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INFLUENCE OF FATS OF VARIOUS ORIGINS ON THE REPRODUCTIVE FUNCTION OF EXPERIMENTAL ANIMALS

Abstract. In Ukraine, about 50–60 thousand men annually turn to specialists with sexual and andrological problems for help. Today, about 80% of men admit that having problems in the sexual sphere seriously affects the quality of life. At the same time, the leading place is occupied by the issue of the safe impact of chronic consumption of dietary fats on reproductive function.

The aim of the study was to study the effect of long-term consumption of dietary fats of various origins on the spermatogenic function of the testes of male rats.

The studies were carried out on 7 month old male rats of the Wistar population after 75 days of consumption of fats of various origins (palm oil, refined sunflower oil, margarine) at a rate of 3 mg/kg. At the end of the experiment, the state of spermatogenesis in a suspension of epididymal spermatozoa was assessed in males.

In experimental animals, it was observed: a decrease in the total concentration of gametes, a deficiency of morphologically normal cells and the emergence of a significant number of abnormal forms of spermatozoa.

Relative to control males, a decrease in the total concentration of gametes was found in the Palm oil group by 22%, in the Margarine group – by 30.2%, in the Refined sunflower oil group – by 34%. A decrease in the total number of gametes led to a deficit of morphologically normal forms in all experimental animals. In the “Palm oil” group the shortage was 25%, in the “Margarine” group – 24%, in the “Refined sunflower oil” group – 36% relative to the control animals. When determining the percentage of mobile gametes, there was found deficiency only in the “Margarine” group. It was 20% relative to the indicators of control males. But the percentage of abnormal forms of gametes in the group “Margarine” remained at the level of indicators of the control males. And in the groups “Palm oil” and “Refined sunflower oil” an excess of this was found indicator by 67% and 61%, respectively.

The experimental data obtained indicate that the consumption of dietary fats leads to disruption of all the constituent stages of spermatogenesis in adult male rats.

Key words: spermatogenesis, male rats, palm oil, refined sunflower oil, margarine.

INTRODUCTION

Inadequate and unbalanced nutrition causes the greatest number of various diseases, including reproductive [2; 4; 5; 6]. Analysis of recent studies has shown that in Ukraine as a result of malnutrition during 1990–2008, the incidence of endocrine diseases and metabolic disorders has doubled [1], which in turn contribute to hypofertility.

In today's world there are many factors that negatively affect the male reproductive system. Among those that impair reproductive health – stress, alcohol consumption, smoking, chronic diseases, etc. are of great importance [3; 13].

Researchers have proven the important role of nutrition in the functioning of the reproductive system [4; 8; 9]. It is important to change the usual diet, composition and amount of individual ingredients, which can affect the urge and duration of sexual intercourse. It is known that there are foods that can even weaken or, conversely, enhance sexual function (so-called "aphrodisiacs") [9, 10].

An important provoking factor in the development of reproductive disorders is the intake of excess fat from food [3].

It is known that obesity leads to erectile dysfunction [2], which, in turn, can lead to male infertility [7; 12]. More severe consequences for the reproductive potential of men has a state of obesity on the background of type 2 diabetes [10].

Modern cooking products, especially fast food, are increasingly high in fat. Therefore, the consumption of fats and oils has increased significantly, and at the same time, as a rule, their fatty acid composition is not taken into account. However, it should be noted that there is a difference in the physiological function of individual fatty acids [3].

All fatty acids are divided into saturated (in the radical all chemical bonds are single) and unsaturated (when the radical has one or more double bonds). It is known that such unsaturated fatty acids as linoleic, linolenic are not synthesized in the human body and come to him only with food, so they are called essential. However, they play an important role, in particular, are part of biomembranes, and are also able to be converted into biologically active compounds that have the properties of regulators of physiological processes [10]. None of the dietary fats fully satisfies the physiological needs of man and its fatty acid composition is far from the ideal formula for a fatty diet. Thus, sunflower oil, which is most often consumed in Ukraine, contains 60% of linoleic acid from the total amount of fatty acids, but there are no other essential fatty acids. Milk fat has a large amount of palmitic acid (C16: 0) – 26–29%, while the recommended content of this acid should not exceed 15% [2]. Among vegetable oils, a special place is occupied by palm oil, the consumption of which increases every year, although it contains up to 50% palmitic acid.

In turn, it is known that the consumption of excessive amounts of fat causes obesity, which often leads to the development of atherosclerosis, metabolic syndrome and type 2 diabetes, as well as disturbs the balance of sex hormones [1]. Diet with excessive fat intake is the cause of development of dysbacteriosis in rats [3], negative changes in the liver and serum [1].

The authors of other studies conducted on rats aged 1, 3, 6 and 12 months, confirm that from an early age to 12 months of life, the composition of fatty acids in the liver of animals is closely related to the fatty component of the diet [7].

This was most evident when consuming fats containing increased amounts of palmitic acid (palm oil and butter). Also, the analysis of modern scientific literature shows that refined

palm oil and palm oil, which has been subjected to heat treatment, have a negative impact on human health, in particular on its immune and cardiovascular systems [9].

The pathogenic effect of saturated fatty acids, especially palmitic (C16: 0), and excess of linoleic acid in food (C18: 2), which is the basis of the vast majority of vegetable oils (sunflower, corn, soybean) other researchers declare [9].

Linoleic acid is a source of prostaglandins, which are involved in the regulation of blood pressure, the activity of the blood coagulation system, inflammatory reactions, as well as able to interfere with the process of carcinogenesis.

However, an excess of linoleic acid can have a negative impact, as a person's need for linoleic fatty acid is very limited (not more than 6–7 g / day) [2].

Data on the effect of palm oil on the reproductive function of experimental animals are currently ambiguous. Thus, intake of palm oil from fresh, frozen or pasteurized palm juice for two months improves fertility in male Wistar rats. However, its consumption over a long period (more than two months) leads to necrotic changes in the testicles. It should also be noted that the intake of oil from pasteurized palm juice has a negative effect on reproductive performance in males [11].

Analysis of the literature showed that prolonged consumption of vegetable fats in rats for 6 weeks leads to an increase in body weight in the dynamics and the emergence of alimentary obesity 2–3 degrees. It is shown that the consumption of excessive amounts of fat affects the quality of sperm, reducing sperm motility, without affecting other parameters of sperm. In turn, the change in sperm motility causes a slight decrease in fertility potential. These data suggest that a diet high in fat may impair the fertility of male rats [10].

The effect of chronic consumption of edible vegetable oils of various kinds (natural, hydrogenated, refined) and origin (palm or sunflower) on the reproductive function of men, in particular on the state of spermatogenesis, remains unclear. Given the heterogeneity of the human population, as well as the limited ability to study the state of human spermatogenesis in terms of ethical issues, it is more appropriate to investigate these issues in the experiment.

The aim of the study: was to examine the effect of long-term consumption of dietary fats of various origins on the spermatogenic function of the testes of male rats.

MATERIALS AND METHODS

The experiment and keeping of animals were carried out in accordance with the national "General ethical principles of animal experiments", approved by the V National Congress of Bioethics (Kyiv, 2013). The experiments were performed on 28 adult male rats weighing 230 ± 50 g of the Wistar line at the age of 7 months. Before the experiment, males were randomly divided into 4 groups: 1st group – control; 2nd – "Palm oil"; 3rd – "Refined sunflower oil"; 4-a – "Margarine". To simulate the effect of vegetable fats on the body of the animal for 75 days additionally received vegetable fats at the rate of 3 mg / kg of rat weight daily, while the control group received a standard feed compiled in accordance with feed rates for laboratory animals (grain products, granulated feed, fresh vegetables) [3]. The first experimental group received a standard feed with the addition of refined sunflower oil; the second experimental group – with the addition of palm oil; the third experimental group – the addition of margarine. Experimental groups of animals additionally received vegetable fats at the rate of 3 mg / kg of rat weight daily. According to the literature, this dose leads to metabolic changes [11]. The study used refined sunflower oil and margarine from Ukrainian

producers; palm oil of the Delta Wilmar brand. Animals of all groups were kept in standard vivarium conditions with free access to water.

At the end of the experiment in males evaluated the state of spermatogenesis in a suspension of epididymal sperm. To do this, sperm were obtained from the cut tail of the epididymis by washing for two minutes in 2 ml of 0.9% sodium chloride solution. The amount of epididymal sperm in 1 ml of suspension, their motility and the percentage of pathological forms were determined according to the generally accepted method, using Goryaev's camera and light microscope Biolamps [7]. Gamete motility was expressed as the percentage of motile cells (per 200 sperm examined), the percentage of abnormal forms was determined per 200 cells examined, taking into account the shape of the head, neck, middle and tail of mature sperm, and the presence of cytoplasmic droplets around the head and tail of sperm as a sign of their immaturity. The concentration of morphologically normal cells [7]. was calculated. The probability of differences between groups was assessed using the analysis package Excel 2003 and Statistika 6.0 and using parametric and nonparametric methods (Mann-Whitney U-test, Q Dana).

DISCUSSION

Analysis of the functional status of epididymal sperm showed violations of quantitative and qualitative indicators of spermogram in all experimental groups. Thus, a decrease in the total concentration of gametes was found: in the group "Palm oil" by 22%, in the group "Margarine" – by 30.2%, in the group "Refined sunflower oil" – by 34% relative to control males ($P \leq 0,05$; Table 1).

Table 1

Spermogram parameters ($\bar{x} \pm Sx$; $n = 7$)

Indicator	Group			
	Control	Palm oil	Margarine	Refined sunflower oil
Sperm concentration, mln/ml	50,71±3,3	39,57±1,2*	35,43±2,1*	33,43±3,2*
Mobility, %	81,29±2,6	72,00±5,8	65,00±3,7*	84,14±2,9
Pathological forms, %	5,14±1,0	8,57±1,1*	5,00±0,7	8,29±0,9*
Concentration of morphologically normal sperm, mln/ml	48,16±3,3	36,21±1,3*	36,43±4,12*	30,71±3,05*
* – Statistically significant differences relative to the control group ($P < 0,05$).				

Decreased total sperm concentration in experimental animals may be associated with androgen deficiency. This is consistent with the literature on the violation of spermatogenesis in such cases, at least its androgen-dependent stages [5; 6; 7].

In turn, the decrease in the total number of gametes led to a deficit of morphologically normal forms in all experimental animals. In the group "Palm oil" the deficiency was 25%, in the group "Margarine" – 24%, in the group "Refined sunflower oil" – 36% relative to control animals ($P \leq 0.05$; see Table 1). This may be due to testosterone deficiency and, as a consequence, disruption of androgen-dependent stages of spermatogenesis, testicular

cretory function. After all, the leading hormone of spermatogenesis is testosterone. To ensure the continuity of spermatogenesis at the physiological level requires a high intratesticular concentration of androgen. Its content in the body can be several times higher than in the blood of the general circulation [1].

When determining the percentage of mobile gametes, their lack was detected only in the group “Margarine”. It was 20% relative to control males ($P \leq 0.05$; see table. 1). It is known that the motor activity of sperm cells is an energy-dependent and energy-intensive process and largely depends on the concentration of ATP. The main suppliers of ATP in sperm cells are respiration (about 90% of all energy) and glycolysis (about 10% of energy required) [11]. However, the process of β -oxidation of fatty acids can also be a supplier of energy for the synthesis of ATP in sperm cells. After all, it was found that the gametes themselves are able to modulate lipid metabolism [1]. Therefore, the decrease in motor activity of sperm in males of the group “Margarine” may be a consequence of energy deficiency caused by excessive load of margarine fats.

Instead, the percentage of abnormal gamete forms in the Margarine group remained at the level of control males. And in the groups “Palm oil” and “Refined sunflower oil” exceeded this indicator by 67% and 61%, respectively, ($P \leq 0.05$; see Table 1).

It is possible that spermiogenesis may have been affected by a change in the direction of hormonal processes. This caused the appearance of a significant number of abnormal sperm, which reduced the concentration of morphologically normal gametes.

CONCLUSIONS

An unbalanced diet with an excessive content of palm oil, margarine and refined sunflower oil leads to disruption of all components of the spermatogenesis of adult male rats. This is manifested by a deterioration in the parameters of the spermogram: a decrease in the total concentration of gametes, a deficiency of morphologically normal cells and the appearance of a significant number of abnormal forms of sperm.

It is believed that excessive fat diet can lead to metabolic disorders that can affect the processes of spermatogenesis. This is due to abnormalities in gene expression and contributes to the disruption of protein synthesis, usually enzymes that control key stages of metabolism. These disorders may be due to the absence of the product of the enzymatic reaction or the accumulation of a suitable substrate. That is, the increase in the number of abnormal forms of gametes may be due to changes in gene expression under the influence of excessive intake of hydrogenated and refined fats, which is unnatural and not physiological for this species. This caused the appearance of a significant number of abnormal forms and reduced the concentration of morphologically normal gametes. In the future, this can lead to reduced fertility and the development of infertility.

Prospects for further research: given the ever-increasing consumption of oils in the world and the integration of Ukraine into the world economy, there is a need for scientific and experimental studies of the impact of oils on the reproductive state of the human body during their use.

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АНОТАЦІЯ

ВПЛИВ ЖИРІВ РІЗНОГО ПОХОДЖЕННЯ НА РЕПРОДУКТИВНУ ФУНКЦІЮ ЕКСПЕРИМЕНТАЛЬНИХ ТВАРИН

Щорічно в Україні близько 50–60 тисяч чоловіків звертаються до спеціалістів із сексуальними та андрологічними проблемами. Сьогодні близько 80% чоловіків визнають, що проблеми в сексуальній сфері серйозно впливають на якість життя. При цьому провідне місце посідає питання про безпечний вплив хронічного вживання харчових жирів на репродуктивну функцію.

Мета дослідження – вивчити вплив тривалого споживання харчових жирів різного походження на сперматогенну функцію сім'яників самців щурів.

Дослідження проводили на 7-місячних щурах-самцях популяції Wistar після 75-денного споживання жирів різного походження (пальмова олія, рафінована соняшникова олія, маргарин) з розрахунку 3 мг/кг. Наприкінці експерименту у самців оцінювали стан сперматогенезу в суспензії сперматозоїдів епідидиму.

У піддослідних тварин спостерігалось: зниження загальної концентрації гамет, дефіцит морфологічно нормальних клітин та поява значної кількості аномальних форм сперматозоїдів.

У контрольних самців виявлено зниження загальної концентрації гамет у групі пальмової олії на 22%, у групі маргарину – на 30,2%, у групі соняшnikової рафінованої олії – на 34%. Зменшення загальної кількості гамет призвело до дефіциту морфологічно нормальних форм у всіх піддослідних тварин. У групі «олія пальмова» нестача становила 25%, у групі «маргарин» – 24%, у групі «олія соняшnikова рафінована» – 36% відносно контрольних тварин. При визначенні відсотка рухомих гамет дефіцит виявлено лише в групі «маргарин». Він становив 20% відносно показників контрольних самців. Але відсоток аномальних форм гамет у групі «маргарин» залишився на рівні показників контрольних самців. А в групах «олія пальмова» та «олія соняшnikова рафінована» виявлено перевищення цього показника на 67% та 61% відповідно.

Отримані експериментальні дані свідчать про те, що споживання харчових жирів призводить до порушення всіх етапів сперматогенезу у дорослих самців щурів.

Ключові слова: сперматогенез, самці щурів, пальмова олія, рафінована соняшnikова олія, маргарин.