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Review Article

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Effects of Bleomycin, Selenium, and Vitamin E on Male Fertility: A Review

Hosny A. Ibrahim[1], Shimaa I. Shalaby[2], Ahmed A. Hassan[3], Rasha Mahmoud M.A. Hebishy[4*], Eman M.A. Abdel Ghani[1]

[1]Pharmacology Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44519, Egypt.
[2]Physiology Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44519, Egypt.
[3]Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44519, Egypt.
[4]Faculty of Veterinary Medicine, Zagazig University, Egypt.

***Correspondence**
Rasha Mahmoud M.A. Hebishy
Faculty of Veterinary Medicine, Zagazig University, Egypt.
E-mail address: rashamahmoud073@gmail.com

Abstract

Bleomycin (BL) is a glycopeptide antibiotic derived from the bacterium Streptomyces verticillus that is commonly used in the treatment of human cancer. However, BL has been linked to several cases of male infertility in humans and animals. Vitamin E requires vascular transport to the liver after absorption, which is primarily facilitated by tocopheroltransfer protein. Vitamin E acts as a peroxyl radical scavenger as an antioxidant, preventing free radical propagation in tissues by reacting with them to form a tocopheryl radical, which is reduced by a hydrogen donor and returns to its reduced state. Because of its solubility, it is incorporated into cell membranes, protecting them from oxidative damage. Vitamin E plays critical roles in improving reproductive efficiency and mitigating the negative effects of several reproductive toxicants. Selenium is a trace element found in soil, water, and certain foods. It is thought to be an essential component that actively participates in a variety of metabolic pathways and plays a variety of important roles in the body. Among these functions are participation in various enzyme systems and anti-oxidative cellular processes. Selenium is required for the detoxification of harmful metals from the human body, foetal breathing, energy transfer reactions, and sperm cell formation. It is thought that a lack of selenium contributes to male infertility because it causes degradation in the testicular tissues, which leads to impaired active sperm motility as the first sign of impending infertility. In this review, we will summarize the recent findings related to BL-adverse effects of male fertility and the beneficial effects of selenium and vitamin E on the reproduction and male fertility.

KEYWORDS
Bleomycin, Selenium, Vitamin E, Male fertility

INTRODUCTION

Because of early detection and improved cancer treatment protocols, the number of men surviving cancer at a young age has increased dramatically in the last 20 years; today, more than 75% of young cancer patients are long-term survivors. Quality of life has become an important consideration for both pediatric and adult cancer patients. Leukemia, Hodgkin's lymphomas, and testicular germ cell tumors are the most common cancers in people of reproductive age. Fertility is frequently impaired following chemotherapy and radiation therapy (Dohle 2010). Bleomycin (BL) is a glycopeptide antibiotic derived from the bacterium *Streptomyces verticillus* that is commonly used to treat cancer in humans. However, BL is reported to be associated with several cases of male infertility in humans and animals (Amirshahi *et al.*, 2014). In this review we will try to throw the light on the recent reports about the adverse effects of BL or its combination with other anticancer medications on male fertility.

Selenium (Se) is a crucial mineral for healthy spermatogenesis, spermatozoa motility, and testicular development. By acting as an antioxidant through the Se-dependent enzyme glutathione peroxidase, selenium primarily protects cellular membranes and organelles against oxidative damage in both humans and animals (Moslemi and Tavanbakhsh, 2011). A little deviation, either a shortfall or an excess of Se in the testis, results in improper development of the male reproductive tissue, which is dependent on an appropriate amount of Se in the testis. Selenium is a component of selenoproteins such as GPx1, GPx3, mGPx4, cGPx4, and GPx5, which protect spermatozoa from oxidative damage as they mature. In contrast, selenoproteins like mGPx4 and snGPx4 are structural elements of mature spermatozoa. Se and selenoproteins thus guarantee spermatozoa's survival and offer defense against reactive oxygen species. Selenoprotein gene knockout studies have shown that their absence during spermatogenesis results in defective spermatozoa, which in turn impairs the quality of the semen and fertility (Ahsan *et al.*, 2014). Here, we will summarize the recent findings of the beneficial effects of Se in the treatment and prevention of male infertility.

In the presence of other lipid rich foods, vitamin E is absorbed through the intestine. Following absorption, vitamin E requires vascular transport to the liver, which is facilitated primarily by tocopheroltransfer protein. The water-soluble end product carboxyethylhydroxychroman is formed after one cycle of CYP4F2/CYP3A4dependent hydroxylation followed by five cycles of subsequent oxidation. All known hepatic metabolites can be conju-

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gated and excreted in the faeces or urine (Schmölz *et al*., 2016). As an antioxidant, vitamin E acts as a peroxyl radical scavenger, preventing free radical propagation in tissues by reacting with them to form a tocopheryl radical, which is reduced by a hydrogen donor and returns to its reduced state. It is incorporated into cell membranes due to its solubility, which protects them from oxidative damage (Gokce *et al*., 2011).

Because the membranes of germ cells and spermatozoa are highly vulnerable to oxidation due to their high amount of PUFA, vitamin E is a significant lipophilic chain-breaking antioxidant that protects tissue polyunsaturated fatty acids (PUFA) from peroxidation (Bolle *et al*., 2002). 15% of couples experience infertility, and in 30% of these couples, the problem is due to abnormalities in the male partner, which is known as male infertility. Numerous cases of male infertility that were once thought to be idiopathic are now being linked to oxidative damage brought on by an increase in reactive oxygen species. Oral vitamin E supplementation is a suggested treatment for this kind of infertility (Wen 2006).

Bleomycin reported impairment in male fertility

Bleomycin has been used in the treatment of several cancer types either alone or in a combination with other chemotherapeutic agents such as etoposide, and cis-platinum. In this regard, the therapeutically appropriate dose levels of bleomycin, etoposide, and cis-platinum (BEP) (0.75, 7.5, and 1.5 mg/kg) were administered in three cycles of 21 days each to adult male and female Sprague-Dawley rats. At the conclusion of the course of treatment, sperm characteristics, fertility, serum hormone levels, testicular histopathology, and expression of PCNA and transferrin were assessed. BEP resulted in tubular atrophy, decreased sperm count, increased abnormalities, and decreased sperm motility. increased inhibin B levels, but decreased transferrin, FSH, and testosterone (Kilarkaje *et al*., 2013).

Histones are replaced by transition proteins and then by protamines during spermiogenesis, resulting in a very condensed sperm DNA structure that is absolutely critical for normal sperm function. It was demonstrated that, despite a 9-week recovery period, mature sperm from rats treated for 9 weeks with the testicular cancer drugs (BEP) have lower levels of protamine 1 and an upregulation of specific histones, indicating a problem with histone eviction. We show that histone removal regulators are increased in elongating spermatids after recovery; however, Ac-H4 and gammaH2AX histones remain elevated in elongating spermatids or caudal epididymal spermatozoa 9 weeks after BEP treatment. This suggests that chromatin remodelers and effector proteins that react to histone removal are involved. A decrease in SMARCE1 expression in elongating spermatids could explain the persistent retention of histones in cauda epididymal sperm 9 weeks after BEP treatment was stopped. Surprisingly, proteins involved in protamine 1 translational control and posttranslational processing are also significantly elevated 9 weeks after BEP treatment, implying that histone eviction may dictate DNA availability for protamine binding. Males mated to control females 9 weeks after BEP treatment have smaller litter sizes; additionally, the profile of gene expression in their pups' developing testes is altered. Changing the proportion of histones to protamine in mature spermatozoa reduces male fecundity, with epigenetic changes potentially jeopardising normal progeny development (Maselli *et al*., 2013).

In another report, the effects of BL on the sperm parameters and malondialdehyde (MDA) generation were assessed in rats. When compared to the control group, BL considerably increased the amount of immature sperm, sperm with damaged DNA, and MDA concentration. Conversely, BL dramatically decreased the number of sperm, their viability, and their motility (Amirshahi *et al*., 2014).

Telomeres, which are specialized structures at the physical ends of chromosomes, are important in the maintenance of genetic stability as well as the response of somatic cells to anticancer drugs. Bleomycin, etoposide, or cisplatin (drugs used to treat testicular cancer) or cyclophosphamide (an anticancer agent and immunosuppressant) affected telomeres in the male germ line. Bleomycin, etoposide, cisplatin, or 4-hydroperoxycyclophosphamide were used to treat C18-4 spermatogonial cells (4OOH-CPA, a preactivated analogue of cyclophosphamide). As measured by gamma-H2AX immunofluorescence, all four anticancer drugs caused a significant increase in DNA damage in C18-4 cells. Interestingly, after treatment with bleomycin, cisplatin, and 4OOH-CPA, but not etoposide, the gamma-H2AX signal was localised to telomeres. Exposure to cisplatin and 4OOH-CPA reduced mean telomere lengths, the intensity of the telomere fluorescence in situ hybridization signal, telomerase activity, and the expression of the telomerase enzyme mRNA components, Tert and Terc, but not bleomycin or etoposide. Thus, while all four anticancer drugs caused DNA damage in this spermatogonial cell line, etoposide had no effect on telomeres, and only the two alkylating agents, cisplatin and 4OOH-CPA, caused telomere dysfunction. This telomere dysfunction could lead to infertility and developmental defects in children (Liu *et al*., 2014).

Chromomycin A3 and immunofluorescence labelling were used to measure sperm protamination and ubiquitination in rats given the medicines bleomycin, etoposide, and cisplatin (BEP). In comparison to the control group, the mean percentage of ubiquitinated sperm was considerably higher in the BEP group. However, in comparison to the control group, the mean percentage of sperm protamination considerably decreased in the BEP group (Hashemi *et al*., 2018).

In another study, the effects of combined cancer treatment modalities (radiotherapy and chemotherapy) on mouse sperm chromatin in vivo were examined. A total of 48 mice were divided into 12 groups: three irradiations (1, 2, and 4 Gy), two drugs Actinomycin-D (ACTD) and Bleomycin (BLM), three ACTD/irradiation, three BLM/irradiation, and one control. Before being exposed to X-rays, mice were given an intra-testicular injection of 7g/25 g Actinomycin-D and Bleomycin. Mice were sacrificed 48 hours after irradiation, and the epididymis and testes were removed. The alkaline comet assay was used to assess sperm DNA damage. Furthermore, the morphology and motility of sperms were studied under a microscope. The results showed that when combined with irradiation, the drug had a slight but not significant effect on sperm DNA damage. There was a significant difference in DNA sperm damage between the experimental and control groups, but no significant differences in sperm morphology. When compared to the controls, DNA damage increased dramatically in the drug+4Gy group (Saghaei *et al*., 2019).

Young people have a high incidence rate of testicular cancer. The survival rate of individuals with testicular cancer has increased with the co-administration of BEP. Even while BEP is among the most successful treatments for testicular cancer, it has a serious adverse effect on the reproductive system and ultimately results in infertility. The purpose of this study was to assess how BEP affected testicular shape, chromatin condensation, and sperm parameters. Forty male rats were employed in this experiment, and their spermatogenesis was monitored twice (i.e. 18 weeks). For three cycles of three weeks, BEP was administered to

the rats in BEP at the recommended levels (0.75, 7.5, and 1.5 mg/kg). We measured sperm parameters after 18 weeks and used aniline blue staining to detect increased histone in sperm chromatin. As well as testicular structure and germ line cells using periodic acid-Schiff staining. Normal sperm morphology, motility, and concentration were all significantly reduced after BEP therapy, and rat sperm chromatin condensation and testicular tissue were also changed (Razavi *et al*., 2019).

One of the most prevalent cancers in young men is testicular cancer. Since chemotherapy can harm both healthy and malignant cells, we looked into the effects of chemotherapy on rat chromatin integrity and testicular histomorphometry. The testicular histology, sperm DNA methylation, ubiquitination, DNA fragmentation, and protamination were further evaluated using immunofluorescence after the male rats (n = 40) were treated with BEP at the appropriate dose levels of BEP (0.75, 7.5, and 1.5 mg/kg) for 9 weeks. Testicular degeneration was caused by a considerable increase in ubiquitination, DNA fragmentation, and worldwide DNA methylation and protamination following BEP therapy (Khadivi *et al*., 2020).

Strong chemotherapy medication, BL, has catastrophic effects on spermatogenic activity and raises the possibility of infertility in cancer survivors. The BL-treated Balb/c mice group got 10 mg/kg BL every day for 35 days, BL was intraperitoneally administered. Results revealed that BL significantly reduced sperm count, viability, morphology, maturation, and progressive movement, testosterone, the ratio of testis weight to body weight, the number of spermatogonia, spermatocytes, spermatids, and Sertoli cells per tubule, and expression of Bcl2l1 and Bcl2l1/Bax, while significantly increasing non-progressive movement and immotile sperm, intermediate and immature (Yaghutian Nezhad *et al*., 2021).

There is growing concern that certain cytotoxic cancer treatment regimens may negatively impact male fertility and spermatogenesis. Bleomycin, etoposide, and cisplatin chemotherapy regimen's produced testicular damage is still unknown. The purpose of the current study was to investigate rats' testicular damage caused by BEP. In one cycle of 21 days, 0.33 clinically relevant dose levels of BEP (.5 mg/kg bleomycin, 5 mg/kg etoposide, and 1 mg/kg cisplatin) were intraperitoneally (i.p.) administered into adult male Wistar rats (n = 10/group). At the conclusion of the study, sperm characteristics, testosterone levels, testicular stereology, levels of nitric oxide (NO), malondialdehyde (MDA), and total antioxidant capacity (TAC), as well as the expression of genes linked to apoptosis such as Bcl2, Bax, Caspase-3, p53, and TNF- (using real-time PCR and immunohistochemistry), were assessed. According to the sperm count, motility, viability, and morphology, the acquired data indicated severe spermatogenetic aberrations. Significant distortions were seen in testicular stereology, histology, and testosterone levels. Additionally, BEP-evoked apoptosis and -induced testicular injury through production of oxidative stress, apoptosis, and inflammation provide evidence for BEP-associated gonadotoxicity and male sub/infertility (Moradi *et al*., 2021).

The researchers (Razavi *et al*., 2021) looked into the effects of bleomycin, etoposide, and cisplatin (BEP) on rat sperm chromatin condensation, DNA damage, and spermatogenesis. Aniline blue and acridine orange staining were used to assess sperm chromatin condensation and DNA damage, respectively. The results show that after 9 weeks of BEP exposure, the mean percentage of sperms with excessive histone and DNA damage increased significantly when compared to the control group. BEP treatment significantly reduced the mean number of spermatogonia, primary spermatocytes, leydig cells, and testicular histology properties, according to testicular histomorphometric analysis.

Effects of selenium and vitamin E supplementation on male fertility

Vitamin E and selenium are very necessary micronutrients for the normal functions of the reproductive system. The zona binding test shows that vitamin E taken orally greatly enhances the *in vitro* performance of human spermatozoa (Kessopoulou *et al*., 1995). Likely, a study by Keskes-Ammar *et al*. (2003) was conducted to examine the effects of vitamin E and selenium supplementation on sperm parameters and lipid peroxidation. The study involved 54 willing and infertile males who provided blood samples for high-performance liquid chromatography analysis of the serum vitamin E level and provided sperm samples for spermiogram and for spectrophotometric measurement of a lipid peroxidation marker, the malondialdehyde (MDA). The trial was open and randomized. Over the course of three months, 28 males received daily supplements of vitamin E (400 mg) and selenium (225 g). The same amount of vitamin B (4,5 g/day) was administered to the remaining 26 patients. There were only 20 patients who completed their treatment and came back for a control analysis. Sperm motility and viability were inversely linked with semen MDA levels, and sperm MDA concentrations were significantly lower than those in seminal plasma. Vitamin E and selenium supplements, as opposed to vitamin B administration, resulted in a considerable drop in MDA concentrations and an improvement in sperm motility. The findings support the use of vitamin E and selenium in the treatment of male infertility because they have protective and advantageous effects on the quality of semen.

Selenium is a trace element that can be found in soil, water, and some foods. It is thought to be an essential component that participates actively in a number of metabolic pathways and is thought to play a number of significant roles in the body. These functions include taking part in several enzyme systems and anti-oxidative cellular processes. Selenium plays a function in the manufacture of deoxyribonucleic acid (DNA) and ribonucleic acid, which is essential for maintaining the integrity of muscle cells and red blood cells (RNA). The detoxification of harmful metals from the human body, foetal breathing, energy transfer reactions, and sperm cell formation have all been found to depend on selenium. It is believed that a selenium shortage may contribute to male infertility because it causes degradation in the testicular tissues, which in turn causes active sperm motility impairment as the first sign of imminent infertility (Oguntibeju *et al*., 2009).

In a study by Safarinejad and Safarinejad (2009) the effectiveness of selenium and/or N-acetyl-cysteine in enhancing the characteristics of infertile men's sperm as well as the relationships between the quality of sperm and the levels of selenium and N-acetyl-cysteine in seminal plasma were investigated. 468 infertile men with idiopathic oligo-asthenoteratospermia participated in the study. They were randomly assigned to receive 200 g of selenium orally daily, 600 mcg of N-acetyl-cysteine orally daily, 200 g of selenium plus 600 mcg of N-acetyl-cysteine orally daily, or a similar regimen of placebo (control group) for 26 weeks, The serum levels of testosterone, estradiol, follicle-stimulating hormone, luteinizing hormone, prolactin, inhibin B, selenium, and N-acetylcysteine were measured from the blood samples these individuals provided. Semen samples were also collected for regular semen analysis, seminal plasma selenium measurement, and N-acetyl-cysteine testing. Serum follicle-stimulating hormone dropped in response to selenium and N-acetyl-cysteine therapy, although serum testosterone and inhibin B increased. Treatment with selenium and N-acetylcysteine dramatically improved all semen parameters. N-acetyl-cysteine and selenium administration had additively positive benefits. The levels of selenium

and N-acetylcysteine in seminal plasma and semen parameters showed a strong positive connection. The total of selenium and N-acetyl-cysteine concentrations was shown to be strongly correlated with mean sperm concentration (r = 0.67, p = 0.01), sperm motility (r = 0.64, p = 0.01), and percent normal morphology (r = 0.66, p = 0.01).

Selenium and vitamin E have been tested for their efficacy in increasing infertile men's semen parameters and conception rates. The 690 infertile men with idiopathic asthenoteratospermia who participated in the trial received daily supplemental vitamin E (400 units) and selenium (200 g) for at least 100 days. The cases ranged in age from 20 to 45, with a mean age of 28.5 and a median age of 30. For at least a year, these instances experienced male factor infertility (primary or secondary). Infertility lasted for an average of 10 years and 1 year, respectively. With a mean of 2.5 years, the median period from infertility diagnosis to treatment was 1 year. In comparison to no therapy, it was shown that 10.8% (75 instances) of spontaneous pregnancies and 52.6% (362 cases) of overall sperm motility, morphology, or both improved (95% confidence interval: 3.08 to 5.52). After 14 weeks of combined therapy, 253 cases (36.6%) had no response to treatment. Semen examinations of cases before and after therapy showed a mean difference of 4.3%, with a standard deviation of 4.29. Oral Se and vitamin E combination therapy was successful in treating asthenospermia, asthenoteratospermia, and inducing spontaneous conception (Moslemi and Tavanbakhsh, 2011). Besides, a study was conducted to measure the serum levels of testosterone, zinc, and selenium in infertile men visiting a reproductive clinic in Nnewi. Between the ages of 25 and 55, we studied 50 males who appeared to be infertile (subjects) and 20 males who appeared to be in good health and fertile (controls). While the serum level of testosterone was evaluated using enzyme immunoassay methods, the serum levels of zinc and selenium were measured using an atomic absorption spectrophotometer. When comparing the two groups, the results revealed that there were substantial differences in the mean serum levels of testosterone, selenium, and zinc. A strong negative association was found between testosterone and zinc in the serum, a strong positive correlation between serum testosterone and selenium, and a high positive correlation between serum zinc and selenium in the infertile guys, according to the findings. Therefore, we draw the conclusion that testosterone, zinc, and selenium blood levels are related in infertile guys and should be considered while examining male infertility cases (Oluboyo *et al*., 2012). Furthermore, Ahsan *et al*. (2014) stated that deviation from the recommended dietary Se levels, whether above or below, may result in a variety of spermatozoa defects that impact motility and fertility. Selenium may also make you more libido. To sustain male reproductive function and prevent infertility, diet should contain the recommended amount of selenium. Moreover, the levels of zinc, selenium, glutathione peroxidase activity, and antioxidant status in the following populations of men were compared: men with severe prostate inflammation (>106 white blood cells in prostate secretion; n = 29), men with severe leukocytospermia (>106 white blood cells in semen; n = 31), men with mild inflammation (0.2-1 M white blood cells in semen or prostate secretion; n = 24), men with non-reduced levels of antioxidative activity, selenium, and zinc were found in the seminal plasma of male partners in infertile couples. Most noteworthy, regardless of the degree of inflammation, decreased selenium levels were visible in all patient groups. Therefore, selenium supplementation may be advantageous for these patients (Türk *et al*., 2014).

Numerous human disorders, including oligoasthenozoospermia syndrome, are greatly influenced by oxidative stress. So, in men with oligoasthenozoospermia, the efficacy of antioxidant co-supplementation therapy employing vitamins C, E, and CoQ10 was assessed. With regard to antioxidant therapy, 169 infertile males with oligoasthenozoospermia got 120 mg/day of coenzyme Q10, 80 mg/day of vitamin C, and 40 mg/day of vitamin E. Following vitamin C, vitamin E, and coenzyme Q10 treatment, there were noticeable improvements in some semen characteristics, including sperm concentration and motility (Kobori *et al*., 2014).

When male mice were given Vitamin E, their thyroxin levels increased significantly more than control mice. In male mice fed vitamin E, histomorphological examination revealed a significant increase in the diameter of seminiferous tubules. Female mice's oestradiol and thyroxin levels, on the other hand, showed no significant changes in either the control or treated groups. However, the ovaries of mice fed vitamin E had a greater number of follicles of various stages than control mice. The findings highlighted vitamin E's promoting action on mouse reproductive functions, which can be used to treat infertility in humans and animals (Mustari *et al*., 2022).

CONCLUSION

This review confirmed the BL-induced adverse effects on male fertility. Such adverse effects can be significantly reduced when vitamin E, and selenium supplements are provided on a regular basis.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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