2022, Scienceline Publication
World's Veterinary Journal
World Vet J, 12(3): 296-303, September 25, 2022
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2022.wvj37

Effect of Gadolinium Orthovanadate Nanoparticles on Male Rabbits' Reproductive Performance under Oxidative Stress

Vsevolod Koshevoy[1]*, Svitlana Naumenko[1], Pavlo Skliarov[2], Kateryna Syniahovska[1], Galina Vikulina[1], Volodymyr Klochkov[3], and Svitlana Yefimova[3]

[1]*Department of Veterinary Surgery and Reproductology, Faculty of Veterinary Medicine, State Biotechnological University, 44 Alchevskich Str., Kharkiv, 61002, Ukraine*
[2]*Department of Surgery and Obstetrics of Farm Animals, Faculty of Veterinary Medicine, Dnipro State Agrarian and Economics University, 25 Serhii Efremov str., Dnipro, 49600, Ukraine*
[3]*Department of Nanostructured Materials named after Y.V. Malyukin, Institute of Scintillation Materials of NAS of Ukraine, 60 Nauky ave., Kharkiv, 61072, Ukraine*
*Corresponding author's Email: koshevoyvsevolod@gmail.com

ORIGINAL ARTICLE
pii: S232456822003 7-12
Received: 16 July 2022
Accepted: 02 September 2022

ABSTRACT

Oxidative stress as a leading factor of male infertility requires correction with modern pharmacological agents, particularly redox-active nanoparticles, to improve sperm quality and hormonal balance. The current experimental study aimed to investigate the effect of orthovanadate nanoparticles of rare earth elements, particularly Gadolinium, with pronounced redox properties on the reproductive function of male rabbits under oxidative stress. A total of 36 mature male *Hyla* rabbits were divided into three groups of intact control (n = 12) and two experimental groups, including rabbits ubder oxidative stress (n = 12), induced by the introduction of tert-Butyl hydroperoxide, and those under oxidative stress plus hydrosol of gadolinium orthovanadate nanoparticles (NPs, n = 12) intake for 14 days. There were four rabbits per three replicates in each group. Animals of all groups were kept on the same diet and had free access to water. The use of NPs led to an improvement in sperm quality indicators. There was an improvement in motility and ejaculate volume indicators (by 14.6% and 39.2%, respectively), a reduction of the content of morphologically abnormal sperm by 26.7%; normalization of sex hormones, an increase in the level of total testosterone (by 113%) with a decrease in 17-β-estradiol (by 16.5%). This sex hormones improvement led to an increase in the androgen saturation of the rabbit's body (free androgen index at the end of the experiment was 36.5%). The obtained changes were accompanied by a decrease in the oxidative load, as evidenced by a reduced content of diene conjugates and thio-barbituric acid-reactive compounds in the blood serum of rabbits by 30.4% and 26.8%, compared to the control. At the same time, there was an increase in the antioxidant potential, especially its glutathione link – the activity of glutathione peroxidase and glutathione reductase (by 42.5% and 34.2%, respectively), and the content of reduced glutathione increased by 62.3%, compared to the indicators before the introduction of NPs. The results of the study confirmed the effectiveness of using gadolinium orthovanadate NPs to correct the reproductive function of males under oxidative stress.

Keywords: Gadolinium orthovanadate, Male rabbits, Nanoparticles, Oxidative stress, Reproductive performances

INTRODUCTION

Male reproductive function is one of the most vulnerable to negative factors, both external and endogenous (Sharma et al., 2021). Infertility is noted as a consequence of unbalanced feeding, non-compliance with sanitary conditions and the mode of male use, metabolic disorders, especially vitamin and mineral ones, inflammatory, and infectious processes (Skliarov et al., 2020). The influence of such various factors is marked by an oxidative imbalance in the body of animals, which leads to a decrease in the reproductive capacity and fertilization potential of sperm (Koshevoy et al., 2021). The lack of an adequate response to the increase in the synthesis and accumulation of toxic radicals leads to oxidative stress (OS), which is the cause of male infertility in 95% of cases, while infectious processes and inflammation account for only 5% of cases (Agarwal et al., 2018). The causes of infertility cases in domestic animals are similar to human male infertility. Its main factors include Vitamin A deficiency as well as lack of Zinc and Selenium (Koshevoy et al., 2021; Skliarov et al., 2021).

Taking into account the pathogenesis of male infertility and developing the means for its correction, their pronounced antioxidant effect is of significant importance (Barik et al., 2019). In addition, nanostructures, such as nanoparticles (NPs), nanocontainers, and nanotubes, have attracted great attention in modern scientific research. Thus, NPs based on vanadates of rare earth elements, in particular Gadolinium and Yttrium, exhibit redox-active properties (Koreneva et al., 2016; Maksimchuk et al., 2021). The high antioxidant activity of these NPs makes it possible to use

To cite this paper: Koshevoy V, Naumenko S, Skliarov P, Syniahovska K, Vikulina G, Klochkov V, and Yefimova S (2022). Effect of Gadolinium Orthovanadate Nanoparticles on Male Rabbits' Reproductive Performance under Oxidative Stress. *World Vet. J.*, 12 (3): 296-303. DOI: https://dx.doi.org/10.54203/scil.2022.wvj37

them as geroprotectors (Nikitchenko et al., 2021a). The positive effect of gadolinium orthovanadate NPs on the reproductive function of male rats with reproductopathy and experimental prostatitis has been proven (Belkina et al., 2017; Karpenko et al., 2020). Therefore, the aim of the study was to investigate the effect of gadolinium orthovanadate NPs on the dynamics of sperm quality, sex hormones, and oxidative/antioxidant balance in male rabbits affected by OS.

MATERIALS AND METHODS

Ethical approval

The present study was affirmed by the Ethics Committee of State Biotechnological University in Kharkiv, Ukraine (ethical approval No. 7-07 of May 7, 2022). All manipulations with animals were carried out in accordance with the European Convention for the protection of vertebrate animals used for experimental and scientific purposes (2006) and the General ethical principles of animal experiments adopted by the First National Congress on Bioethics (Kyiv, Ukraine, 2001).

Study design

A total of 36 adult male *Hyla* rabbits with the age of 29 weeks and a weight of 3.54 ± 0.05 kg were included in the present study. The rabbits were obtained from a private farm (Kharkiv region, Ukraine) free of charge. Animals were kept in the vivarium of the Department of Veterinary Surgery and Reproductology SBTU, Kharkiv, Ukraine. Before the start of the experiment, the animals were kept in the preparatory period without using pharmacological adaptogens. All animals were randomly divided into three experimental groups. The first group (n = 12) induced OS with tert-Butyl hydroperoxide (tBHP). The second group of animals (n = 12) received the combination of tBHP-induced OS and gadolinium orthovanadate NPs, and the third group was considered as a control group (n = 12). There were four rabbits per three replicates in each group. For the animals in the first experimental group (OS), the state of OS was simulated by administering tBHP in a dose equivalent to 1:10 LD_{50} (3.7 mg/kg body weight) for 14 days, according to Fatemi et al. (2014). After a two-week of tBHP intake, male rabbits in the second experimental group (OS+NPs) were orally administered the hydrosol of gadolinium orthovanadate NPs activated by europium at a dose of 0.05-0.10 mg/kg of live weight for 14 days. The control group received the same volume of distilled water. The concentrated feed and fresh tap water were available *ad libitum*. The rabbits were housed in a well-ventilated room at 25 ± 1°C and with a relative humidity of 55 ± 5% with a regular 12 hours light/12 hours dark cycle. The basal diet and its feeding values are indicated in Table 1.

Table 1. Composition of diet ingredients for 29-week-old male *Hyla* rabbits

Ingredient	Content
Amount of feed units (g/kg)	215
Root crops (g/kg)	190
Maize grain (g/kg)	70
Wheat bran (g/kg)	215
Bagasse (g/kg)	20
Meadow hay (g/kg)	70
Bean hay (g/kg)	60
Salt, (g/kg)	1.0
Phosphorus (g/kg)	1.0
Calcium (g/kg)	1.5
Carotene (mg/kg)	2.0
Digestible protein (per 100 g of feed in g)	14.0
Digestible energy (MJ/kg)	2.28

Gadolinium orthovanadate nanoparticles preparation

In the present research, $GdVO_4$: Eu^{3+} NPs were used. The hydrosol of gadolinium orthovanadate NPs doped with europium ions $Gd_{0.9}Eu_{0.1}VO_4$ was synthesized as previously described. To a mixture of aqueous 1 mol gadolinium chloride (0.4 ml) and 1 mol europium chloride (0.05 ml) 49.55 ml of H_2O bidi stilled water added and then dropwise: first 37.5 ml disodium ethylenediaminetetraacetic acid (EDTA) solution and second 37.5 ml of Na_3VO_4 (pH = 10.5). After vigorous stirring, the mixture was heated in a water bath under a reflux condenser for 24 hours at 100°C. The resulting colorless transparent solution under side illumination should scatter light (Tyndall's cone). The cooled solution was dialyzed against water for 24 hours to remove excess ions with a change of water every 6 hours. The NPs were characterized using a TEM-125K transmission electron microscope (Selmi, Ukraine). Nanoparticles of $GdVO_4$: Eu^{3+} have a spindle shape and are 8 × 25 nm in size (Figure 1). The concentration of NPs in the hydrosol was determined from the absorption spectra at 279 nm on a Specord 200 spectrophotometer (Jena, Germany, Klochkov et al., 2012).

To cite this paper: Koshevoy V, Naumenko S, Skliarov P, Syniahovska K, Vikulina G, Klochkov V, and Yefimova S (2022). Effect of Gadolinium Orthovanadate Nanoparticles on Male Rabbits' Reproductive Performance under Oxidative Stress. *World Vet. J.*, 12 (3): 296-303. DOI: https://dx.doi.org/10.54203/scil.2022.wvj37

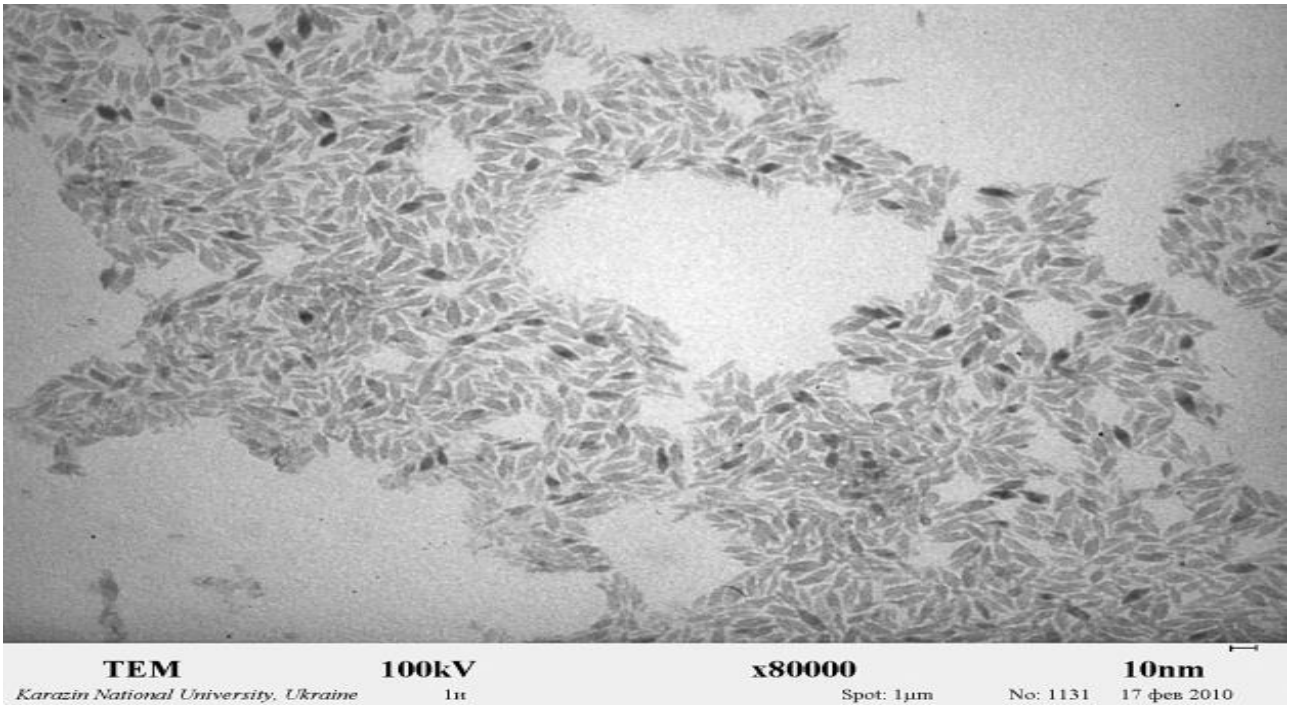

Figure 1. Electron microscopic view of the solid phase of the $GdVO_4$: Eu^{3+} colloidal solution with a size of 8×25 nm (Klochkov et al., 2012). TEM: Transmission electron microscopy

Sperm collection and evaluation

Sperm quality was assessed on days 55, 70, and 85. Males were accustomed to the artificial vagina for sperm collection prior to the experiment. Semen volume (ml), sperm motility, the number of motile sperm in the ejaculate, concentration, and percentage of sperm content with morphological abnormalities were evaluated by commonly used methods (Menon et al., 2011). The obtained ejaculates were evaluated immediately after collection. The volume of ejaculation was measured using a graduated test tube. The evaluation of live and morphologically abnormal sperm was carried out by counting 200 germ cells stained with eosin-nigrosine and expressing the obtained value as a percentage, and the number of motile sperm was counted in several fields of view using a light microscope (Zeiss, Germany) for eyepiece ×10, objective ×10. The concentration was calculated using a camera (Horyaev, Ukraine).

Blood samples collection and biochemical assay

Blood samples were taken from the lateral saphenous veins on days 15, 30, and 45 of the study (Moore et al., 2015). Pharmacological preparations for sedation and anesthesia of animals were not employed at the time of blood sampling. In the next step, 2 ml of blood samples were taken at the same time throughout the study in tubes with separating gel (BD Vacutainer®, RF).

The content of OS markers in blood serum was estimated using spectrophotometric methods in order to determine the concentrations of diene conjugates (based on the value of the molar extinction coefficient for conjugated dienes of polyunsaturated higher fatty acids wavelength $\lambda = 233$ nm) and thiobarbiturate acid-reactive compounds (based on the binding of malondialdehyde with thiobarbituric acid with the formation of a stable trimethylene complex at a wavelength of $\lambda = 532$ nm).

Antioxidants (enzymes and non-enzyme) were spectrophotometrically determined following Vlizlo (2012). Therefore, superoxide dismutase activity was calculated by the degree of reaction inhibition by the enzyme to reduce nitro blue tetrazolium in the presence of nicotinamide adenine dinucleotide and phenazine methosulfate (at $\lambda = 540$ nm). Catalase activity was determined based on the ability of hydrogen peroxide to form a stable complex with ammonium molybdate, color intensity at $\lambda = 410$ nm. Glutathione peroxidase activity (GSH-Px) was measured based on the oxidation rate of the reduced glutathione in the presence of tBHP in the color reaction with 5,5-dithiobis-2-nitrobenzoic acid (at $\lambda = 412$ nm). Glutathione reductase activity (GSH-Rd) was calculated by reducing the content of nicotinamide adenine dinucleotide phosphate at 37°C for 1 minute (at $\lambda = 340$ nm), and finally, reduced glutathione was assessed by the Butler method using Ellman's reagent (at $\lambda = 412$ nm).

Sex hormones and free androgen index

The content of Testosterone-estradiol-binding globulin (TEBG) was assessed by ELISA immunoassay to establish the dynamics of changes in the hormonal background, the level of sex hormones in the blood serum – total testosterone and 17β-estradiol with the help of standard sets of reagents ELISA Kit (LifeSpan BioSciences, USA) according to the

To cite this paper: Koshevoy V, Naumenko S, Skliarov P, Syniahovska K, Vikulina G, Klochkov V, and Yefimova S (2022). Effect of Gadolinium Orthovanadate Nanoparticles on Male Rabbits' Reproductive Performance under Oxidative Stress. *World Vet. J.*, 12 (3): 296-303. DOI: https://dx.doi.org/10.54203/scil.2022.wvj37

instructions on the immune enzyme analyzer Stat Fax 303 plus (Awaraness Technology, USA). The Free androgen index (FAI) was calculated by taking into account the obtained data. The amount of total testosterone in blood serum was divided by the content of TEBG. The obtained values were expressed in percentages.

Statistical analysis

All data obtained during the study were processed statistically using Microsoft EXCEL software. To determine the effect of gadolinium orthovanadate nanoparticles on sperm quality, hormonal levels, and redox status of rabbits, a statistical analysis of these changes in the OS+NPs group with the OS group and the control group was performed. The obtained data from the rabbits of the control and experimental groups were analyzed using a one-way analysis of variance (ANOVA). The significant differences among means at probability were examined by Duncan's Multiple Range Test. The data in the tables were presented as mean ± standard error means (SEM). The differences between groups were considered statistically significant at $p < 0.05$.

RESULTS

Effect of $GdVO_4$: Eu^{3+} nanoparticles on sperm quality

Table 2 shows the results of the rabbit sperm quality assessment. Thus, in the rabbits of the experimental group, induced OS caused a decrease in the main characteristics of the ejaculate, in particular, the semen volume by 28.4%, the number of live sperm by 15.0%, sperm motility by 14.6%, and the concentration of sperm by 8.4% ($p < 0.05$). However, the content of morphologically abnormal cells increased significantly by 44.8%, compared to the control group ($p < 0.05$).

Positive dynamics of changes in the reproductive function of rabbits were observed using gadolinium orthovanadate NPs. For example, there was a significant increase in the volume of ejaculation by 17.0% and 34.0% on days 70 and 85, respectively, compared to the OS group ($p < 0.05$). At the same time, a significant increase in the content of live and motile sperm in the ejaculate was noted on day 70 by 15.5% and 8.7%, and on day 85 by 18.8% and 13.2%, respectively ($p < 0.05$). The concentration of germ cells underwent minor changes on days 70 and 85 of the study and increased by 3.8% and 4.7%, respectively ($p < 0.05$). A positive trend toward a significant decrease in the content of morphologically abnormal spermatozoa was noted on day 70 (15.0%). In comparison, on day 85 of the study, it was lower than that of the OS group by 25.6% ($p < 0.05$).

Table 2. Qualitative indicators of ejaculates in *Hyla* male rabbits

Experimental group (Mean±SEM) / Parameters	Control	OS	OS+NPs		
			Day 55	Day 70	Day 85
Semen volume (ml)	$0.74±0.03^{a}$	$0.53±0.02^{ab}$	$0.51±0.02^{ac}$	$0.62±0.03^{b}$	$0.71±0.02^{bc}$
Sperm concentration ($\times10^6$ sperm cell/ml)	$296.42±2.03^{a}$	$271.64±1.39^{ab}$	$272.98±1.34^{abc}$	$281.94±1.78^{bc}$	$284.40±1.94^{c}$
Sperm motility (%)	84.40	72.10	71.20	78.40	81.60
Live sperm (%)	86.30	73.40	75.60	84.80	87.20
Abnormal sperm (%)	14.30	20.70	21.00	17.60	15.40

[a,b,c] Means within the same row followed by different superscripts are significantly different ($p < 0.05$). OS: Oxidative stress, OS+NPs: Oxidative stress plus nanoparticles, SEM: Standard error means

Effect of gadolinium orthovanadate nanoparticles on sex hormones

In rabbits of the OS group, the total testosterone significantly decreased by 56.8%, while there was a significant increase in 17-β-estradiol and TEBG by 25.2% and 41.2%, respectively, compared to the control group ($p < 0.05$). As a result of such changes, the value of the free androgen index (FAI) was stable at 12.6%. The results of the effect of gadolinium orthovanadate NPs on the hormonal balance in male rabbits are shown in Table 3.

Table 3. Levels of sex hormones and the state of androgen saturation of the *Hyla* male rabbits

Experimental group (Mean±SEM) / Parameters	Control	OS	OS+NPs		
			Day 15	Day 30	Day 45
Total testosterone (nmol/l)	$4.21 ± 0.17^{a}$	$1.82 ± 0.07^{ab}$	$1.84 ± 0.06^{ac}$	$2.37 ± 0.11^{b}$	$3.89 ± 0.14^{c}$
17-β estradiol (nmol/l)	$1.31 ± 0.05^{a}$	$1.64 ± 0.06^{ab}$	$1.61 ± 0.05^{c}$	$1.58 ± 0.04$	$1.37 ± 0.03^{c}$
TEBG (nmol/l)	$10.24 ± 0.33^{a}$	$14.46 ± 0.61^{ab}$	$14.12 ± 0.58^{ac}$	$13.23 ± 0.47^{b}$	$10.67 ± 0.36^{c}$
FAI (%)	41.1	12.6	13.0	17.9	36.5

[a,b,c] Means within the same row followed by different superscripts are significantly different ($p < 0.05$). OS: Oxidative stress, OS+NPs: Oxidative stress plus nanoparticles, TEBG: Testosterone-estradiol binding globulin, FAI: Free androgen index, SEM: Standard error means

To cite this paper: Koshevoy V, Naumenko S, Skliarov P, Syniahovska K, Vikulina G, Klochkov V, and Yefimova S (2022). Effect of Gadolinium Orthovanadate Nanoparticles on Male Rabbits' Reproductive Performance under Oxidative Stress. *World Vet. J.*, 12 (3): 296-303. DOI: https://dx.doi.org/10.54203/scil.2022.wvj37

The influence of NPs on the hormonal balance in the body of rabbits was ambiguous. For example, a significant increase of total testosterone in blood serum by 30.2% and 113% on days 30 and 45, respectively ($p < 0.05$) was accompanied by slight fluctuations in the level of 17-β-estradiol and TEBG, which tended to decrease on day 30 after the use of NPs and was lower than the indicators of the OS group by 16.5% and 26.2%, respectively on day 45 ($p < 0.05$). It should be noted that the dynamics of changes within the OS+NPs group confirm the effectiveness of the applied NPs. For example, on day 45 of the study the level of total testosterone increased by 111%, and 17-β-estradiol decreased by 14.9%, while the number of TEBG was significantly lower by 24.4% of the indicators before the introduction of NPs ($p < 0.05$).

Effect of $GdVO_4$: Eu^{3+} nanoparticles on oxidative/antioxidant balance in blood serum

The dynamics of the prooxidant-antioxidant system in rabbits are shown in Table 4. In the rabbits of the OS group, changes in the oxidative balance were observed with an increase in the oxidative load. Thus, the content of the primary products of lipoperoxidation diene conjugates (DC) was significantly higher by 57.9% of the indicators of the control group, and thiobarbituric acid-reactive compounds (TBA-RC), among which malondialdehyde with pronounced toxic properties increased by 48.3% ($p < 0.05$). In addition, a significant decrease in the antioxidant defense system activity was established in superoxide dismutase activity by 40.6% and catalase by 23.0% ($p < 0.05$). A significant decrease in the thiol-disulfide link pool reduced glutathione content by 37.2% and the activity of glutathione peroxidase and glutathione reductase by 28.7% and 20.8%, respectively ($p < 0.05$). The dynamics of antioxidant protection and peroxidation processes with NPs underwent positive changes. The number of DC decreased on days 30 (14.7%) and 45 (33.0%), respectively ($p < 0.05$), compared to the OS group. While the elimination of TBA-RC was noted (a tendency to decrease on day 30), it was 27.9% lower than the OS group on day 45 ($p < 0.05$).

The antioxidant potential under the influence of gadolinium orthovanadate NPs was normalized: an increase in Superoxide dismutase and catalase activity was noted on day 30 by 25.4% and 14.0%, respectively ($p < 0.05$), and on day 45 of the experiment by 53.3% and 26.4%, respectively ($p < 0.05$). It should be noted that a special feature of the used NPs action was the restoration of the pool of the glutathione link of antioxidant protection. On day 30 of the study, GSH content significantly increased by 37.6% ($p < 0.05$). The activity of GSH-Px and GSH-Rd on day 15 in OS + NPs group significantly increased by 31.1% and 10.7%, respectively ($p < 0.05$), compared to the OS group. On day 30 of the study, these indicators in OS + NPs group were significantly higher than the OS group as GSH, GSH-Px, and GSH-Rd increased by 66.7%, 56.8%, 32.6%, respectively ($p < 0.05$). At the same time, the obtained indicators on day 45 in OS + NPs group exceeded the data of the control group GSH-Px activity by 11.8% ($p < 0.05$), GSH-Rd activity tended to increase by 5.1%, and GSH content by 4.6%.

Table 4. Dynamics of oxidative/antioxidant status in male *Hyla* rabbits

Experimental group (Mean±SEM) / Parameters	Control	OS	OS+NPs		
			Day 15	Day 30	Day 45
DC (μmol/l)	1.21 ± 0.04^{a}	1.91 ± 0.06^{ab}	1.84 ± 0.05^{ac}	1.63 ± 0.04^{b}	1.28 ± 0.04^{bc}
TBA-RC (μmol/l)	0.87 ± 0.03^{a}	1.29 ± 0.03^{ab}	1.27 ± 0.03^{ac}	1.14 ± 0.03^{b}	0.93 ± 0.03^{bc}
Catalase activity (μmol H_2O_2/min/mg protein)	83.27 ± 1.21^{a}	64.15 ± 1.42^{ab}	63.06 ± 1.36^{abc}	73.10 ± 1.44^{b}	81.10 ± 0.89^{bc}
SOD activity (U/mgHb)	12.14 ± 0.13^{a}	7.21 ± 0.17^{ab}	7.46 ± 0.26^{abc}	9.04 ± 0.11^{b}	11.05 ± 0.09^{bc}
GSH (μmol/l)	7.12 ± 0.38^{a}	4.47 ± 0.28^{ab}	4.59 ± 0.34^{ac}	6.15 ± 0.34^{b}	7.45 ± 0.47^{bc}
GSH-Px (μmol/min×mg protein)	15.12 ± 0.24^{a}	10.78 ± 0.17^{ab}	11.86 ± 0.19^{ac}	14.13 ± 0.20^{b}	16.90 ± 0.22^{bc}
GSH-Rd (μmol/min×mg protein)	2.36 ± 0.04^{a}	1.87 ± 0.03^{ab}	1.84 ± 0.04^{ac}	2.07 ± 0.05^{b}	2.48 ± 0.03^{bc}

[a,b,c] Means within the same row followed by different superscripts are significantly different ($p < 0.05$). OS: Oxidative stress, OS+NPs: Oxidative stress plus nanoparticles, DC: Diene conjugates, TBA-RC: Thiobarbiturate acid-reactive compounds, SOD: Super oxide dismutase, GSH: Reduced glutathione, GSH-Px: Glutathione peroxidase, GSH-Rd: Glutathione reductase, SEM: Standard error means

DISCUSSION

The effectiveness of using gadolinium orthovanadate NPs, their antioxidant properties, and the effect on the reproductive function of male rabbits affected by OS is unique. Tert-Butyl hydroperoxide-induced OS caused a decrease in the main characteristics of the ejaculate (including motility and number of motile sperm in the ejaculate, concentration, and semen volume percentage of sperm content with morphological abnormalities). It is caused by a significant oxidative load on the reproductive system and a physiologically low antioxidant potential of sperm (Palani, 2018). The use of gadolinium orthovanadate NPs improved the sperm quality indicators (including sperm motility and life sperm) of male rabbits and their hormonal balance. The decrease in the number of morphologically abnormal sperm can be explained due to the antioxidant activity of the used NPs, as the decrease in their number leads to an improvement in the sexual function of rabbits (Vasicek et al., 2014). Similar changes in rabbit sperm quality were observed with the use of plant material

To cite this paper: Koshevoy V, Naumenko S, Skliarov P, Syniahovska K, Vikulina G, Klochkov V, and Yefimova S (2022). Effect of Gadolinium Orthovanadate Nanoparticles on Male Rabbits' Reproductive Performance under Oxidative Stress. *World Vet. J.*, 12 (3): 296-303. DOI: https://dx.doi.org/10.54203/scil.2022.wvj37

(turmeric), leading to an increase in their mass, which can be economically effective, as reported by Okanlawon et al. (2020). The improvement of sperm quality indicators of spotted thistle and rosemary in rabbits was experimentally confirmed by Attia et al. (2017). Moreover, Yousef (2005) reported the possibility of using acacia leaves and their positive effect on sperm quality and hormonal balance. The obtained results showed the uniqueness of the established properties of gadolinium orthovanadate NPs, as most metal compounds in nanoform could negatively affect the physiology and metabolism of sperm. For example, the oxidizing and inflammatory effect of silver NPs on rabbit sperm cannot be eliminated even by anti-inflammatory agents and Vitamin E (Collodel et al., 2020).

Oxidative stress has a negative effect on the balance of sex hormones and reduces the amount of androgens in the male bodies due to a violation of the synthetic function of Leydig cells and the ways of regulating reproductive function, in particular, the hypothalamic-pituitary-gonadal and hypothalamic-pituitary-adrenal axis (Appasamy et al., 2007; Darbandi et al., 2018). The negative dynamics of hormonal balance were investigated by Mohammed et al. (2016). The use of boldenone undecylenate increases rabbits' growth indicators (body weight, skeletal muscle volume); however, it causes a decrease in their fertility. On the other hand, Asadi et al. (2017) confirmed an increase in changes in the balance of sex hormones (total testosterone) due to the use of molybdenum nanoparticles and their influence on the dynamics of enzyme activity in the liver (cytochrome p450) in male rats. Biologically active substances that increase rabbits' immunological resistance during cultivation are plants of the Amaranth family (Molina et al., 2018). Ghomsi et al. (2017) indicated similar changes in biochemical indicators after adding *Moringa Oleifera* leaf to the diet of rabbits, which has an antioxidant-like effect that improves the lipid profile and immune status of animals (Salem et al., 2020). Oxidative stress was characterized as a state of intensification of peroxidation processes, accumulation of active forms of Oxygen, Nitrogen, Sulfur and/or reduction of antioxidant potential (Otasevic et al., 2020). Similar changes in blood serum of male rabbits were reported by Yousef et al. (2004) for the use of isoflavonols. However, their use can lead to negative changes in sperm quality and testosterone concentration (Abo-Elsoud et al., 2019).

Some researchers used folic acid to correct male human infertility caused by chromium-VI (Yousef et al., 2006). Scarlata and O'Flaherty (2020) indicated the leading role of enzymes in the antioxidant defense system to maintain reproductive capacity. Nikitchenko et al. (2021b) confirmed the effects of gadolinium orthovanadate NPs by activating the GSH-dependent antioxidant system in male rats. Barati et al. (2020) also indicated the effectiveness of antioxidant therapy as a pathogenetic method in male infertility in humans, and drew attention to the effect of OS correction on sperm motility. Generally, the complex action of gadolinium orthovanadate NPs as fertility correctors is similar to the complex action of Zinc oxide NPs plus Thyme oil in the study by Abdel-Wareth et al. (2020). The findings indicated improved sperm quality, testosterone concentration, and nutrient digestibility in male rabbits. At the same time, similar to the NPs used, high serum antioxidant defense potential was proven in gold nanorods in the experiment by Mehanna et al. (2022).

CONCLUSION

The use of NPs of orthovanadates of rare earth elements, in particular, Gadolinium has a positive effect on the reproductive function of male rabbits by reducing the oxidative load and increasing their antioxidant potential, which leads to an increase in the volume of ejaculate and the number of motile and live spermatozoa, an increase in the concentration of germ cells in the ejaculate, and reduces effectively the content of morphologically abnormal sperm. In addition, as a result of exposure to NPs, the balance of sex hormones in the male rabbits' blood serum was normalized and the androgen saturation of their body increased. Thus, gadolinium orthovanadate nanoparticles is effective for the correction of sperm quality, the balance of sex hormones, and the dynamics of peroxidation processes in male rabbits under OS. The obtained changes indicate the presence of a complex effect of gadolinium orthovanadate NPs on the reproductive function of male rabbits under oxidative stress, and a detailed study might make it possible to substantiate the mechanism of the action of these NPs as correctors of reproductive ability. Future studies should be conducted to investigate the effect of gadolinium orthovanadate nanoparticles on the preservation of the sperm DNA structure and their fertilizing ability, as well as expanding the data on the prooxidant-antioxidant system in the tissue of the testes of male rabbits.

DECLARATIONS

Acknowledgments

The study was carried out within the framework of the agreement on scientific and practical cooperation between the teams of the authors of the article (No. 48 of July 22, 2020). The research was performed without any financial support. This work was part of an initiative topic with state registration "Development and implementation of innovative methods and solutions using modern information technology tools in veterinary reproduction" (state registration number 0114U005415).

To cite this paper: Koshevoy V, Naumenko S, Skliarov P, Syniahovska K, Vikulina G, Klochkov V, and Yefimova S (2022). Effect of Gadolinium Orthovanadate Nanoparticles on Male Rabbits' Reproductive Performance under Oxidative Stress. *World Vet. J.*, 12 (3): 296-303. DOI: https://dx.doi.org/10.54203/scil.2022.wvj37

Authors' contribution

Vsevolod Koshevoy and Svitlana Naumenko designed the experiment, wrote the article, and discussed it. Pavlo Skliarov helped with the field study, collected data, and conducted the statistical analysis. Kateryna Sinyagovska and Galina Vikulina helped with laboratory analyses and tabulation of experimental data. Volodymyr Klochkov and Svitlana Yefimova helped with experiment application, and manuscript writing. All authors have read and approved the final version of the manuscript for publication in the present journal.

Competing interests

The authors declare that they have no competing interests.

Ethical considerations

Ethical issues under current regulations, including plagiarism, consent to publication, misconduct, data fabrication and/or falsification, double posting and/or submission, and redundancy, have been verified by the authors and warranted against the aforementioned violations.

REFERENCES

Abdel-Wareth AAA, Al-Kahtani MA, Alsyaad KM, Shalaby FM, Saadeldin IM, Alshammari FA, Mobashar M, Suleiman M, Ali A, and Taqi MO et al. (2020). Combined supplementation of Nano-Zinc oxide and thyme oil improves the nutrient digestibility and reproductive fertility in the male Californian rabbits. Animals, 10(12): 2234. DOI: https://www.doi.org/10.3390/ani10122234

Abo-Elsoud MA, Hashem NM, Nour El-Din A, Kamel KI, and Hassan GA (2019). Soybean isoflavone effects in rabbits: Effects on metabolism, antioxidant capacity, hormonal balance and reproductive performance. Animal Reproduction Science, 203: 52-60. DOI: https://www.doi.org/10.1016/j.anireprosci.2019.02.007

Agarwal A, Rana M, Oiu E, AlBunni H, Bui AD, and Henkel R (2018). Role of oxidative stress, infection and inflammation in male infertility. Andrologia, 50(11): e13126. DOI: https://www.doi.org/10.1111/and.13126

Appasamy M, Muttukrishna S, Pizzey AR, Ozturk O, Groome NP, Serhal P, and Jauniaux E (2007). Relationship between male reproductive hormones, sperm DNA damage and markers of oxidative stress in infertility. Reproductive Biomedicine Online, 14(2): 159-165. DOI: https://www.doi.org/10.1016/s1472-6483(10)60783-3

Asadi F, Mohseni M, Dadashi Noshahr K, Soleymani FH, Jalilvand A, and Heidari A (2017). Effect of molybdenum nanoparticles on blood cells, liver enzymes and sexual hormones in male rats. Biological Trace Element Research, 175(1): 50-56. DOI: https://www.doi.org/10.1007/s12011-016-0765-5

Attia YA, Hamed RS, Bovera F, Abd El-Hamid A, Al-Harthi MA, and Shahba HA (2017). Semen quality, antioxidant status and reproductive performance of rabbits bucks fed milk thistle seeds and rosemary leaves. Animal Reproduction Science, 184: 178-186. DOI: https://www.doi.org/10.1016/j.anireprosci.2017.07.014

Barati E, Nikzad H, and Karimian M (2020). Oxidative stress and male infertility: Current khowledge of pathophysiology and role of antioxidant therapy in disease management. Cellular and Molecular Life Scienes, 77(1): 93-113. DOI: https://www.doi.org/10.1007/s00018-019-03253-8

Barik G, Chaturvedula L, and Bobby Z (2019). Role of oxidative stress and antioxidants in male fertility: An interventional study. Journal of Human Reproductive Science, 12(3), 204-209. DOI: https://www.doi.org/10.4103/jhrs.jhrs_135_18

Belkina IO, Smolenko NP, Klochkov VK, Malukin YuV, Chistyakova EE, Karpenko NA, and Karachentsev YuI (2017). The assessment of gadolinium orthovanadate nanoparticles value for neonatally-induced reproductive disease in male rats. International Journal of Physiology and Pathophysiology, 8(4): 299-307. DOI: https://www.doi.org/10.1615/IntJPhysPathophys.v8.i4.20

Collodel G, Mattioli S, Moretti E, Cerretani D, Micheli L, Fiaschi AI, Menchetti L, Brecchia G, and Castellini C (2020). Oxidative and/or inflammatory trust induced by silver nanoparticles in rabbits: effect on vitamin E or NSAID administration on semen parameters. Mediators of Inflammation, article number: 6664062. DOI: https://www.doi.org/10.1155/2020/6664062

Darbandi M, Darbandi S, Agarwal A, Sengupta P, Durairajanayagam D, Henkel R, and Sadeghi MR (2018). Reactive oxygen species and male reproductive hormones. Reproductive Biology and Endocrinology, 16(1): 87. DOI: https://www.doi.org/10.1186/s12958-018-0406-2

Fatemi N, Sanati MH, Shamsara M, Moayer F, Zavarehei MJ, Pouya A, Sayyahpour F, Ayat H, and Gourabi H (2014). TBHP-induced oxidative stress alters microRNAs expression in mouse testis. Journal of Assisted Reproduction and Genetics, 31(10): 1287-1293. DOI: https//www.doi.org/10.1007/s10815-014-0302-4

Ghomsi MOS, Enow JT, Etchu AK, Tientcheu BL, Enamou G, Chouengouong TM, Mongo BG, and Bayemi PH (2017). Effect of *Moringa oleifera* leaf meal on the growth, carcass, hematology and biochemical parameters of rabbits. SOJ Veterinary Sciences, 3(3): 1-5. DOI: https://www.doi.org/10.15226/2381-2907/3/3/00133

Karpenko NO, Korenieva YeM, Chystiakova EYe, Smolienko NP, Bielkina IO, Kustova SP, Boiko MO, Kavok NS, Medvedovska NV, and Klochkov VK (2020). The studying of the prostateprotective and spermmodulating effects of the nanoparticles based on rare-earth elements oxides. Problems of Endocrine Pathology, 73(3): 109-118. DOI: https://www.doi.org/10.21856/j-PEP.2020.3.14

Klochkov VK, Grigorova AV, Sedyh OO, and Malyukin YV (2012). Characteristics of $nLnVO_4:Eu^{3+}$ (Ln = La, Gd, Y, Sm) sols with nanoparticles of different shapes and sizes. Journal of Applied Spectroscopy, 79(5): 726-730. DOI: https://www.doi.org/10.1007/s10812-012-9662-7

To cite this paper: Koshevoy V, Naumenko S, Skliarov P, Syniahovska K, Vikulina G, Klochkov V, and Yefimova S (2022). Effect of Gadolinium Orthovanadate Nanoparticles on Male Rabbits' Reproductive Performance under Oxidative Stress. *World Vet. J.*, 12 (3): 296-303. DOI: https://dx.doi.org/10.54203/scil.2022.wvj37

Koreneva EM, Karpenko NA, Smolenko NP, Belkina IO, Chistyakova EYe, Selyukova NY, Karachentsev YuI, Klochkov VK, Yefimova SL, and Kavok NS (2016). The influence of gadolinium orthovanadate and cerium dioxide nanoparticles on spermogram of adult male rats with neonatal induced disorders of reproductive function. Problems of Endocrine Pathology, 55(1): 48-55. DOI: https://www.doi.org/10.21856/j-PEP.2016.1.07

Koshevoy V, Naumenko S, Skliarov P, Fedorenko S, and Kostyshyn L (2021). Male infertility: Pathogenetic significance of oxidative stress and antioxidant defence (review). Scientific Horizons, 24(6): 107-116. DOI: https://www.doi.org/10.48077/scihor.24(6).2021.107-116

Maksimchuk PO, Hubenko KO, Seminko VV, Karbivskii VL, Tkachenko AS, Onishchenko AI, Prokopyuk VY, and Yefimova SL (2021). High antioxidant activity of gadolinium-yttrium orthovanadate nanoparticles in cell-free and biological milieu. Nanotechnology, 33(5): 055701. DOI: https://www.doi.org/10.1088/1361-6528/ac31e5

Mehanna ET, Kamel B, Abo-Elmatty DM, Elnabtity SM, Manmoud MB, Abdelhafeez MM, and Abdoon ASS (2022). Effect of gold nanoparticles shape and dose on immunological, haematological, inflammatory, and antioxidants parameters in male rabbit. Veterinary World, 15(1): 65-75. DOI: https://www.doi.org/10.14202/vetworld.2022.65-75

Menon AG, Thundathil JC, Wilde R, Kastelic JP, and Barkema HW (2011). Validating the assessment of bull sperm morphology by veterinary practitioners. The Canadian Veterinary Journal, 52(4): 407-408. Available at: https://pubmed.ncbi.nlm.nih.gov/21731095

Mohammed HH, Badawi ME, El-Tarabany MS, and Rania M (2016). Effects of boldenone undecylenate on growth performance, maintenance behavior, reproductive hormones and carcass traits of growing rabbits. Polish Journal of Veterinary Sciences, 19(2): 245-251. DOI: https://www.doi.org/10.1515/pjvs-2016-0031

Molina E, González-Redondo P, Moreno-Rojas R, Montero-Quintero K, Chirinos-Quintero N, and Sánchez-Urdaneta A (2018). Evaluation of haematological, serum biochemical and histopathological parameters of growing rabbits fed *Amaranthus dubius*. Journal of Animal Physiology and Animal Nutrition, 102(2): e525-e533. DOI: https://www.doi.org/10.1111/jpn.12791

Moore DM, Zimmerman K, and Smith SA (2015). Hematological assessment in pet rabbits: blood sample collection and blood cell identification. The veterinary clinics of North America. Exotic animal practice, 18(1): 9-19. DOI: https://www.doi.org/10.1016/j.cvex.2014.09.003

Nikitchenko YV, Klochkov VK, Kavok NS, Averchenko KA, Karpenko NA, Nikitchenko IV, Yefimova SL, and Bozhkov AI (2021a). Anti-aging effects of antioxidant rare-earth orthovanadate nanoparticles in Wistar rats. Biological Trace Element Research, 199(11): 4183-4192. DOI: https://doi.org/10.1007/s12011-020-02531-y

Nikitchenko YV, Klochkov VK, Kavok NS, Karpenko NA, Yefimova SL, Nikitchenko IV, and Bozhkov AI (2021b). Age-related effects of orthovanadate nanoparticles involve activation of GSH-dependent antioxidant system in liver mitochondria. Biological Trace Element Research, 199(2): 649-659. DOI: https://doi.org/10.1007/s12011-020-02196-7

Okanlawon EO, Bello KO, Akinola OS, Oluwatosin OO, Irekhore OT, and Ademolue RO (2020). Evaluation of growth, reproductive performance and economic benefits of rabbits fed diet supplemented with turmeric (Curcuma longa) powder. Egyptian Poultry Science, 40(3): 701-714. DOI: https://www.doi.org/10.21608/EPSJ.2020.115968

Otasevic V, Stancic A, Korac A, Jankovic A, and Korac B (2020). Reactive oxygen, nitrogen, and sulfur species in human male fertility: A crossroad of cellular signaling and pathology. BioFactors, 46(2): 206-219. DOI: https://www.doi.org/10.1002/biof.1535

Palani AF (2018). Effect of serum antioxidant levels on sperm function in infertile male. Middle East Fertility Society Journal, 23(1): 19-22. DOI: https://www.doi.org/10.1016/j.mefs.2017.07.006

Salem MI, El-Sebai A, Elnagar SA, and El-Hady AMA (2020). Evaluation of lipid profile, antioxidant and immunity statuses of rabbits fed *Moringa oleifera* leaves. Asian Australasian Journal of Animal Sciences. DOI: https://www.doi.org/10.5713/ajas.20.0499

Scarlata E and O'Flaherty C (2020). Antioxidant enzymes and male fertility: Lessons from knockout models. Antioxidants & Redox Signaling, 32(8): 569-580. DOI: https://www.doi.org/10.1089/ars.2019.7985

Sharma A, Minhas S, Dhillo WS, and Jayasena CN (2021). Male infertility due to testicular disorders. The Journal of Clinical Endocrinology and Metabolism, 106(2): e442-e459. DOI: https://www.doi.org/10.1210/clinem/dgaa781

Skliarov P, Fedorenko S, Naumenko S, Koshevoy V, and Pelyh K (2021). The development of phyto- and tissue origin medicines for veterinary reproductive issues. Scientific Horizons, 24(8): 15-25. DOI: https://www.doi.org/10.48077/scihor.24(8).2021.15-25

Skliarov PM, Fedorenko SY, Naumenko SV, Onischenko OV, and Holda KO (2020). Retinol deficiency in animals: Etiopathogenesis and consequences. Regulatory Mechanisms in Biosystems, 11(2): 162-169. DOI: https://www.doi.org/10.15421/022024

Vasicek J, Pivko J, and Chrenek P (2014). Reproductive performance of New Zealand White rabbits after depletion of apoptotic spermatozoa. Folia Biologica, 62(2): 109-117. DOI: https://www.doi.org/10.3409/fb62_2.109

Vlizlo VV (2012). Laboratory Methods of Research in Biology, Animal Husbandry and Veterinary Medicine. Lviv: Spolom. 764 p. Available at: https://www.inenbiol.com/index.php/diialnist/publikatsii/knyhy/63-diyalnist/publikaciii/knyhy/349-laboratorni-metody-doslidzhen-u-biolohii-tvarynnytstvi-ta-veterynarnii-medytsyni

Yousef MI (2005). Reproductive performance, blood testosterone, lipid peroxidation and seminal plasma biochemistry of rabbits as affected by feeding Acacia saligna under subtropical conditions. Food and Chemical Toxicology, 43(2): 333-339. DOI: https://www.doi.org/10.1016/j.fct.2004.10.006

Yousef MI, El-Demerdash FM, Kamil KI, and Elaswad FA (2006). Ameliorating effect of folic acid on chromium (VI)-induced changes in reproductive performance and seminal plasma biochemistry in male rabbits. Reproductive Toxicology, 21(3): 322-328. DOI: https://www.doi.org/10.1016/j.reprotox.2005.09.005

Yousef MI, Esmail AM, and Baghdadi HH (2004). Effect of isoflavones on reproductive performance, testosterone levels, lipid peroxidation, and seminal plasma biochemistry of male rabbits. Journal of Environmental Science and Health, Part B, 39(5-6): 819-833. Available at: https://www.tandfonline.com/doi/abs/10.1081/LESB-200030880

To cite this paper: Koshevoy V, Naumenko S, Skliarov P, Syniahovska K, Vikulina G, Klochkov V, and Yefimova S (2022). Effect of Gadolinium Orthovanadate Nanoparticles on Male Rabbits' Reproductive Performance under Oxidative Stress. *World Vet. J.*, 12 (3): 296-303. DOI: https://dx.doi.org/10.54203/scil.2022.wvj37