicles were taken to calculate the number of Leydig, and Sertoli cells. The preparations to count Leydig and Sertoli cells were using Haematoxylin-Eosin (HE), while sperm analysis were counted using the WHO 2010 standard. This research was conducted after getting approval from ethical committee of Faculty of Medicine UNISSULA Semarang.

### **Number of Leydig and Sertoli Cells**

The number of Leydig and Sertoli cells were

**Table 1. Mean of concentration, motility, morphology, number of Leydig, and Sertoli cells count after vitamin C administration**

Variables	Group				p-value
	Nor-G (n = 6)	VC18-G (n = 6)	VC36-G (n = 6)	VC72-G (n = 6)	
Leydig cells ( $\Sigma$ )	3.10 ± 0.74	4.13 ± 0.72	2.40 ± 0.81	0.93 ± 0.42	0.001 *
Sertoli cells ( $\Sigma$ )	6.75 ± 0.86	8.12 ± 0.85	5.12 ± 0.84	2.82 ± 0.35	0.001 *
Spermatozoa Concentration ( $\Sigma$ /ml)	48.94 ± 0.41	48.66 ± 0.55	47.33 ± 1.04	38.36 ± 0.21	0.001 ^
Motility of spermatozoa (%)	63.80 ± 0.37	62.82 ± 0.77	60.68 ± 0.82	54.41 ± 0.36	0.001 *
Morphology of spermatozoa (%)	38.05 ± 0.19	37.08 ± 0.53	35.71 ± 0.75	29.88 ± 0.34	0.001 *

\*: one way anova test, ^: kruskal wallis

obtained by observing on Haematoxylin-Eosin (HE) stained rats testicles slide using an electron microscope Olympus type CX41 with OptiLab camera at 400 times magnification. Leydig and Sertoli cells were calculated at 5 fields of view, then the means of Leydig and Sertoli cells of the testicles were counted.

### Spermatozoa Quality (Concentration, Motility, and Morphology)

#### Spermatozoa Concentrations

Calculating the number of spermatozoa was performed after the cement sample of the epididymis were mixed with homogeneous physiological salts. The solution was then sipped using a haemocytometer up to the mark of 101. Remove a few drops on tissue paper, and then drop it on one side of the glass counter that has been prepared in the microscope. Next, observation with microscope is performed using microscope with 400 times magnification (World Health Organization (WHO), 2010).

#### Spermatozoa Motility

Examination of spermatozoa motility was performed immediately after the cement become homogeneous. Ten mL of cement was dripped onto a glass object, then cover with a glass lid 22 x 22 mm. The observations were performed under an electron microscope with 400 times magnification. The calculation of 200 spermatozoa per replicate in 5 fields of view. The calculation is done after the normal% of sperm difference between two slides is acceptable (World Health Organization (WHO), 2010).

#### Spermatozoa Morphology

Spermatozoa morphological examination was conducted by dripping one drop of sperm on the glass object, then leave to dry. The preparations are then fixated by dipping into a methanol solution for 5 minutes

then dried. After that, dip into 1% safranin solution for 5 minutes. Furthermore, the preparation was rinsed with distilled water and let it dry. The preparation was then stained with Giemsa dye and made into two replication, then observed under an electron microscope with 100x magnification. Seen on 200 spermatozoa per replicate (World Health Organization (WHO), 2010).

### STATISTICAL ANALYSIS

The analysis used were One way anova, followed by Post Hoc LSD test for sperm cell motility variable, morphology, Leydig and Sertoli cell count. As for the number of spermatozoa, Kruskal wallis test was conducted followed by Mann Whitney, considering the data is not homogeneous. The result of analysis is significant if the value of  $p < 0.05$ .

### RESULTS

After administration of high doses vitamin C in male Wistar rats for 20 days, the results obtained as illustrated in Table 1.

Statistical analysis one way anova for the number of Leydig, Sertoli cells, the percentage of motility and good sperm morphology showed a significant difference in between the four groups,  $p < 0.05$ . The same results were obtained from statistical analysis with Kruskal Wallis for the number of spermatozoa, which indicated a significant difference between the four groups,  $p < 0.05$ . To find out which groups is significantly differed, statistical analysis was performed by using Post Hoc LSD and Mann Whitney test as described below.

#### Spermatozoa Concentration

Mann Whitney test results showed that almost all average number of spermatozoa showed significant differences ( $p < 0.05$ ), except the ratio of the average number of spermatozoa between Nor-G group with VC18-G, and between VC18-G group with VC36-G

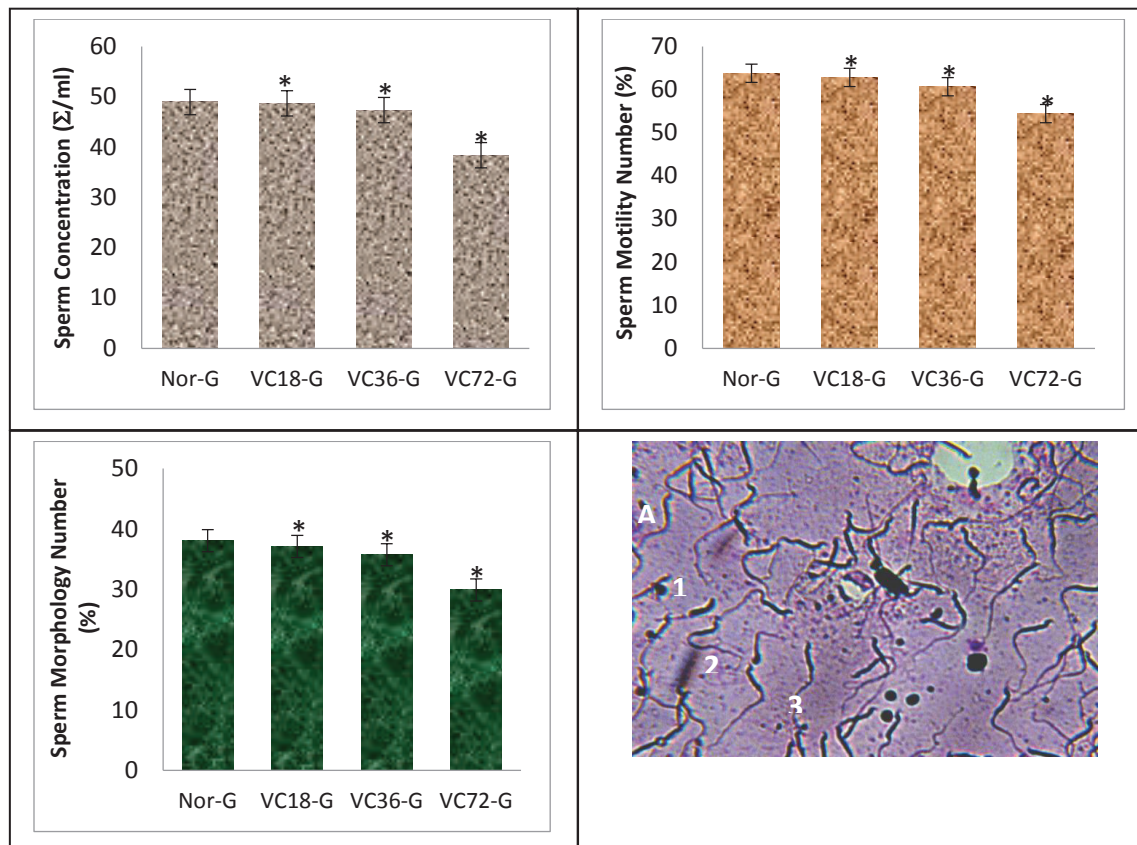


Figure 1. Post Hoc Analysis on Sperm Parameter (Concentration, Motility, and Morphology). \*\*  $p > 0.05$ ; \*  $p < 0.05$  Compared to Nor-G as a Control. A: Sperm Morphology; 1. Short sperm; 2. Normal sperm; 3. Small Head sperm

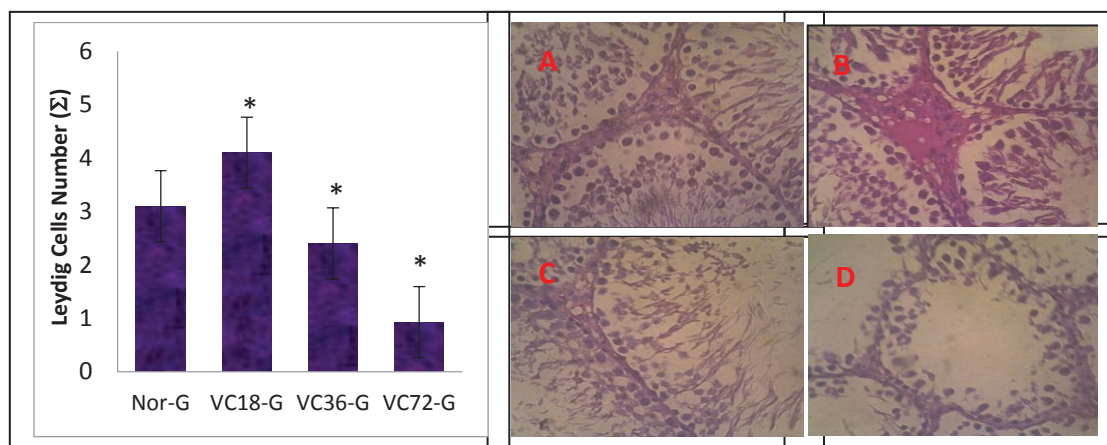


Figure 2. Leydig Cells Number in each Group. A: Nor-G; B: VC-18-G; C: VC36-G; D: VC72-G. \*  $p < 0.05$  Nor-G as a control

group, respectively  $p > 0.05$ . The number of spermatozoa in the VC36-G and VC72-G groups were significantly lower than Nor-G,  $p < 0.05$ . As well as the average sperm count than VC36 VC72-G-G,  $p < 0.05$ . While the mean sperm count in VC36-G even though it is lower than VC18-G, but it is not significant,  $p = 0.065$  (figure 1).

### Spermatozoa Motility

The result of LSD post hoc test showed the mean

percentage for good spermatozoa motility in treatment group (VC18-G, VC36-G, VC72-G) was significantly lower than Nor-G group,  $p < 0.05$ . The percentage of sperm motility at VC36-G and VC72-G were significantly lower than VC18-G,  $p < 0.05$ . Similarly, the motility percentage at VC72 -G were significantly lower than VC36-G,  $p < 0.05$ . Thus, the best percentage of sperm motility was found the lowest in the VC72-G group with  $p < 0.001$ . These results illustrated that

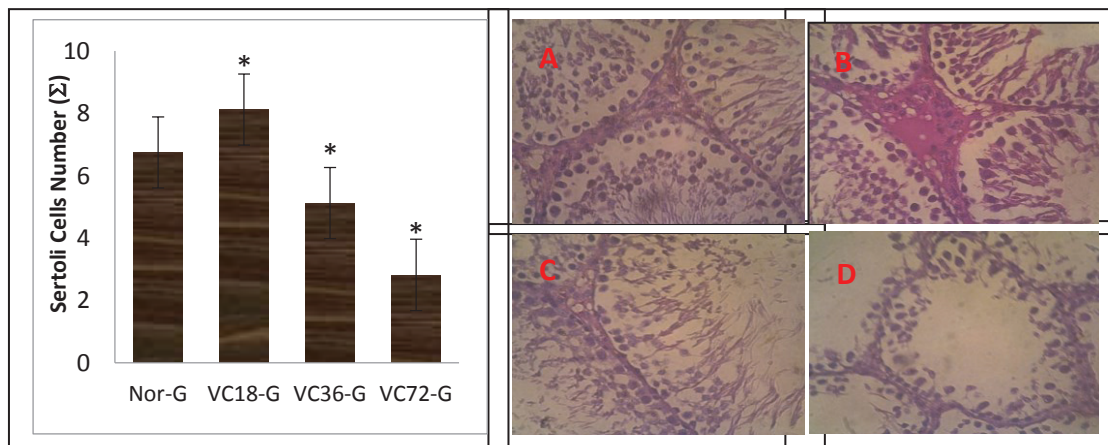


Figure 3. Sertoli Cells Number in each Group. A: Nor-G; B: VC-18-G; C: VC36-G; D: VC72-G. \*  $p < 0.05$  Nor-G as a control

the higher the vitamin C dose administered, the lower the percentage of good sperm motility (dose dependent manner) (figure 1).

### Spermatozoa Morphology

The LSD post hoc results showed that the mean percentage of good spermatozoa morphology in the treatment group (VC18-G, VC36-G, VC72-G) was significantly lower than the Nor-G group,  $p < 0.05$ . The lowest percentage of good sperm morphology and was found in VC72-G,  $p < 0.001$  (Figure 1).

### Leydig cells Number

Post hoc test results LSD showed that the average number of Leydig cells in the VC18-G group was significantly higher than the Nor-G group,  $p < 0.05$ . While in the VC36-G and VC72-G groups, the number of Leydig cells was significantly lower than Nor-G,  $p < 0.05$ . Leydig cells number in the VC72-G group was significantly lower than Nor-G, VC18-G, and VC36-G,  $p < 0.05$  (figure 2).

### Sertoli cells Number

Post hoc test results LSD showed that the average number of Sertoli cells in the VC18-G group was significantly higher than the Nor-G group,  $p < 0.05$ . While in the group VC36-G and VC72-G, the number of Sertoli cells significantly lower than Nor-G,  $p < 0.05$ . The number of Sertoli cells in the VC72-G group was significantly lower than Nor-G, VC18-G, and VC 36-G,  $p < 0.05$  (figure 3).

## DISCUSSION

The results of this study showed the administration of vitamin C with a dose of 18 mg/day or equal to 1000 mg in human could increase the Leydig and Sertoli

cells number in male wistar rats. The results of this study are similar to studies conducted by Takhshid et al. which stated that the administration of 20 mg/kg/day vitamin C for 10 days in rats induced with pesticides Endosulfan, showed improvement in testicular cells following oxidative stress (Takhshid et al., 2012). The results of this study also aligned with the study conducted by Mazloom et al., which showed that the administration of 1000 mg/day vitamin C for 6 weeks in Diabetes mellitus patients could significantly decrease the levels of malondialdehyde (MDA) compared to placebo (Zohreh et al., 2011). Another study reported by Ugalde et al. also showed that administration of 1000 mg/day vitamin C for 6 months in healthy individuals will not cause DNA damage (Ugalde R, Casanueva E, Lozano AM, Torres GC, 2008). Referring to the results of this study and other studies as mentioned before, it can be stated that administration of 18 mg/day vitamin C or equivalent to 1000 mg still has a strong antioxidant effect as a cell protection against oxidative stress attacks experienced by Leydig cells and Sertoli cells or other cells (Qiang ZX, Fan ZY, Wei ZQ, Huai J, 2002)

On the other hand, administration of 36 mg and 72 mg vitamin C per day significantly lowered the number of Leydig and Sertoli cells. The decrease in the number of Leydig and Sertoli cells was caused by oxidative stress from Vitamin C transformation, from antioxidant state into pro oxidant ascorbate. Study by Podmore et al. showed that supplementation of 500 mg vitamin C per day or more, for 7 weeks in healthy individuals was proved to increase the levels of 8-oxoadenin peripheral blood lymphocyte. This results suggested that there has been a mutation of DNA lymphocyte due to oxidative stress, since DNA mutations are DNA damage markers due to endogenous oxygen radical attacks (Podmore et al., 1998). Some

literature also showed that the administration of vitamin C up to 1000 mg/day is the maximum dose, so taking vitamin C with more than 1000 mg/day could cause oxidative stress (Roohi B N, Babaei P, Nia RF, 2008; Zohreh et al., 2011). The results of this study indicated that the administration of vitamin C with a dose of 18 mg/day did not affect the quality of sperm parameter deterioration consisting of concentration, motility, and morphology. On the other hand, vitamin C with dose of 36 mg and 72 mg/day or equivalent with 2000 mg to 3000 mg/day proven to decrease the sperm quality. The results of this study indicated that the increased dose of vitamin C given to rats has a negative correlation with the sperm quality. The higher the dosage of vitamin C that is given the higher the decrease of sperm quality. This is related to changes in vitamin C activity as an antioxidant to ascorbic radicals that could easily attack sperm cell membranes. Study by Chakraborty stated that vitamin C could undergo transformation of anti-oxidants in physiological conditions and become prooxidant in pathological conditions thanks to the Fenton reaction ( $\text{Fe}^{2++} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{OH}\cdot$ ) (Chakraborty et al., 2014; Mojic et al., 2014). The presence of prooxidant or ascorbic radicals in the seminiferous tubules could cause changes in sperm concentration, motility, and morphology due to oxidative stress. The sperm cell membrane is rich in unsaturated fatty acids that it is particularly susceptible to free radical attack, lipid peroxidation, sperm shaping, and apoptosis (Kefer, Agarwal and Sabanegh, 2009; Ko, Sabanegh and Agarwal, 2014). Damage to sperm cells by free radicals can also occur due to antibiotic gentamicin. The results of a study reported by Rahmah et al. mentioned that, administration of gentamicin 5 mg/kgBW/day, intraperitoneally for 10 days could decrease the spermatozoa number significantly. Decline in the sperm count related with elevated levels of superoxide and decline superoxide dismutase, catalase, glutathion peroxidase, and ascorbic acid (Rahmah, 2014). As a result, there is an imbalance between free radicals and antioxidants that can cause oxidative stress (Ko, Sabanegh and Agarwal, 2014). Unfortunately, an examination DNA sperm fragmentation was not performed in this study, so the decline in the quality of sperm parameters from high doses vitamin C administration or not accompanied by DNA damage, as well as its total antioxidant capacity (TAC).

## CONCLUSION

Administration of high-dose vitamin C, 36 mg/day and 72 mg/day in rats or equivalent to 2000 mg and 3000 mg/day in human, may lead to a decrease

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in the number of leydig, sertoli cells and sperm quality assessed by concentration, motility and morphology of spermatozoa in male wistar rats.

## BIBLIOGRAPHY

- Acharya, U. R. et al. (2008) 'Effect of vitamins C and E on spermatogenesis in mice exposed to cadmium', *Reproductive Toxicology*, 25(1), pp. 84–88. doi: 10.1016/j.reprotox.2007.10.004.
- Aitken, R. J. and Roman, S. D. (2008) 'Antioxidant systems and oxidative stress in the testes.', *Oxidative medicine and cellular longevity*, 1(1), pp. 15–24. doi: 10.4161/oxim.1.1.6843.
- Chakraborty, A. et al. (2014) 'Antioxidant and pro-oxidant activity of Vitamin C in oral environment', *Indian Journal Of Dental Research*, 25(4), pp. 499–504.
- Ebesunun, M. O. et al. (2009) 'Ascorbic Acid Supplementation and Spermatogenesis in Nigerian Men', *The Open Clinical Chemistry Journal*, 37, pp. 1874–2416.
- Kefer, J. C., Agarwal, A. and Sabanegh, E. (2009) 'Role of antioxidants in the treatment of male infertility', *International Journal of Urology*, 16(5), pp. 449–457. doi: 10.1111/j.1442-2042.2009.02280.x.
- Ko, E. Y., Sabanegh, E. S. and Agarwal, A. (2014) 'Male infertility testing: Reactive oxygen species and antioxidant capacity', *Fertility and Sterility*. Elsevier Inc., 102(6), pp. 1518–1527. doi: 10.1016/j.fertnstert.2014.10.020.
- Makker, K., Agarwal, A. and Sharma, R. (2009) 'Oxidative stress & male infertility.', *The Indian journal of medical research*, 129(4), pp. 357–367.
- van Meeteren, M. E. et al. (2005) 'Antioxidants and polyunsaturated fatty acids in multiple sclerosis', *European Journal of Clinical Nutrition*, 59(12), pp. 1347–1361. doi: 10.1038/sj.ejcn.1602255.
- Mojic, M. et al. (2014) 'Extracellular iron diminishes anticancer effects of vitamin C: An in vitro study', *Scientific Reports*, 4, pp. 1–8. doi: 10.1038/srep05955.
- Podmore, I. D. et al. (1998) 'Vitamin C exhibits pro-oxidant properties', *Nature*, 392(6676), p. 559. doi: 10.1038/33308.
- Qiang ZX, Fan ZY, Wei ZQ, Huai J, S. H. (2002)

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- 'Effects of endosulfan on the spermatogenesis and oxidative damage in rats', *CJPT*, 16, pp. 391–5.
- Rahmah, H. (2014) *Effects of Vitamin C on Number of Gentamicin-Induced Spermatozoa Mice*. Essay. Syarif Hidayatullah State Islamic University.
- Roohi B N, Babaei P, Nia RF, B. S. (2008) 'Effect of vitamin C supplementation on lipid peroxidation, muscle damage and inflammation after 30-min exercise at 75% VO<sub>2</sub>max', *A Sports Med Phys Fitness*, 48(2), pp. 217–24.
- Sharlip, I. et al. (2002) 'Best practice policies for male infertility', 77, pp. 873–882.
- Singh, A. R. et al. (2013) 'Screening Of Potential Male Contraceptive Drugs From Natural Resources: An Overview', *INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH*, 4(5), pp. 1654–1668.
- Takhshid, M. A. et al. (2012) 'Protective Effects of Vitamins E and C on Endosulfan-Induced Reproductive Toxicity in Male Rats', *Iran J Med Sci*, 37(3), pp. 173–180.
- Ugalde R, Casanueva E, Lozano AM, Torres GC, N. M. (2008) 'High dosage of ascorbic acid and alpha-tocopherol is not useful for diminishing oxidative stress and DNA damage in healthy elderly adults', *Ann Nutr Metab*, 52(2), pp. 167–73.
- Vijayprasad, S., Ghongane, B. B. and Nayak, B. B. (2014) 'Effect of vitamin C on male fertility in rats subjected to forced swimming stress', *Journal of Clinical and Diagnostic Research*, 8(7), pp. 6–9. doi: 10.7860/JCDR/2014/8432.4622.
- World Health Organization (WHO) (2010) 'World Health Organization Laboratory Manual for the Examination and Processing of Human Cement', in, pp. 13–114.
- Zohreh, M. et al. (2011) 'Effect of vitamin C supplementation on postprandial oxidative stress and lipid profile in type 2 diabetic patients', *Mr. J Biol Sci*, 4(19), pp. 900–4.