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THE IMPACT OF ASCORBIC ACID ON SEMEN OVERHAUL IN MALE RABBITS

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ABSTRACT

Ascorbic acid is one of the critical antioxidants. Consequently, It avoids spread of chain responses which activated by free radicals. It is also has the capability to prevention of lipid peroxidation and decreasing of oxidative harm, which hurtful for the layer and damage spermatozoa DNA. The objective of this study was to determine the impact of ascorbic acid (AA, 40mg/kg BW) on semen overhaul and plasma testosterone level in male rabbits every day for 12 weeks. Results showed that treatment with ascorbic acid caused significant increase in the testosterone level, reaction time, ejaculate volume, sperm concentration, total sperm output, sperm motility (%), total motile sperm per ejaculate (TMS), packed sperm volume (PSV), total functional sperm fraction (TFSF), normal and live sperm and semen initial fructose. While, dead sperm, initial hydrogen ion concentration (pH) and TBARS were decreased. In addition, relative weights of testes (RTW) and epididymis (REW) were increased compared to control group.

KEYWORDS

Ascorbic acid, Relative weights, Semen and Rabbits.

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INTRODUCTON

Ascorbic acid is a water-soluble vitamin essential for a number of forms within the human body. As an against oxidant, ascorbic corrosive is an electron benefactor that specifically scavenges free radicals, avoids the era of modern free radicals through its suppressive impacts on the NADPH oxidase (NOX) pathway, and helps within the reusing of other anti-oxidants[1]. The anti-oxidant protective role of ascorbic comes around in scavenging radical species, blocked endothelial cell-derived and induced apoptosis of endothelial cells[2]. In expansion, ascorbic corrosive incorporates a number of impacts on the safe framework, counting control of macrophage work, lessening of provocative go between, and indeed a coordinate bacteriostatic impact at tall concentrations[3]. An ascorbic acid is essential in the generation of endogenous vasopressors and may be important in maintaining vascular vasopressor responsiveness[4]. Ascorbic acid (Vitamin C), is an imperative antioxidant, which normally presents in seminal plasma of teleost

angles, begins from the count calories and its concentration can be directed by dietary treatment[5]. Particularly, ascorbic acid avoids dispersal of chain responses which activated by free radicals. Also, it has the capability of prevention of lipid peroxidation and reducing of oxidative damage, which harmful for the membrane and DNA integrity in thawed spermatozoa[6]. Ascorbic acid or Vit C concentration in seminal plasma surpasses 10 times more than that in blood plasma (364 compared with 40µmol/L)[7]. In semen tests showing ROS movement, ascorbate concentrations within the seminal plasma are essentially diminished[8]. In semen tests showing ROS action, ascorbate concentrations within the seminal plasma are essentially decreased with a pharmacological supplementation of Vitamin C (1g/day), a more than 2-fold increment in plasma ascorbic corrosive concentrations can be accomplished[9]. Additionally, ascorbic corrosive concentrations in seminal plasma are too emphatically related to the rate of morphologically ordinary spermatozoa, and it has been suggested that ascorbic corrosive could be a defensive vitamin within the epididymis[10]. Besides, it has been appeared that ascorbic corrosive ensures human spermatozoa against endogenous oxidative DNA harm[11]. Tall concentration of ascorbic corrosive in human semen plays a key part in keeping up the hereditary keenness of sperm cells by preventing oxidative harm. The decline in ascorbic acid concentration of human semen caused decrease in activities of antioxidant enzymes superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase and increase in levels of hydrogen peroxide and lipid peroxidation in the epididymal sperm[12]. However, many studies have been reported that the decrease in epididymal sperm counts may be due to increased lipid peroxidation[13]. Dabrowski and Ciereszko indicated that the concentration of ascorbic acid in seminal plasma reflected the dietary intake of ascorbic acid, and it caused the increase in sperm quality[14]. In recent study, confirmed that dietary supplementation of ascorbic acid reversed nonspecific sperm agglutination and might facilitate deposition of the metals in semen, which associated with improvement in the physical characteristics of the semen in men[15]. On the other hand, many studies reported that excessive intake of ascorbic acid caused reproductive failure in the male. But, ascorbic acid harmful responses are uncommon at measurements less than 4g/day, because the over doses of Vitamin C are well tolerated in body, and the excessive Vitamin C rapidly eliminated in the urine for 24 h[16]. Tall concentrations of ascorbic acid in seminal plasma may play a part in ensuring sperm from ROS and in keeping up the hereditary astuteness of sperm cells by avoiding oxidative harm to sperm DNA[11]. Testicles and seminal plasma are amazingly touchy to a diminish in body levels of ascorbic corrosive, ascorbic acid deficiency caused a reduction in reproductive performance[17]. Nevertheless, the excessive intake of ascorbic acid has been reported to cause reproductive failure in the male[15].

MATERIAL AND METHODS

In this study vitamin C Solution for oral administration (250mg/ml) was supplied from chemistry department, faculty of science Omar Al-Mokhtar University, El - Beida-Libya. The dosage of AA was 40mg/kg BW each other day[18]. Male New Zealand White rabbits (age of 6 months and initial weight of 2662 ± 72g) were used. Animals were individually housed in cages and weighed weekly throughout 3-months experimental period. Rabbits nourished pellets which comprised of 30% berseem (Trifolium alexandrinum) roughage, 25% yellow corn, 26.2% wheat bran, 14% soybean supper, 3% molasses, 1% $CaCl_2$, 0.4% NaCl, 0.3% blend of minerals and vitamins, and 0.1% methionine. The vitamin and mineral premix per kg contained the taking after IU/gm for vitamins or minerals: vit A-4000,000, vit D3-5000, 000, vit E-16, 7g, K-0.67g, vit B1-0.67g, vit B2-2g, B6-0.67g, B12-0.004g, B5-16.7g, Pantothinc acid-6.67g, Biotein-0.07g, Folic acid-1.67g, Choline chloride-400g, Zn-23.3g, Mn-10g, Fe-25g, Cu-1.67g, I-0.25g, Se-0.033g, and Mg-133.4g (Rabbit premix created by Holland Nourish Connect. Co)[19]. The chemical analysis of the pellets showed that they contained 15.8% crude protein, 11.3% crude fiber, 3.7% ether extract, 7.2% ash, 92.9% organic matter and 62.4% nitrogen free extract % as DM basis.

Ten mature male rabbits were randomly divided into two equal group. Group 1 served as control, while groups 2 was given AA (40 mg/kg body weight). The doses of AA was calculated according to the animal's body weight on the week before dosing. The proper doses of AA for each animal were placed into a syringe that was inserted orally with the help of plastic tube directly into the oesopharyngeal region. Semen collection was done weekly and continued throughout the 12-week experimental period, so 60 ejaculates obtained per treatment. Ejaculates were collected using an artificial vagina and a teaser doe. The volume of each ejaculate was recorded (utilizing a graduated collection tube) after takeoff of the gel mass[20]. A weak eosin solution was used for evaluation of sperm concentration by the improved Neubauer haemocytometer slide (GmbH + Co., Brandstwiete 4, 2000 Hamburg 11, and Germany). Add up to sperm yield calculated by increasing semen

ejaculate volume and semen concentration. Determination of initial fructose concentration in seminal plasma was determined immediately after semen collection according to[21]. Assessments of dead and normal spermatozoa were performed using an eosin-nigrosine blue staining mixture[22]. The percentages of motile sperm were estimated by visual examination under low-power magnification (10×) using light microscope. Add up to number of motile sperm was calculated by duplicating the rate of motile sperm and add up to sperm yield. Reaction time was chosen as the diminutive of subjecting a doe to the buck until the completion of erection; it was measured in seconds. Starting hydrogen particle concentration (pH) was determined immediately after collection using pH cooperative paper (Universalindikator pH 0-14 Merck, Merck KgaA, 64271 Darmstadt, Germany). Packed sperm volume (PSV) was recorded. Add up to useful sperm division (TFSF) was calculated as the item of add up to sperm yield, motility (%), and ordinary morphology (%)[23]. Plasma thiobarbituric acid-reactive substances (TBARS) were measured by the strategy of the relative weight of organs (%) was calculated as g/100g body weight. Serum was obtained by centrifugation of blood samples at 860×g for 20min, and was stored at (-20°C) until used for analysis Testosterone hormone concentration were assayed by using commercial kit that was supplied by Coat - A - Count testosterone RIA, from Diagnostic Systems Laboratories (DSL), from Texas, USA[24].

RESULTS AND DISCUSSION

Table No.1: The changes in body weight (BW), relative testicles weight (RTW) and the concentrations of blood plasma testosterone and thiobarbituric acid-reactive substances (TBARS) all through the 12-week exploratory period of bucks treated with ascorbic acid caused increment ($p<0.05$) in BW and testosterone levels. While, decrease TBARS Treatment with ascorbic acid caused a diminish ($p<0.05$) in relative testicles weights compared with control group. Information on semen ejaculate volume (EV), beginning hydrogen particle concentration (pH), reaction time (RT), packed sperm volume (PSV), sperm concentration (SC), total sperm output (TSO) of rabbits treated with ascorbic acid is displayed in Table No.2. Treatment of rabbits with ascorbic acid essentially increment ($P < 0.05$) the EV, PSV, SC, RT and TSO values compared to control bunch. On the other hand, the values of pH were diminished altogether as a treatment of rabbits with ascorbic acid. The ascorbic acid treatment upgraded the semen volume, RT, pH, sperm concentration and add up to sperm yield in male rabbit (Table No.2). Information in Table No.3) present the cruel values of rabbit sperm motility (SM), total motile sperm (TMS), dead sperm (DS), unusual sperm (AbS), total function sperm fraction (TFSF) and beginning fructose (On the off chance that). Treated male rabbits with ascorbic acid altogether expanded ($P < 0.05$) the SM, TMS, TFSF and on the off chance that values compared to control gather. On the other hand, critical diminish in DS and AbS parameters was watched within the treated rabbits compared with control gather.

DISCUSSION

Ascorbic acid (AA) caused increasing the body weight, relative testes weight and testosterone (Table No.1). This agrees with previous studies showing that AA supplementation stimulated weight gain and testosterone[25-27]. In addition, found that supplementation of Vitamin C to rabbits increased body weight gain and testosterone ($P<0.05$) compared to the control group[28]. Supplementation of AA also showed beneficial effects on RTW (Table No.1), and this is in accordance with the previous study of[26]. On the otherwise, ascorbic acid (AA) decrease TBARS (Table No.1). Vitamin C (ascorbic acid) could be a normal antioxidant, which cleans radicals to erwqdrive out free radical responses[29]. It has been demonstrated that the supplementation of ascorbic acid into the extenders reduces damaging effects of ROS and also develops the motility and viability of mammalian sperm[30,31]. Detailed positive impact of ascorbic corrosive supplementation on pig sperm motility, mitochondrial film conjointly layer keenness. Comes about gotten from this consider demonstrate a positive impact of ascorbic corrosive verbal organization on male richness modulators. The basic part of Vitamin C within the physiology of the testicles shows up to be related with protein digestion system[32,33]. Numerous enzymatic capacities of Vitamin C are fundamental for the typical judgment and work of the testicles, i.e. the union, improvement and support[34]. Furthermore, Vitamin C is a fabulous radical forager due to reductive properties of the ascorbic acid/dehydroascorobic corrosive proportion in natural media and it may invalidate the conceivable spermicidal and genotoxic impact of different free radicals that show up amid cellular digestion system of a few xenobiotic. Illustrated that antioxidant species may act *in vivo* to diminish oxidative harm to DNA, protein and lipids[35,36]. This finding suggested that antioxidant AA may be needed to protect sperm against reactive oxygen species. Therefore, the progressed in semen quality of rabbits treated with AA (Table No.2) can be ascribed to the truth

that this compound is effective antioxidant, and a forager of oxygen free radicals which are toxic by-products of many metabolic processes[37]. In this manner, AA is vital in keeping up the physiological astuteness of testis, epididymis and embellishment organs. Diets deficient in AA were demonstrated to cause the rapid degeneration of the entire reproductive system of male guinea pig[17]. These natural parts of AA in other species may clarify the noteworthy advancement in RTW of male rabbits (Table No.1). Detailed concentrations of AA in semen are eight to-tenfold higher than those in blood[38], suggesting an vital natural part for AA in seminal plasma. Tall concentrations of AA in semen play a key part in keeping up the hereditary astuteness of sperm cells by anticipating oxidative harm to sperm DNA[11,13]. These tall AA concentrations of seminal plasma protect against free radicals which may something else deliver the oxidized nucleotide 8- oxo-deoxyguanosine[13,39]. It is worth noticing that expanded oxidative harm to sperm DNA has been related with moo AA concentrations in seminal liquid[11,39] and infertile guys were found to have moo AA levels in seminal plasma[34]. Studies on guinea pigs showed that AA deficiency adversely affects sperm count, morphology, motility, and viability[17]. In addition, cigarette smoke which contains high concentrations of oxidants was found to deplete tissues and seminal fluid of antioxidants including AA and to decrease semen quality (reduced sperm count and increased number of abnormal sperm cells) in humans[39]. The display think about appeared that organization of AA caused critical enhancements in rabbit sperm characteristics Such changes concur with the already detailed benefits of AA supplementation on sperm quality and male richness[40,26,27]. Also, high level of ascorbic acid is necessary for steroid hormone production and possibly for follicle grown and integrity[41]. According to[42] Luck *et al*. steroid genesis is dependent on ascorbic corrosive, particularly at the hydroxylation step. It is possible that the reason that steroidogenesis is enhanced by ascorbic acid due to its antioxidant properties.

Table No.1: The overall means (±SE) of body weight, relative testes weight and blood plasma testosterone concentration during treatment of male rabbits with ascorbic acid

S.No	Parameters	Animal Groups	
		Control	Ascorbic acid
1	BW (g)	2.823 ± 42^{b}	3.339 ± 22^{a}
2	RTW (g/100g BW)	0.196 ± 0.08^{c}	0.234 ± 0.07^{a}
3	Testosterone (ng/mL)	2.55 ± 0.25^{b}	3.81 ± 0.50^{a}
4	TBARS (nmol/ml)	1.123 ± 0.032^{c}	0.91 ± 0.084^{a}

[abcd] Within row, means with different superscript letters differ significantly ($p < 0.05$)

Table No.2: The overall means (±SE) of semen characteristics during treatment of male rabbits with ascorbic acid

S.No	Parameters	Animal Groups	
		Control	Ascorbic acid
1	Ejaculate volume (ml)	0.62 ± 0.08^{b}	0.88 ± 0.11^{a}
2	PH	7.5 ± 0.97^{b}	7.1 ± 0.92^{c}
3	Reaction time (s)	2.9 ± 0.37^{a}	1.2 ± 0.15^{b}
4	Packed sperm volume (%)	16.1 ± 0.23^{b}	19.1 ± 0.22^{a}
5	Sperm concentration ($\times10^{6}ml^{-1}$)	322 ± 8.1^{b}	377 ± 7.2^{a}
6	Total sperm output ($\times10^{6}$)	241 ± 8.9^{b}	320 ± 10.8^{a}
7	Sperm motility (%)	$72.1. \pm0.6^{b}$	82.1 ± 0.79^{a}
8	Total motile sperm ($\times10^{6}$)	169 ± 6.2^{b}	280 ± 9.9^{a}
9	Dead sperm (%)	24.4 ± 0.6^{b}	15.0 ± 0.49^{c}
10	Normal sperm (%)	85.9 ± 0.79^{b}	91.0 ± 0.60^{a}
11	Total functional sperm fraction ($\times10^{6}$)	150.7 ± 5.69^{b}	241.5 ± 8.90^{a}
12	Initial fructose (mg/dl)	170 ± 1.40^{b}	199 ± 2.1^{a}

[abcd] Within row, means with different superscript letters differ significantly ($p < 0.05$)

CONCLUSION

Ascorbic acid treatment shows up to progress semen quality in male rabbits additionally upgrade the quality of sperm.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Mortensen A, Lykkesfeldt J. Does Vitamin C enhance nitric oxide bioavailability in a tetrahydrobiopterin-dependent manner? *In vitro*, *In vivo* and clinical studies, *Nit Oxi- Bio and Chem,* 36, 2014, 51-57.
2. Amrein K, Oudemans-Van Straaten H M, Berger M M. Vitamin therapy in critically ill patients: Focus on thiamine, Vitamin C, and vitamin D, *Intensive Care Med,* 44(11), 2018, 1940-1944.
3. Pedraza A, Bo O, Golde D W. Vitamin C suppresses TNF alpha-induced NF kappa B activation by inhibiting I kappa B alpha phosphorylation, *Biochemistry,* 41(43), 2002, 12995-13002.
4. Carr A C, Shaw G M, Fowler A A, Natarajan R. Ascorbate-dependent vasopressor synthesis: A rationale for Vitamin C administration in severe sepsis and septic shock? *Critical Care,* 19(1), 2015, 418.
5. Ciereszko A, Dabrowski K. Sperm quality and ascorbic acid concentration in rainbow trout semen are affected by dietary Vitamin C: An across-season study, *Biol. Reprod,* 52(5), 1995, 982-988.
6. Cabrita E, Sarasquete C, Martinez-Paramo S, Robles V, Beirao J, Perez-Cerezales S. Herraez M P. Cryopreservation of fish sperm: Applications and perspectives, *Journal of Applied Ichthyology,* 26(5), 2010, 623-635.
7. Tariq M, Khan M S, Shah M G, Nisha A R, Umer M, Hasan S M, Rahman A, Rabbani I. Exogenous antioxidants inclusion during semen cryopreservation of farm animals, *J. Chem. Pharm. Res,* 7(3), 2015, 2273-2280.
8. Lewis S E M, Sterling E S L, Young I S, Thompson W. Comparison of individual antioxidants of sperm and seminal plasma in fertile and infertile men, *Fertil. Steril,* 67(1), 1997, 142-147.
9. Wen Y, Cooke T, Feely J. The Effect of Pharmacological Supplementation with Vitamin C on Low-Density Lipoprotein Oxidation, *Br. J. Clin. Pharmacol,* 44(1), 1997, 94-97.
10. Thiele J J, Freisleben H J, Fuchs J, Ochsendorf F R. Ascorbic acid and urate in human seminal plasma: Determination and interrelationships with chemiluminescence in washed semen, *Hum. Reprod,* 10(1), 1995, 110-115.
11. Fraga C G, Motchnik P A, Shigenaga M K, Helbock H J, Jacob R A, Ames B N. Ascorbic acid protects against endogenous oxidative DNA damage in human sperm, *Proc. Natl. Acad. Sci. U. S. A,* 88(24), 1991, 11003-11006.
12. Potts R J, Mjefferies T, Notarianni L J. Antioxidant capacity of the epididymis, *Hum. Reprod,* 14(10), 1999, 2513-2516.
13. Dabrowski K, Ciereszko A. Ascorbic acid protects against male infertility in a teleost fish, *Experientia,* 52(2), 1996, 97-100.
14. Harris W A, Harden T E, Dawson E B. Apparent effect of ascorbic acid medication on semen metal levels, *Fertil. Steril,* 32(4), 1979, 455-459.
15. Paul P K, Duttagupta P N. Beneficial or harmful effects of a large dose of Vitamin C on the reproductive organs of the male rat depending upon the level of food intake, *Indian J. Exp. Biol,* 16(1), 1978, 18-21.
16. Weltman A S, Sackler A M, Owens H. Effects of levels of audiogenic-seizure susceptibility on endocrine function of rats, *Physiol. Behav,* 3(2), 1968, 281-284.
17. Chinoy N J, Mehta R R, Seethalakshmi L, Sharma J D, Chinoy M R. Effects of Vitamin C deficiency on physiology of male reproductive organs of guinea pigs, *Int. J. Fertil,* 31(3), 1986, 232-239.
18. Khan P K, Sinha S P. Ameliorating effect of Vitamin C on murine sperm toxicity induced by three pesticides (endosulfan, phosphamidon and mancozeb), *Mutagenesis,* 11(1), 1996, 33-36.
19. AOAC. Official Methods of Analysis (AOAC) Official Method 923.03, *In Association of Official Analytical Chemists International,* 15th Edition, 1990.
20. Smith J T, Mayer D T. Evaluation of sperm concentration by the hemacytometer method; Comparison of four counting fluids, *Fertil. Steril,* 6(3), 1955, 271-275.
21. Mann T. Fructose Content and Fructolysis in Semen, Practical Application in the Evaluation of

Semen Quality, *J. Agric. Sci,* 38(3), 1948, 323-331.
22. Blom E. A one-minute live-dead sperm stain by means of eosin-nigrosin, *Fertil. Steril,* 1, 1950, 176-177.
23. Correa J R, Zavos P M. Preparation and recovery of frozen-thawed bovine spermatozoa via various sperm selection techniques employed in assisted reproductive technologies, *Theriogenology,* 46(7), 1996, 1225-1232.
24. Tappel A L, Zalkin H. Inhibition of lipide peroxidation in mitochondria by Vitamin E, *Arch. Biochem. Biophys,* 80(2), 1959, 333-336.
25. Kawai-Kobayashi K, Yoshida A. Effect of dietary ascorbic acid and vitamin e on metabolic changes in rats and guinea pigs exposed to PCB, *J. Nutr,* 116(1), 1986, 98-106.
26. Salem M H, Kamel K I, Yousef M I, Hassan G A, El-Nouty F D. Protective role of ascorbic acid to enhance semen quality of rabbits treated with sublethal doses of aflatoxin B1, *Toxicology,* 162(3), 2001, 209-218.
27. Yousef M I, Abdallah G A, Kamel K I. Effect of ascorbic acid and Vitamin E supplementation on semen quality and biochemical parameters of male rabbits, *Anim. Reprod. Sci,* 76(1-2), 2003, 99-111.
28. Montero D, Marrero M, Izquierdo M S, Robaina L, Vergara J M, Tort L. Effect of Vitamin E and C Dietary Supplementation on Some Immune Parameters of Gilthead Seabream (Sparus Aurata) Juveniles Subjected to Crowding Stress, *Aquaculture,* 171(3-4), 1999, 269-278.
29. Martinez-Paramo S, Diogo P, Dinis M T, Herraez M P, Sarasquete C, Cabrita E. Incorporation of ascorbic acid and α-tocopherol to the extender media to enhance antioxidant system of cryopreserved sea bass sperm, *Theriogenology,* 77(6), 2012, 1129-1136.
30. Thuwanut P, Chatdarong K, Techakumphu M, Axner E. The effect of antioxidants on motility, viability, acrosome integrity and DNA Integrity of frozen-thawed epididymal cat spermatozoa, *Theriogenology,* 70(2), 2008, 233-240.
31. Jeong Y J, Kim M K, Song H J, Kang E J, Ock S A, Mohana Kumar B, Balasubramanian S, Rho G J. Effect of α-tocopherol supplementation during boar semen cryopreservation on sperm characteristics and expression of apoptosis related genes, *Cryobiology,* 58(2), 2009, 181-189.
32. Pena F J, Johannisson A, Wallgren M, Rodriguez Martinez H. Antioxidant supplementation in vitro improves boar sperm motility and mitochondrial membrane potential after cryopreservation of different fractions of the ejaculate, *Anim. Reprod. Sci,* 78(1-2), 2003, 85-98.
33. Flier J S, Underhill L H, Levine M. New concepts in the biology and biochemistry of ascorbic acid, *N. Engl. J. Med,* 314(14), 1986, 892-902.
34. Dawson E B, Harris W A, Teter M C, Powell L C. Effect of ascorbic acid supplementation on the sperm quality of smokers, *Fertil. Steril,* 58(5), 1992, 1034-1039.
35. Machlin L J, Bendich A. Free radical tissue damage: Protective role of antioxidant nutrients 1, *FASEB J,* 1(6), 1987, 441-445.
36. Sierens J, Hartley J A, Campbell M J, Leathem A J C, Woodside J V. Effect of phytoestrogen and antioxidant supplementation on oxidative DNA damage assessed using the comet assay, *Mutat. Res. - DNA Repair,* 485(2), 2001, 169-176.
37. Pszczola D E, Katz F, Giese J. Research trends in healthful foods, *Food Technol,* 2000.
38. Jacob R A, Pianalto F S, Agee R E. Cellular ascorbate depletion in healthy men, *J. Nutr,* 122(5), 1992, 1111-1118.
39. Fraga C G, Motchnik P A, Wyrobek A J, Rempel D M, Ames B N. Smoking and low antioxidant levels increase oxidative damage to sperm DNA, *Mutat. Res. - Fundam. Mol. Mech. Mutagen,* 351(2), 1996, 199-203.
40. Dawson E B, Harris W A, Rankin W E, Charpentier L A, Mcganity W J. Effect of ascorbic acid on male fertility, *Ann. N. Y. Acad. Sci,* 498(1), 1987, 312-323.
41. Murray A A, Molinek M D, Baker S J, Kojima F N, Smith M F, Hillier S G, Spears N. Role of ascorbic acid in promoting follicle integrity and survival in intact mouse ovarian follicles *In vitro*, *Reproduction,* 121(1), 2001, 89-96.
42. Luck M R, Jeyaseelan I, Scholes R A. Minireview: Ascorbic acid and fertility, *Biology of Reproduction,* 52(2), 1995, 262-266.

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