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The peculiarities of hormonal background in boars under correction of reproductive capacity by gadolinium orthovanadate nanoparticles

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Determination of the influence of redox nanomaterials on the body of males is of great interest to researchers, because the basis of the pathogenesis of male infertility is oxidative stress. A necessary condition in the development of such drugs is the absence of toxic effects on sexual function. It has been proved that nanomaterials based on oxides of rare earth elements have a positive effect on reproductive capacity and, at the same time, they are not gonadotoxic. A comprehensive approach is of particular importance in order to justify their use as a means of reproductive capacity correction. Taking into account the toxicity parameters the mechanisms of their action need to be studied at the biochemical, morphological and clinical levels. The positive effect of gadolinium orthovanadate nanoparticles on the quality of boar sperm (especially in terms of motility and the number of motile sperm in the ejaculate) with an effective reduction of the oxidative load on the body have been shown. The aim of this study was to determine the effect of these nanoparticles on the hormonal background of males with a decreased reproductive capacity under oxidative stress. Thus, the administration of hydrosol of gadolinium orthovanadate nanoparticles for 14 days revealed a normalization of sex hormones – an increase in total testosterone, in particular, on the 15th day of the study – by 22.6 % ($P < 0.01$), and on the 30th day – by 77.4 % ($P < 0.001$) compared with the group of animals before the administration. There was a decrease in the level of 17 β -estradiol on the 30th day – by 25.0 % ($P < 0.01$), which almost reached the values of the control group. A decrease in the content of globulin testosterone-estradiol-binding in boars of the experimental group, in particular, on the 15th day of the experiment – by 13.0 % ($P < 0.05$), and on the 30th day – by 26.8 % ($P < 0.001$) was determined, which, in turn, led to an increase in androgen saturation of the animal body – the index of free androgens at the end of the study was 43.2 %. The results are explained by the properties of the nanoparticles. Correcting oxidative stress, they increase the antioxidant potential, thereby normalizing the activity of endocrine glands and ways of regulating the germ-endocrine function of the gonads. The prospect of further research is to elucidate the effect of the correction of decreased reproductive capacity in boars under oxidative stress by nanoparticles of oxides of rare earth elements on the morphological state of the gonads.

Key words: nanomaterials, sex hormones, free androgen index, reproductive function.

Introduction

Actuality of the problem. There are many exogenous and endogenous factors that cause excessive synthesis of reactive oxygen species (ROS). Due to their excess over the antioxidant potential of cells oxidative stress (OS) occurs. In turn, OS adversely affects the reproductive function of males and leads to a partial-temporary decrease. It has a direct or indirect action on the hypothalamic-pituitary-gonadal axis (HPG) and/or disrupts its connection with other hormonal axes. If the imbalance between oxidants (ROS) and antioxidants predominates in the direction of oxidants, there is an OS that exposes cells and the body to stress. As a result, excessive amount of ROS can cause lipid peroxidation (LPO), destroy DNA, RNA, and disrupt the function of proteins in sperm and other gonadal cells (Wang et al., 2017; Darbandi et al., 2018; Rehman et al., 2020).

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It is known that ROS reduces the level of male sex hormones and disturbs the hormonal balance that regulates reproductive function (Appasamy et al., 2007; Olukole et al., 2020). OS resulting from increased ROS synthesis or decreased antioxidant availability can cause LPO in Leydig cells and germ cells, damage lipoproteins, aggregation and protein fragmentation, and inhibit steroidogenic enzymes (Wang et al., 2017).

Analysis of recent research and publications. ROS in the male reproductive system disrupts the balance between oxidants and antioxidants. After ROS generation the hypothalamic-pituitary-adrenal axis (HPA) is activated and releases corticosterone in response to stress. In response to the action of corticosterone between HPG and HPA there is an interaction that adversely affects the secretion of luteinizing hormone (LH) by the anterior pituitary gland. Decreased LH level is not able to stimulate Leydig cells to produce enough testosterone. Instead, a decrease in follicle-stimulating hormone reduces the release of androgen-binding protein (ABP) from Sertoli cells, and thus causes a decrease in testosterone level in the male body (Darbandi et al., 2018; Goma et al., 2020).

In addition, OS leads to a decrease in insulin secretion in the pancreas, which, in turn, has a negative effect on the release of T3 from the thyroid gland, and in general, on the biosynthesis of testosterone. Obesity-induced ROS synthesis affects adipocytes causing excessive leptin synthesis, which together with insulin reduces the release of T3 and, as a consequence, inhibits the functioning of the male gonads. Leptin produced by adipocytes also inhibits the release of gonadoliberin from the hypothalamus (Amjad et al., 2019; Khodamoradi et al., 2020; Turan & Öztekin, 2020).

It is known that testicular estradiol and inhibin segregate intensively under OS, thereby inhibiting the release of testosterone. This is due to the effect of ROS on aromatase activity, which causes an increase in estradiol. Decreased testosterone levels cannot regulate spermatogenesis to produce full-fledged sperm. Such level is not able to maintain the functioning of additional gonads, which play an important role in sperm maturing. As the main regulator of the manifestation of sexual reflexes, testosterone causes them to slow down. Thus, impaired endocrine reproductive function leads to a decreased reproductive capacity in males (Makary et al., 2018; Sharma et al., 2021).

Contradictory data on the effectiveness of antioxidant therapy of male infertility by nanoparticles (NPs) of various chemical composition and their effect on the level of sex hormones and the morphological state of the gonads motivate researchers to seek effective and safe means. Thus, with the administration of NPs based on gold, silver and molybdenum, which are widely used in medical practice, the initiation of OS in gonads and pathological effect on their structural organization are observed. Instead, zinc oxide NPs caused an increase in sperm quality, sex hormone content and normalization of antioxidant potential (Asadi et al., 2017; Barati et al., 2020; Goma et al., 2020; Liu et al., 2020; Olugbodi et al., 2020).

Aim of the study was to evaluate the effect of gadolinium orthovanadate nanoparticles on the hormonal back-

ground of boars by correcting their reproductive capacity under oxidative stress.

Research objectives:

1. To determine the dynamics of the level of sex hormones in the blood serum of boars with a decreased reproductive capacity under oxidative stress and by the correction of gadolinium orthovanadate nanoparticles.

2. To establish the content of testosterone-estradiol-binding globulin and the index of free androgens as indicators of androgen saturation of boars by correcting their reproductive capacity with gadolinium orthovanadate nanoparticles.

Material and methods

The work was performed in the laboratories of the Department of Veterinary Reproductology at Kharkiv State Zooveterinary Academy (from 01.09.2021 – the Department of Veterinary Surgery and Reproductology of State Biotechnological University) and the Department of Nanostructured Materials named after Y. V. Malyukin at the Institute of Scintillation Materials of the National Academy of Sciences of Ukraine (Kharkiv).

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 General ethical principles of animal experiments adopted by the First National Congress on Bioethics (Kyiv, Ukraine, 2001).

Groups of animals were formed according to the sperm quality indicators and the content of OS markers. They were divided into two groups. The sperm quality in males of the control group (n = 5) met the standards, and in the experimental group (n = 5) it was reduced, especially in terms of sperm motility and the number of motile sperm in ejaculate. At the same time, intensification of peroxidation processes towards the content of OS markers in blood serum was noted (Koshevoy et al., 2021).

Nanoparticles (NPs) of oxides of rare earth elements synthesized at the Department of Nanostructured Materials at the Institute of Scintillation Materials of the National Academy of Sciences of Ukraine under the agreement on scientific and practical cooperation (№ 48 from 22.07.2020) were used to correct the OS. Males of the experimental group were orally administered the hydrosol of gadolinium orthovanadate NP activated by europium with a size of 25×8 nm grain size at a dose of 0.0125 mg per kg of live weight for 14 days. It has been studied that gadolinium orthovanadate NP belong to the toxicity class IV – low-toxic compounds, that makes it possible to use them in biomedical studies (Koreneva et al., 2016).

Blood for research was taken according to the generally accepted method on the 1st, 15th and 30th day of the experiment. To establish the dynamics of changes in hormonal background, the level of sex hormones in the blood serum – total testosterone and 17β-estradiol and the content of testosterone-estradiol-binding globulin (TEBG) was assessed by immunochemiluminescence. The free androgen index (FAI) was calculated from the obtained data. For this purpose, the amount of total testosterone in

blood serum was divided by the content of TEBG. The obtained value was expressed in percentages.

All digital data obtained during the study were processed statistically using Microsoft EXCEL. Student's criterion was used to determine the probability of differences between mean values.

Results and discussion

The hormonal background in boars of the experimental group – with reduced reproductive capacity was characterized by a decrease in sex hormones. Thus, the level of total testosterone before the administration of NPs was lower by 49.7 % ($P < 0.001$) of the control group, and the level of 17β -estradiol – by 47.7 % ($P < 0.001$), which is shown in table 1.

Table 1

Dynamics of hormonal background in boars with the introduction of gadolinium orthovanadate nanoparticles ($M \pm m$, $n = 5$)

№	Indicator	Groups of animals			
		Control	Experimental		
			Before administration	15 th day	30 th day
1	Total testosterone level, nmol/l	16.7 ± 0.52	8.4 ± 0.33***	10.3 ± 0.31*** ¹	14.9 ± 0.44* ²
2	The level of 17β -estradiol, nmol/l	1.3 ± 0.05	1.92 ± 0.08***	1.71 ± 0.07**	1.44 ± 0.06 ¹

Notes: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ – statistically significant changes in relation to the control group; ¹ $P < 0.01$; ² $P < 0.001$ – statistically significant changes in the group of animals before administration

The content dynamics of testosterone-estradiol-binding globulin (TEBG) and the index of free androgens (FAI) in boars with the administration of gadolinium orthovanadate nanoparticles is shown in Fig. 1. Thus, in boars of the experimental group before the administration

of NPs an increase in the content of TEBG by 38.9 % ($P < 0.001$) compared with animals of the control group was determined. Taking into account the level of total testosterone and the amount of TEBG it can be proved that FAI in this group of animals was 49.3 %.

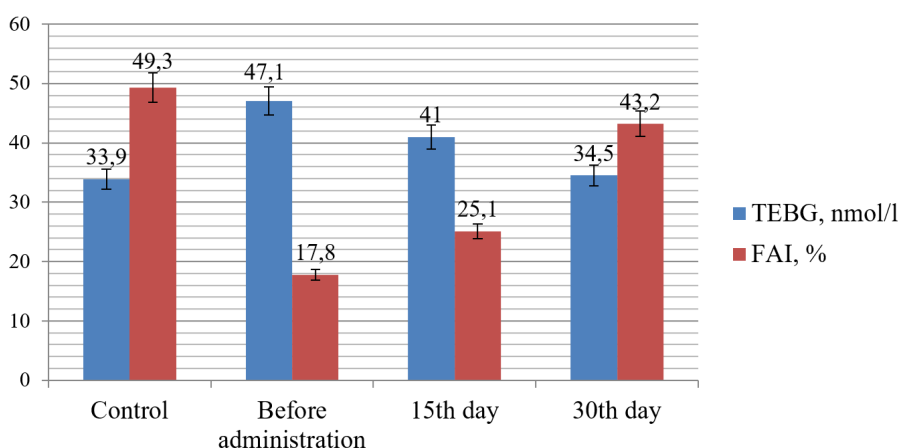


Fig. 1. Dynamics of the content of testosterone-estradiol-binding globulin and free androgen index in boars with the administration of gadolinium orthovanadate nanoparticles ($M \pm m$, $n = 5$)

The administration of the hydrosol of gadolinium orthovanadate NPs contributed to the normalization of the hormonal background in boars of the experimental group. Thus, on the 15th day of the experiment we observed an increase in the level of total testosterone in the blood serum of males by 22.6 % ($P < 0.01$), instead, the level of 17β -estradiol tended to decrease. At the same time, a decrease in the content of TEBG by 13.0 % ($P < 0.05$) was found, which, in turn, contributed to the increase of FAI to 25.1 %.

Restoration of hormonal background values in animals with full reproductive capacity occurred on the 30th day of the experiment – testosterone levels in boar blood serum increased by 77.4 % ($P < 0.001$) compared with the group before the administration of NPs and almost reached the control group ($P < 0.05$). On the contrary, there was a

decrease in the indicators of 17β -estradiol level (by 25.0 %, $P < 0.01$) and the content of TEBG (by 26.8 %, $P < 0.001$). The obtained data show that the FAI in the group of boars on the 30th day of the experiment was 43.2 %, i.e. almost reached the normative values.

The obtained changes in animals of the experimental group before the administration of NPs are due to the influence of OS, which causes a decrease in testosterone synthesis due to the damage in Leydig cells damage and other endocrine structures such as the anterior pituitary (Turner et al., 2005; Wang et al., 2017). It may be associated with an increase in the number of immature forms of sperm due to the impact on the production of male sex hormones that correlate with spermatogenesis.

Systemic hormones (testosterone, estradiol) are able to regulate the antioxidant capacity of testicular cells. Ap-

parently, some hormones such as testosterone have an antioxidant effect that protects sperm and gonadal cells from the damage caused by ROS. There is a proven negative relationship between levels of testosterone, estradiol, free thyroxine in blood serum and DNA damage in sperm (Makary et al., 2018).

Thus, it was found that the administration of NPs helped to reduce the oxidative load on the body and restore sperm quality (Koshevoy et al., 2021). Our research results show that the administration of gadolinium orthovanadate NP causes the restoration of hormonal activity of the testes and other endocrine glands. This is due to their high redox activity and antioxidant properties shown in Wistar rats (Nikitchenko et al., 2021a; 2021b). Similar results have been reported by researchers using alternative antioxidant therapies for male infertility, such as NPs of zinc oxide, melatonin or coenzyme Q₁₀ (Banihani, 2018; Obembe, 2019; Goma et al., 2020; Zhao et al., 2021).

Conclusions

The positive influence of the correction method of reproductive ability in boars by gadolinium orthovanadate nanoparticles on the hormonal balance of the body is proved:

1. With the administration of gadolinium orthovanadate NPs normalization of sex hormones – an increase in total testosterone, in particular, on the 15th day of the study – by 22.6 %, and on the 30th day – by 77.4 % compared to the group of animals before administration was determined. In turn, there was a decrease in the level of 17β-estradiol on the 30th day – by 25.0 %, which almost reached the values of the control group.

2. There was a decrease in the content of TEBG in boars of the experimental group, in particular, on the 15th day of the experiment – by 13.0 %, and on the 30th day – by 26.8 %, which, in turn, led to an increase in androgenic saturation of the body of animals – FAI at the end of the study was 43.2 %.

The prospect of further research is to elucidate the influence of the correction of the decreased reproductive capacity in boars under oxidative stress by gadolinium orthovanadate NPs on the morphological state of the gonads.

Conflict of interest

The authors declare that there is no conflict of interest.

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