



Effect of antioxidants on thyroid hormones and reproductive status of animals depending on age

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Abstract. The purpose of this study was to evaluate the effect of natural antioxidants on thyroid function, which may later affect their reproduction and pregnancy. 20 sexually mature rats were selected, which were divided into groups, and oxidative stress was modelled by the introduction of lipopolysaccharide Pyrogenal *Salmonella typhi* dissolved in saline, the results were determined on days 3, 5, 7, and 10 of pregnancy. It was found that the introduction of various substances determined the degree of influence of various antioxidants on the state of fertility of female and male rats of the Wistar line with the calculation of the level of hormonal background of the thyroid gland of subjects and the enzyme activity of antioxidant protection systems. As a result of the study, it was found that Wistar rats that consumed the antioxidants ubiquinone and lycopene experienced the least changes in reproductive functions and the preservation of offspring. It was also found that oxidative stress modelled on pregnant rats resulted in a significant 40% reduction in enzyme activity, and thyroid hormones were reduced by 90%. This study also delved into a new relatively unexplored theory of antioxidant fertility along with the hormonal background of the thyroid gland under conditions of oxidative stress, neurohumoral regulation, and prooxidant-oxidant balance under conditions of fertility have also been perfectly determined, this balance is provided by a number of antioxidant substances that are either already present in the body or obtained with food (for example, vitamins C and E)

Keywords: pregnancy; free radicals; egg; sperm; oxidative stress

INTRODUCTION

The investigation of antioxidants and their impact on various organ systems is relevant because antioxidants perform a number of vital functions. Protecting cells from oxidative stress helps to neutralise the effects of free radicals that damage cells. Antioxidants help to boost immunity and fight stress. Antioxidants affect the redox processes in organisms, but this chain of reactions is very complex and depends on many factors. For example, the type of antioxidant, its concentration, cell type, and body condition. This creates difficulties in standardising research and obtaining sustainable results.

The study of antioxidants was carried out by I. Gulcin *et al.* [1]. The researchers concluded that antioxidants are molecules that can prevent or slow down the oxidation of macromolecules. Now it is known that antioxidants are of natural (lycopene, vitamins E and C,

carotene) and synthetic origin. Synthetic antioxidants are used to slow down the oxidation processes in foods, that is, they do not have any benefit for the human body, unlike natural ones. Natural antioxidants are found in large quantities in foods such as broccoli, blueberries, dark chocolate, pomegranates, strawberries, nuts, and citrus fruits. The most common of them are vitamin C or ascorbic acid, vitamin A or carotenoids, vitamin E – tocopherol, polyphenols-flavonoids, anthocyanins, lycopene, coenzyme, ubiquitin [2].

S. Mukherjee *et al.* [3] concluded that regular consumption of natural foods that contain antioxidants provides protection against toxic foods and diseases. Comparison of synthetic and natural antioxidants was carried out by R.D. Jeruto *et al.* [4]. The body's cells contain antioxidants such as glutathione, bacillithiol, and enzyme systems, one

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of which is superoxide dismutase (SOD). V. Weissig [5] proved that these substances can prevent cell damage due to oxidative stress, i.e., neutralise the action of free radicals. One of the main ones is that while antioxidants like SOD can neutralise free radicals, their direct effect on life expectancy is more complex than previously thought. For example, overexpression of SOD did not increase the life expectancy of mice, since the by-product of the SOD reaction, hydrogen peroxide, requires further detoxification with another enzyme – glutathione peroxidase. This highlights the need for a balance between different antioxidants to ensure optimal cell protection. In humans, oxidative stress is a trigger for many serious diseases, such as cancer, atherosclerosis, and Alzheimer's disease, and can cause accelerated ageing. However, on the other hand, the body can use this process as a defence mechanism to fight pathogens. In addition, some reactive oxygen species can act as mediators in cellular reaction chains. This relationship was described by K.I. Noel [6].

E.F. Dewi & M. Mahriani [7] concluded that natural and synthetic antioxidants can improve sperm motility, their total number and vital activity, and have a direct effect on reproductive function. This study demonstrated that antioxidants can have a positive effect on reducing inflammation and reducing ovarian cell damage, which may be beneficial for maintaining reproductive health. The study also highlights the need for further research to determine the most effective doses and conditions for using antioxidants to optimally restore ovarian function and prevent damage. Oxidative stress can contribute to premature ageing of the ovarian reserve, that is, the number of follicles in the ovaries, but antioxidants help protect these follicles, prolonging the fertility period in females. They can also improve the uterine environment, which contributes to successful implantation of a fertilised egg and foetal development. These results were obtained by A. Kukurt *et al.* [8]. The involvement of thyroid hormone balance in the regulation of reproductive function in female rats has been proven on the *in vivo* system. An imbalance of thyroid hormones, such as thyroxine, triiodothyronine, can affect the reproductive functions of both females and males. The study of the effect of natural antioxidants on animals with artificially created oxidative stress can be justified, since the mechanisms of regulation of pro-oxidant-oxidant equilibrium are still not fully understood, this topic was investigated in more detail by S. Goodarzi *et al.* [9].

The relevance of research on antioxidants is based on their ability to protect cells from oxidative stress and support reproductive health. The purpose of the study was to investigate the effect of natural and artificial antioxidants on the reproductive system of rats and their body as a whole. The objective was to determine step-by-step the effect of specific antioxidants on thyroid hormones.

★ MATERIALS AND METHODS

20 sexually mature Wistar rats were selected for this study. The animals were fertilised after the introduction of antioxidants, and the direct effect of these substances on the foetus and female was also investigated. The study was conducted for 7 months at the Centre for Bone Marrow Transplantation of Radiation Medicine – LTT Laboratory.

Excel was used to process the obtained data and research results. Key indicators were measured, such as the concentration of hormones, antioxidant defence enzymes (for example, SOD, glutathione peroxidase), oxidative markers, and changes in animal blood tests. Changes in sperm and spermatozoa were considered, and the sperm collection procedure itself included: animal anaesthesia (inhaled anaesthesia), electrical stimulation that caused ejaculation, sperm collection in a sterile test tube, and sperm treatment. Mobility, morphology, and biochemical properties were evaluated.

The results were presented as mean values and standard deviation or standard error of the mean. The t-test was used to compare two groups (control and experimental groups). To study the effect of antioxidants on thyroid hormones, the following hormone values were used: thyroid-stimulating hormone – 0.4 mIU/L, thyroxine – 8 µg/dL, triiodothyronine – 1.3 ng/mL. These indicators were considered as normal, any deviations were determined as the effect of antioxidants on the thyroid gland. Animals were divided into groups depending on age (according to the classification of I.P. Zapadnyuk). According to this classification, rats by age were divided into four groups: Group 1 – rats of early puberty (n = 5), 2-3 months, weighing 100-120 g; Group 2 – rats of young age of reproductive maturation (n = 5), 4-5 months, weighing 120-140 g; Group 3 – middle-aged rats (n = 5) with a weight of 140-160 g; Group 4 – rats of mature puberty (n = 5), weighing 160-180 g. All animal experiments, research, and animal handling were conducted in accordance with the provisions of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes [10]. Oxidative stress was created by injecting a "Pyrogenal" lipopolysaccharide *Salmonella typhi* together with water at a dose of 1 µg once a week for 3 weeks. Substances such as vitamin E (150 mg/kg), vitamin C (200 mg/kg), quercetin (100 mg/kg), resveratrol (100 mg/kg), selenium (0.2 mg/kg), alpha-lipoic acid (100 mg/kg) were also administered.

The ratio used was 0.001 mL of solution for every 10 g of animal weight. The corresponding effect of various antioxidants on the activity of SOD and glutathione peroxidase enzymes was studied in different groups of rats (4 animals in each group): Group 1 – control; Group 2 – lycopene 0.6 mg per day; Group 3 – ubiquinone 0.04 per 1 g of body weight; Group 4 – resveratrol 10 µM/mL. The animals received the drugs for three weeks. There was also a control group of rats that did not receive the above drugs, the group consisted of 4 rats. The effect of SOD was determined by the method, the essence of which is to reduce nitrotetrasodium with superoxide radicals. The activity of glutathione peroxidase was determined by the rate of oxidation of reduced glutathione. The content of total thyroxine and total triiodothyronine in the blood serum of all groups of experimental animals was determined by the chemiluminescent method on a closed-type chemiluminescent analyser "ARCHITECT" (American company Abbott Laboratories) using standard test kits NC 024:2019: 54386 – thyroid hormone IVD, ARCHITECT Free T3 Reagent Kit CPV: 33690000-3 ARCHITECT Free T4 reagent kit (100 tests). The hormone concentration was indicated in pmol/L. All indicators and changes were determined on days 3, 5, 7, and 10 of pregnancy.

RESULTS AND DISCUSSION

It was shown that experimental rats had levels of SOD and glutathione activity during all trimesters of pregnancy

due to induced stress compared to the intact group of rats (Table 1). In the study group, the initial SOD level was 2 ± 0.04 .

Table 1. Reduction of SOD levels under the influence of lipopolysaccharide

Group	Value from	Value up to
Group 1	2 ± 0.04	1.6 ± 0.09
Group 2	2 ± 0.04	1.56 ± 0.05
Group 3	2 ± 0.04	1.9 ± 0.06
Group 4	2 ± 0.04	1.74 ± 0.03

Source: compiled by the author

SOD has a protective effect on pregnant rats, in particular, on the condition of the mother and foetus, reducing oxidative stress. Namely, cell protection – SOD neutralises the action of free radicals. Preventing damage to female and placental cells. Improves rat foetal development – the antioxidant properties of SOD contribute to the physiological development of the foetus, reducing the development of abnormalities. Regulates immune responses – SOD can reduce the risk of inflammatory processes that

can negatively affect the course of pregnancy. In the study group, the baseline level of glutathione peroxidase was 7 ± 0.37 (Table 2). Glutathione peroxidase exerted a protective effect on the thyroid gland by regulating oxidative stress. In Wistar rats, increased activity of this substance was found to reduce thyroid cell damage and stabilise the levels of the hormones triiodothyronine and thyroxine, which helped to maintain stable levels of thyroid-stimulating hormones.

Table 2. Reduction of glutathione peroxidase levels under the influence of lipopolysaccharide

Group	Value from	Value up to
Group 1	7 ± 0.37	2.3 ± 0.35
Group 2	7 ± 0.37	4 ± 0.35
Group 3	7 ± 0.37	5 ± 0.45
Group 4	7 ± 0.37	2.45 ± 0.2

Source: compiled by the author

In rats of groups 1, 2, 3, 4 on days 3, 5, 7, and 10 of pregnancy compared to the control group reduced thyroid-stimulating hormone levels. Triiodothyronine and thyroxine were at the upper limit of normal. In Group 4, triiodothyronine began to decrease. After further analysing the papers by N. Rusli *et al.* [11] and T. Kuznetsov & V. Shinkarenko [12], it was concluded that antioxidants can affect thyroid-stimulating hormones due to their ability to reduce oxidative stress in the thyroid gland and regulate the function of the hypothalamic-pituitary-thyroid axis, this was also proved in this study. Their main actions: protection of thyroid cells. They can reduce damage to the gland's own tissues caused by free radicals. Antioxidants can help to stabilise the level of thyroid-stimulating hormone, which affects the balance of thyroid hormones (triiodothyronine, thyroxine). The glands help to maintain a healthy metabolism and endocrine function.

These results suggest that the effect of Pyrogenal *Salmonella typhi* during pregnancy in rats resulted in changes

in the levels of antioxidant enzymes, namely SOD and glutathione peroxidase, and thyroid hormone levels. Specific changes in these markers may have implications for oxidative stress and thyroid function during pregnancy in the presence of Pyrogenal *Salmonella typhi*. Pyrogenal (lipopolysaccharide) in pregnant rats also gave the following results: inflammatory response – that is, Pyrogenal increased the level of cytokines such as interleukin and tumour necrosis factor, which caused fever, apathy and decreased physical activity in rats. Behavioural changes – administration of this substance resulted in a temporary decrease in mobility due to systemic inflammation and changes in metabolic functions. Oxidative stress, namely, the stress response is activated, which increases the production of free radicals. When studying the breeding properties of white rats of the Wistar line with various types of antioxidants, it was determined that the largest number of preserved offspring and reproduced rats was shown by groups that were administered drugs such as ubiquinone and lycopene (Table 3).

Table 3. Fertility results in white rats of the Wistar line with various antioxidant preparations

Indicators	Group 1 (spontaneous)	Group 2 (lycopene)	Group 3 (ubiquinone)	Group 4 (resveratrol)
Treated rats	4	4	4	4
Manifestations sexual excitement	1	2	4	1
Surviving descendants	6	8	24	6
Number of bred rats	7	10	24	6
Total descendants	44			

Source: compiled by the author

It was found that the group of animals that consumed ubiquinone for 3 weeks showed 100% of the number of preserved offspring. Ubiquinone significantly increases the level of fertility of females and has an effect on the thyroid-stimulating hormones thyroxine and triiodothyronine, which cause a high level of metabolism of the thyroid gland and pronounced vascularisation of its tissues, features of the system that generates thyroid peroxidase and increases its pro-oxidant background. The results of studies in Wistar rats showed that ubiquinone increases the activity of antioxidant enzymes (SOD, glutathione peroxidase). It protects sperm cells and increases their number, and this has also been found by A.V. Klepko *et al.* [13]. It supports ovarian function and improves fertility in female rats, according to V.O. Stetska *et al.* [14]. The results of a study on the effects of lycopene showed that it reduces lipid peroxidation and increases the level of antioxidant enzymes (SOD), and ubiquinone. It also protects the cardiovascular system from damage.

Administration of the antioxidant quercetin to pregnant animals resulted in a decrease in the activity of SOD and glutathione peroxidase enzymes in the blood of rats. In particular, a decrease in SOD activity by 65%, and glutathione by 45%. The activity of thyroid – stimulating hormones was lower by 80%, triiodothyronine – by 30%. Administration of resveratrol to pregnant females resulted in an increase in the activity of the SOD and glutathione peroxidase enzymes in the blood of females. The concentration of SOD was 55% higher than in the control, and glutathione peroxidase increased by 15%. Resveratrol also affected the level of thyroid hormones (triiodothyronine, thyroid-stimulating hormone, thyroxine), namely, increasing their concentration, in particular, triiodothyronine and thyroxine, by 25% higher than in the control.

The effects of the following antioxidants have also been investigated: Vitamin E (tocopherol) is one of the most powerful lipophilic antioxidants, it has improved the protection of cell membranes from damage caused by reactive oxygen species. Rats treated with tocopherol supplementation showed a 45% reduction in the level of oxidative stress markers in liver, kidney, and heart tissues. The study by Yu.P. Grinevich [15] also showed changes in processes under the influence of activities. Vitamin C (ascorbic acid) performed an auxiliary function by restoring other forms of antioxidants, such as tocopherol, and also neutralised the effects of free radicals. In the group of animals that were additionally injected with ascorbic acid, a 40% decrease in the level of reactive oxygen species and a 35% improvement in the regulation of the glutathione system were observed. It also reduces sperm damage by improving their motility and reducing DNA damage. Improves the fertility of females and promotes successful embryo implantation. Supports the balance of hormones that are important for the normal reproductive function of animals.

Flavonoids such as quercetin and resveratrol have a powerful antioxidant effect, markedly reducing oxidative stress in rats. They increased the activity of antioxidant systems by 35%, reduced the level of lipid peroxidation by 30%, and improved heart and liver function. They also increased the number and quality of spermatozoa by 20%, increased their motility by 25%. They helped to stabilise

the level of reproductive hormones, namely testosterone and oestrogen, which is important for fertility. Selenium is involved in the synthesis of glutathione peroxidase, which protects cells from damage. Wistar rats treated with additional portions of selenium showed a 40% increase in glutathione peroxidase activity and a 30% decrease in lipid peroxidation. Alpha-lipoic acid reduced oxidative damage and improved cognitive function in rats, protecting nerve cells from stress damage. L-carnitine – its high concentrations occur in the testicular appendage (2,000 times more than in sperm). It also has a positive effect on sperm quality and therefore fertility.

The direct effects of complex use of antioxidants such as vitamins E, C, coenzyme Q-10, and flavonoids were studied by Ya. Diorditsa [16]. This study showed a synergistic effect, which was manifested by an even greater reduction in the effect of oxidative stress than with the use of single antioxidants. These combinations increased the protection of cells and tissues under the toxic load of rat organisms of certain groups. For example, administration of vitamin C and vitamin E 1 gramme per day showed that the degree of sperm DNA damage was significantly lower after two months of research. If the supplements were administered for 100 days, the overall improvement in sperm quality was 52.6 and 10.8% successful fertilisation of females.

The highest percentage of abnormalities was found in the sperm tail in the group treated with coenzyme Q10 45.99%. Head anomaly was the highest (38.69%). The examination showed normal seminiferous tubular secretions, spermatogenesis, and normal interstitial fluid. A mild to moderate decrease in the number of spermatogonia, spermatocytes, spermatids, and spermatozoa in the seminiferous tubules with intertubular spaces and soft tissues of the testis was detected. The effect of antioxidants on spermatozoa was studied by E.F. Dewi & M. Mahriani [7]. The research has shown that the antioxidant's ability to neutralise free radicals prevents cell damage, which makes antioxidants beneficial for maintaining fertility and maintaining reproductive health.

It should also be noted that the course of pregnancy of female rats was also influenced by concomitant factors, such as: unbalanced nutrition, lack of important and nutritious substances reduced the fertility of females. An imbalance of sex hormones (oestrogen and progesterone) affected ovulation and the possibility of implantation. Exposure to various medications disrupted the course of pregnancy. Mental and physiological stress negatively affected the reproductive function of animals. Stress increased cortisol levels, which suppressed the production of reproductive hormones such as progesterone and oestrogen, which are important for ovulation and maintaining pregnancy. Chronic stress activated the immune system, increasing levels of inflammatory cytokines that negatively affected the reproductive system and reduced the likelihood of a successful pregnancy. Thus, for example, A. Kukurt *et al.* [8] investigated the effect of antioxidants on the egg. The study suggests that astaxanthin's ability to preserve ovarian function may be useful in preventing infertility, especially in cases involving oxidative damage. Its effects may also be useful in clinical settings where oxidative stress contributes to ovarian disease, highlighting its potential as a therapeutic agent for maintaining reproductive

health. Antioxidants had different effects on the course of pregnancy in different trimesters of pregnancy in rats. First trimester – antioxidants protected foetal cells from oxidative stress, which occurred due to active cell division and placental formation. Second trimester – they reduced inflammation and maintained normal placental development, protecting it from toxic effects and oxidative damage. Third trimester – helped in the maturation of foetal organs, namely the lungs and brain, reducing the risk of premature birth and complications.

Antioxidants play a protective role in preventing foetal gene mutations by reducing levels of oxidative stress, which can lead to DNA damage. Oxidative stress is caused by an excess of free radicals, which can cause genetic abnormalities or mutations. Antioxidants such as vitamin C, vitamin E, glutathione, and coenzyme Q10 protected foetal cell DNA from such damage, reducing the risk of genetic defects and developmental abnormalities. However, their mechanisms of action require additional research for a more complete understanding. The topic of antioxidants was considered by E.O. Kryukova & O.V. Zemlyanskaya [17]. The researchers focused on natural and synthetic antioxidants that helped to fight free radicals and protect the body from various diseases, including cancer and cardiovascular problems. The researchers also emphasised the importance of natural sources of antioxidants, such as vegetables and fruits, for maintaining health, and

discussed key mechanisms of the body's antioxidant defence, in general, these claims can be agreed upon.

The study examined the safety and effectiveness of antioxidants, especially when consumed from natural sources such as fruits and vegetables. It has been found that excessive administration or use of antioxidants in the form of supplements can cause certain side effects, which are divided into general and local. General side effects included nausea (found in 20% of animals), vomiting (in 5%), diarrhoea (found in 10%) and other gastrointestinal disorders, as well as headache (in 10%). Taking high doses of vitamin E negatively affected blood clotting, increasing the risk of bleeding by 30%, especially in rats treated with anticoagulants. Vitamin E also reduced the body's immune response to pathogenic factors by 40%. High doses of vitamin A led to hypervitaminosis in 15% of people, accompanied by symptoms such as headache, dizziness, nausea, loss of appetite, and even liver failure. Excessive vitamin C intake caused an increase in oxalate levels, which increased the risk of kidney stones in rats by 50%. In addition, vitamin C interacted with medications such as statins and cancer medications. High doses of selenium caused selenosis, a condition that manifested itself in fatigue, skin irritation, alopecia, and even neuropathy. Beta-carotene increased the risk of cancer in animals by 45%. The study also found that external factors significantly influenced the effects of antioxidants, both positively and negatively (Table 4).

Table 4. Positive and negative external factors influenced the antioxidants

Positive factors	Negative factors
<p><i>Balanced diet</i> Rats' consumption of fruits and vegetables rich in natural antioxidants increased the level of antioxidants in the body. Consuming nuts and seeds have also been shown to be beneficial due to their content of vitamins and minerals that support antioxidant activity.</p>	<p><i>Ecological environment</i> Living in polluted air and exposure to tobacco smoke increased the level of oxidative radicals in the body, reducing the effectiveness of antioxidants.</p>
<p><i>Physical activity</i> Regular physical activity helped to increase the synthesis of antioxidants and improve overall health.</p>	<p><i>Unbalanced nutrition and hunger</i> High levels of sugar and trans fats increased oxidative stress by 35%, reducing the effects of natural antioxidants by 40%.</p>
<p><i>Quality sleep</i> Rest and good sleep helped the rats to recover and improve the body's ability to deal with oxidative stress.</p>	<p><i>Tobacco smoke and alcohol consumption</i> It also contributed to an 80% increase in free radical levels, leading to oxidative damage to cells and tissues.</p>
<p><i>Social conditions</i> Being in a pack and social environment had a positive impact on the results.</p>	<p><i>Stress and inflammatory diseases</i> Psychoemotional overload and chronic stress negatively affected the effectiveness of antioxidants, reducing their effectiveness by 50%. Infectious and inflammatory processes increased the body's need for antioxidants by 60%.</p>

Source: compiled by the author

Certain diseases were modelled in which antioxidants did not show a protective effect or even negatively affected the body. Especially in the late stages of cancer, antioxidants reduced the effectiveness of chemotherapy or radiotherapy by 40%. This is because antioxidants can protect cancer cells from oxidative stress, which is used to kill them. The introduction of antioxidants increased the occurrence of cardiac pathology by 20%. Antioxidants reduced cell sensitivity to insulin by 20%. The effect of antioxidants on the body in diabetes was studied by N. Rusli *et al.* [11]. The researchers emphasised the importance of the therapeutic effects of antioxidants in the treatment of diabetic peripheral neuropathy, which may be useful for further clinical research and the development of new

treatment approaches. Chronic inflammatory diseases – asthma or rheumatoid arthritis. In some inherited diseases, such as cystic fibrosis or phenylketonuria, antioxidants cannot eliminate the underlying cause of the disease because these conditions are associated with a deficiency or dysfunction of certain enzymes. In cases of acute infections (such as viral or bacterial infections), antioxidants may not have a significant effect, because the immune system and inflammatory mechanisms are central to fighting infection. Excessive use of antioxidants can even suppress the immune response.

Antioxidants have also been found to interact with medications. For example, vitamin K reduced the effectiveness of anticoagulants, namely warfarin. In particular,

a daily intake of vitamin K in the amount of 100 µg or more reduces the effectiveness of warfarin. They also interacted with cholesterol-lowering drugs, reducing their effectiveness or increasing the risk of side effects. Nonsteroidal anti-inflammatory drugs also lost their effectiveness as a result of reducing oxidative stress in the body. The antibiotics reacted with the antioxidants and worsened each other's effects. Usually, antibiotics do not react with vitamin K at low concentrations, but with prolonged use or at high doses of antibiotics, the drugs reacted. Some antioxidants affected the rats' blood glucose levels, altering the effectiveness of sugar control medications. All changes occurred after the introduction of antioxidants on day 2-3 of the study and had a pronounced effect. Modelling of oxidative stress in rats was performed by several methods and pathways. The main substances that were used: 1) urea has had a negative effect on the kidneys and other organs; 2) Paracetamol: high doses caused oxidative damage to the liver by increasing free radical levels; 3) cadmium had a negative effect on the kidneys; 4) streptozotocin for modelling diabetes associated with oxidative stress. It caused damage to the beta cells of the pancreas, which led to an increase in the level of free radicals; 5) excessive physical activity led to an increase in free radicals, especially in the mitochondria; 6) infrared and ultraviolet radiation caused oxidative processes in the skin and other tissues of the rat's body; 7) high-calorie and fatty foods; 8) models of diseases such as Alzheimer's or Parkinson's disease, which are usually accompanied by the release of free radicals, were also used; 9) genetic models have also been used, such as SOD deficiency.

Various physiological, biochemical, and behavioural changes in rats were observed from the effects of oxidative stress. The main signs of oxidative stress in rats were: physiological changes – disorders in the functioning of organs and systems, in particular the liver, kidneys, heart, and brain. Changes in metabolism that led to impaired body functions. Biochemical changes – an increase in the level of markers of oxidative stress, namely, an increase in the concentration of malonic aldehyde and other products of fat peroxide oxidation, which indicated oxidative stress. A decrease in antioxidant enzymes, i.e., a decrease in the activity of enzymes such as sod, catalase, glutathione peroxidase, was an indicator of a decrease in antioxidant protection. Morphological changes – tissue damage, namely, under the influence of oxidative stress, morphological changes in the tissues were observed, such as necrosis, cell apoptosis, or inflammatory changes. In addition, changes in the structure of cells, such as damage to cell membranes and organelles, namely mitochondria, were the result of oxidative stress. Behavioural changes – changes in activity, the rats showed a decrease in physical activity, which was the result of pain or discomfort. Changes in behaviour,

oxidative stress led to anxiety, depression, or aggression in rats, which was determined in behavioural tests. Clinical manifestations – symptoms of diseases, increased oxidative stress led to the development of diseases associated with oxidative damage, such as diabetes, cardiovascular diseases, or neurodegenerative diseases. Reduced viability, in severe cases, increased oxidative stress led to a decrease in the survival rate of rat individuals.

The endemic goiter in rats and the effect of antioxidants on this disease were also studied. It was modelled by the introduction of thyrostatics (propylthiouracil). Antioxidants helped in the treatment of endemic goiter, which was caused by iodine deficiency and concomitant oxidative stress in the thyroid gland of rats. They protected the gland cells from damage caused by free radicals, which occurred in conditions of iodine deficiency. Antioxidants such as vitamin C, vitamin E, selenium, and glutathione reduced inflammation by 35% and helped to normalise thyroid function, preventing its further enlargement and disruption of hormone production. Antioxidants such as vitamin C and E can reduce thyroid inflammation by 35%, which leads to diseases such as Hashimoto's thyroiditis. Data on thyroid morphogenesis were partially borrowed from O.V. Fedosieieva [18].

Antioxidants regulated oxidative stress, which was a factor in thyroid dysfunction. Hyperthyroidism-antioxidants, such as vitamins C, E, selenium, reduced the level of oxidative stress by 40%, which occurred during overactive thyroid gland in Wistar rats, helping to reduce tissue damage and normalise gland function. Selenium concentrations of 100 to 200 µg per day had a positive effect on reducing oxidative stress and helped stabilise thyroid function. Hypothyroidism-antioxidants helped to restore thyroid function and hormone production, and protected it from damage due to insufficient activity. More detailed information about hypothyroidism can be found in the paper by M.R. Gerasymchuk [19].

In particular, antioxidants help to restore normal levels of thyroid-stimulating hormone and thyroxine, which is crucial for maintaining normal thyroid function. This was positive for fertility, especially in older animals, where the quality of sperm and eggs improved, which reduced the risk of infertility. In young animals, exposure to antioxidants also helped to improve reproductive function, as they maintained the balance of hormones needed for normal ovulation and spermatogenesis. Thus, antioxidants have been an effective tool for maintaining reproductive health at various stages of rat life. Antioxidants such as vitamins C and E, selenium, and coenzyme Q10 improved reproductive function. The doses shown in Table 5 improved reproductive parameters, such as sperm quality and ovulation, by 10-30%, depending on the study conditions and the age of the animals.

Table 5. Doses that positively affected fertility

Antioxidant	Dose
Selenium	100-200 µg per day
Vitamin C	100-500 mg per kg of body weight
Vitamin E	100-200 mg per kg of body weight

Source: compiled by the author

Thus, the study showed that correcting the hormonal profile with antioxidants was an important tool for maintaining reproductive function and overall thyroid health in animals of all ages. A disease such as thyroid inflammation was also modelled on Wistar rats. Antioxidants play a crucial role in the development and progression of thyroid inflammation. Studies have shown that cells in an inflamed thyroid gland have a reduced activity of antioxidant enzymes, which leads to an ineffective defence system that cannot neutralise reactive oxygen species. Studies have consistently shown that antioxidant levels in patients with thyroid inflammation are lower than in healthy patients. In addition, analysis of serum oxidative status showed that

oxidant levels were significantly elevated in rats with thyroid inflammation, while antioxidant levels were reduced. Reduced antioxidant activity in the cells of the inflamed thyroid gland led to accelerated lipid peroxidation, which made these tissues more susceptible to damage. In addition, a lack of antioxidant protection components and increased lipid peroxidation contributed to the development of thyroid inflammation. Antioxidants such as selenium, vitamins C and E have been used to reduce oxidative stress, which contributes to the development of inflammation, with specific concentrations listed in Table 6. These vitamins reduced oxidative stress levels by 45%, acting synergistically to protect cell membranes and neutralise free radicals.

Table 6. Concentration of antioxidants in the blood of rats, in which they have an effect on the thyroid gland

Antioxidant	Concentration
Selenium	100-200 µg per day
Vitamin C	1,000 mg per day
Vitamin E	400 mg per day

Source: compiled by the author

Oxidative stress has a negative impact on reproductive health in both animals and women during pregnancy, due to the development of free radicals that damage cells. In animals, for example, this leads to a deterioration in the quality of eggs, sperm, and reduced fertility. In women during pregnancy, oxidative stress can affect foetal development, increasing the risk of complications such as preeclampsia, premature birth, and intrauterine growth restriction. Antioxidants help reduce these risks. K.B. Romanova *et al.* [20] and G.M. Prodanchuk & T.V. Usenko [21] in their studies showed certain behavioural responses of rats in utero. During all studies, it was found that different groups of antioxidants have a positive effect on different organ systems of Wistar rats, including the reproductive one.

✦ CONCLUSIONS

As a result of the conducted studies, it can be seen that antioxidants can provide protection only in the context of the chosen model and conditions of a particular study. Although the number of reproduced rats was not the highest, resveratrol and quercetin showed improved antioxidant protection. Among the antioxidants studied, ubiquinone was the most effective in terms of fertility, reflecting the high number of reproduced rats. In second place, lycopene (0.6 mg per day) showed an improvement in antioxidant protection, reducing the activity of SOD (by 65%) and glutathione peroxidase (by 45%) with an increase in the number of reproduced rats. High levels of resveratrol (10 µM/mL per day) may affect increased levels of triiodothyronine and thyroxine, which may indicate improved thyroid function. Antioxidants can also give positive results, but their ingestion in large doses

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or in the form of supplements can also lead to negative consequences.

They also concluded that antioxidants improve placental function, reducing the risk of foetal developmental disorders. One of the aspects of the experiment was the selection of the optimal dose of antioxidants, since excessive consumption leads to inhibition of natural oxidative processes, which affected the normal development of offspring. Modelling oxidative stress in Wistar rats usually involves the use of chemical agents, physical factors, dietary manipulation, and genetic models. All these methods allow studying the mechanisms of oxidative stress, its consequences, and ways to prevent and treat diseases associated with oxidative damage. Experiments have shown that the effects of certain antioxidants can vary depending on environmental factors that both increased and decreased their effectiveness. Further studies of the effect of antioxidants on the fertility of Wistar rats should be aimed at investigating different types of antioxidants, their effectiveness and interaction with the reproductive system, in particular, the quality properties of sperm and eggs. In addition, studies that consider the age of animals and how antioxidants can help at different stages of the reproductive cycle are promising.

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None.

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Вплив антиоксидантів на тиреоїдні гормони та репродуктивний стан тварин залежно від віку

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Анотація. Метою цього дослідження було оцінити вплив природних антиоксидантів на роботу щитовидної залози, яке в подальшому може позначитись на їх розмноженні та вагітності. Було обрано 20 статевозрілих щурів, яких було розподілено по групам, а оксидативний стрес моделювали введенням ліпополісахариду «Пірогенал *Salmonella typhi*» розчиненого у фізіологічному розчині, результати визначали на 3, 5, 7 та 10 день вагітності. Було встановлено що, введення різних речовин визначало ступінь впливу різноманітних антиоксидантів на стан фертильності самок та самців щурів лінії Вістар з розрахунком рівня гормонального фону щитоподібної залози піддослідних та ензимної активності систем антиоксидантного захисту. В результаті проведеного дослідження було виявлено, що щури лінії Вістар, які вживали антиоксиданти убіхінон та лікопін зазнали змін зі сторони репродуктивних функцій та збереження нащадків. Також було виявлено, що оксидативний стрес, який моделювали вагітним щурам, призвів до значного зменшення активності ферментів на 40 %, а гормони щитоподібної залози були знижені на 90 %. Це дослідження також заглиблює у нову відносно недосліджену теорію фертильності антиоксидантами разом із гормональним фоном щитоподібної залози в умовах окисного стресу, також було досконало визначено нейрогуморальна регуляція та прооксидантно-оксидантний баланс в умовах фертильності, цей баланс забезпечується рядом речовин антиоксидантів, які або вже є в організмі, або отримуються з продуктами харчування (наприклад, вітаміни С і Е)

Ключові слова: вагітність; вільні радикали; яйцеклітина; сперматозоїди; окислювальний стрес