

COMPARATIVE CHARACTERISTICS OF THE UTERUS OF FEMALE WHITE OUTBREED RATS BEFORE AND AFTER LONG-TERM TAKEN OF ENERGY DRINK

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Abstract:

This work is devoted to the study of the effect of energy drinks on the histochemical structure of the uterus using the example of female albino rats. The research methods chosen were staining with Schiff's reagent and a positive Schiff (PAS) reaction of the structure of the uterus after long-term use of an energy drink.

As a result of the study, it was revealed that there is a proliferation and an increase in the amount of type III collagen. (Reticular fibers) in the uterine tissue of white outbred rats.

Keywords: Schiff (PAS) reaction, Schiff's reagent, histochemical study, uterus, white mixed breed rats, type III collagen.

Introduction

Relevance

Energy drinks are a special type of carbonated drinks, which are positioned by their manufacturers as such, which increase physical activity and improve performance when consumed. Energy drinks first appeared on the market in the 80s of the XX century. Drinks of this class became particularly popular in the 2000s of the XXI century, as evidenced by increased sales volumes [Malinauskas B.M and co-authors 2007].

Energy drinks consist of components that have long been known to medicine. So, without exception, all "energy drinks" contain caffeine. In industry, it is obtained in three ways: by isolation from roasted coffee beans, which contain 0.75 - 1.5% caffeine; extraction from tea dust, i.e. ground tea leaves containing 1.5 - 3.5% caffeine; extraction from cola nuts, containing about 2% caffeine. In addition, it can be obtained chemically from uric acid or by methylation of theobromine. It is synthetic, cheaper caffeine that manufacturers include in the composition of energy drinks. Another component of energy cocktails is taurine. Some manufacturers add extract of mate leaves, damiana, lemongrass of the Far East, ginseng.

There are vitamins in "energetics" that are directly related to the energy metabolism of the body: ascorbic acid, B1, B2, VZ, B5, B6, B12, niacin. Moreover, some vitamins are introduced in the amount of the required daily dose of an adult, which is not bad at all. In general, the purpose of these drinks is to increase the very energy metabolism and vitality of the body.

Addiction to energy drinks lies in the properties of their main component - caffeine:

- High doses of caffeine can lead to depletion of nerve cells.
- The effect of caffeine (as well as other psychostimulants) largely depends on the type of higher nervous activity.
- Caffeine weakens the effect of sleeping pills and narcotic drugs, increases the reflex excitability of the spinal cord, stimulates the respiratory and vasomotor centers.
- Cardiac activity under the influence of caffeine increases, myocardial contractions become more intense and become more frequent.

In the amount contained in 2-3 cans of energotonic, drunk within a short time, caffeine causes anxiety, insomnia, irritability and headaches. However, although this has not yet been fully proven, high-dose caffeine consumption over time can cause coronary heart disease, high blood pressure and some birth defects in offspring. [Tkachenko A.V., Makovkina D.V. 2017].

Acute caffeine poisoning gives early symptoms of anorexia, tremor and confusion. Severe intoxication can cause delirium, seizures, supraventricular and ventricular tacharrhythmias, hypokalemia and hyperglycemia. Chronic intake of high doses of caffeine can lead to nervousness, irritability, anger, constant tremors, muscle twitching, insomnia and hyperreflexia. Taurine is a synthetic analogue of caffeine, which is cheaper and is added to energy drinks in huge quantities:

Like caffeine, taurine helps to improve energy processes, but the safety of taurine for patients under 18 years of age has not been established, that is, the likelihood of adverse reactions and its effect on metabolism are unpredictable. Most expert opinions of medical specialists unequivocally state the harmful effects of such drinks on human health. However, the only thing that specialists and society have managed to achieve today is the mandatory application of warning labels on banks. A number of studies also unequivocally indicate the connection between the use of energy drinks and weak alcoholic beverages with future addiction to strong alcoholic beverages and so-called "heavy" drugs.

Numerous facts of exacerbation of psychiatric diseases in people who have abused energy drinks have been documented [Cerimele J.M and co. 2010]. In some cases, their use by patients suffering from epilepsy provoked the development of seizures [Yamada-Takeda M et al. 2019], including, in one case, against the background of their two-year absence. Machado-Vieira R. et al. a case of the development of a manic episode in a 36-year-old patient suffering from bipolar disorder was described after he consumed three cans of Red Bull overnight [Machado-Vieira R et al. 2001]. Cerimele J.M. et al. the fact

of the development of acute psychosis in a patient with schizophrenia was stated [Cerimele J.M et al. 2010].

The danger of consuming excessive amounts of energy drinks by healthy people in the context of psychiatric pathology is confirmed by the following facts. Iyadurai S.J., Chung S.S. documented an episode with the appearance of seizures without a history of epilepsy [Iyadurai S.J. et al. 2007]. And Goruglu Y. et al. A case of acute psychosis in a young man who had no previous psychiatric history was described [Goruglu Y et al. 2014].

Thus, Vivekanandarajah A. et al. [Vivekanandarajah A et al. 2011] described a case of acute hepatitis in a 22-year-old girl who consumed about 10 cans of drink a day daily for two weeks (brand not specified). In the same year, Apestegui C.A. et al. [Apestegui C.A et al. 2011] described a case of cholestatic hepatitis in a patient with a transplanted liver who drank 15 cans of Red Bull within three days. In both cases, the authors of the cited publications linked the hepatotoxicity of beverages with a high content of vitamin B3 in them.

The purpose of the study

To determine the effect of prolonged consumption of energy drinks on the histochemical structure of the uterus in female white mongrel rats using the methods of staining with Schiff reagent and positive CHIC reaction.

Materials and methods of research

For the research, white unborn female rats aged 5-6 months weighing 130-140 g were selected. 5-6-month-old white mongrel rats were kept in standard vivarium conditions with relative humidity (50-60%), temperature (19-22 ° C) and light regime (12 hours of darkness and 12 hours of light).

In order to prevent infectious diseases in the vivarium and ensure their absence of infectious diseases, laboratory animals were quarantined for 21 days and observed during these days, their temperature was measured and their weight was checked several times during these days. The increase was monitored. During this period, they did not have any symptoms of the disease, their temperature was within the normal range (38.5-39.5 ° C), appetite disorders and other external changes were not detected. On the 10th day, the animals were removed from the experiment under ether anesthesia in compliance with the rules of euthanasia and autopsy material was collected for subsequent histological examination (small intestine). The autopsy material was labeled, fixed in 10% buffered formalin and subjected to histological examination using generally accepted histological techniques. To study the morphological parameters of organs of laboratory animals, research methods widely used in experimental studies (anatomical dissection) were used. All histological preparations were scanned using a trinocular microscope HL-19 (China) with software. The slices were stained with Schiff reagent.

The result and discussions

The rat uterus in its structure has paired horns, a double body and a neck. The uterine horns fuse in the caudal direction, forming the body and neck, but their cavities remain separated from each other by a septum and open into the vagina with two independent openings. It should be noted that in the basic textbooks on veterinary medicine and monographs on developmental biology in rats, according to the basic classification of queens, the latter is described as two-part, which means the fusion of bodies with the formation of a single cervical opening. Upon a comprehensive study of this issue, it becomes clear that the rat uterus should be classified as a double one. The body of the uterus is an undivided area between the horns and the cervix. It is located in the abdominal cavity dorsal to the bladder and ventral to the rectum. The cervix is a small thick-walled area between the body of the uterus and the vagina. It distinguishes the vaginal and supravaginal parts.

Histological examination revealed that the wall of the horns, body and cervix consists of three membranes: endometrium (mucosa), myometrium (muscle) and perimetry (serous) (Fig. 1).

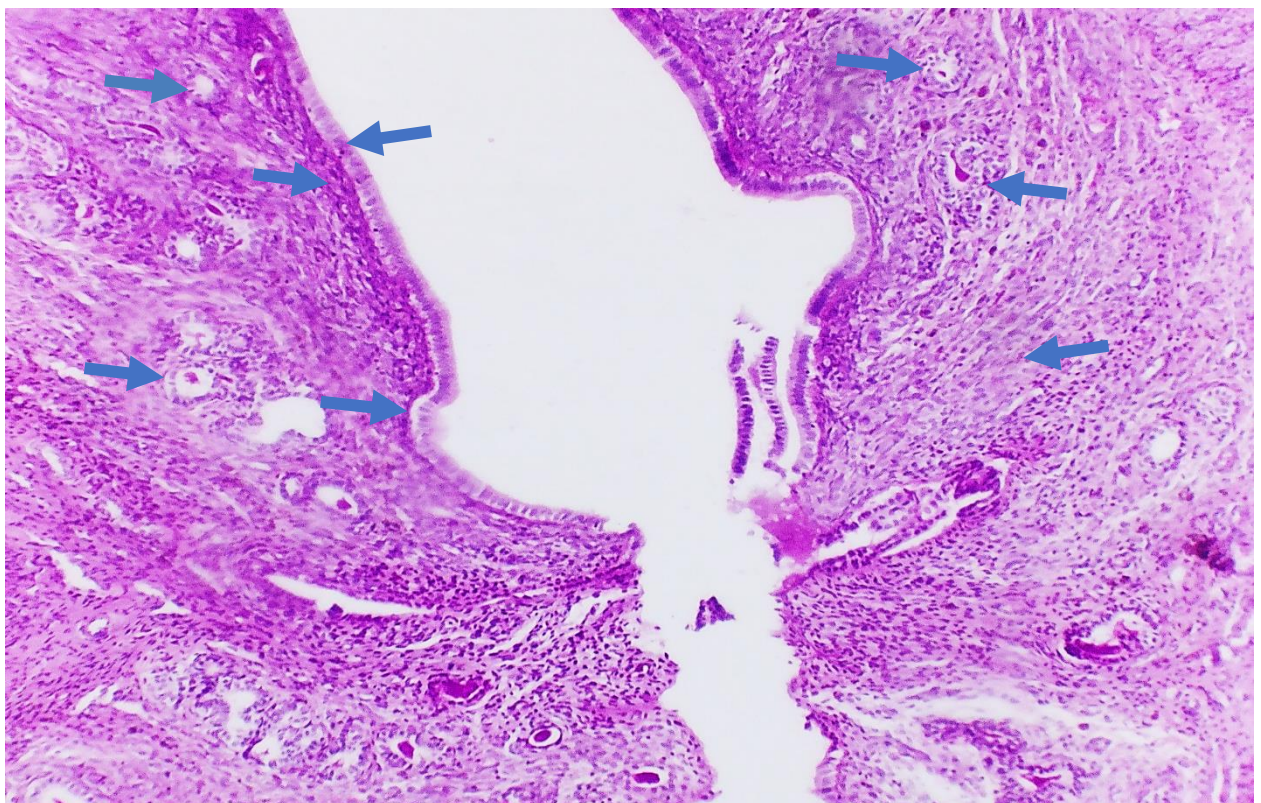


Fig. 1 Morphological structure of the uterus of a white mongrel rat before prolonged consumption of energy drinks (norm) Staining with Schiff reagent and hematoxylin. The magnification is 10x20. 1- endometrium; 2- own plate of the mucous membrane (stroma of the endometrium); 3- uterine glands; 4- muscle layer.

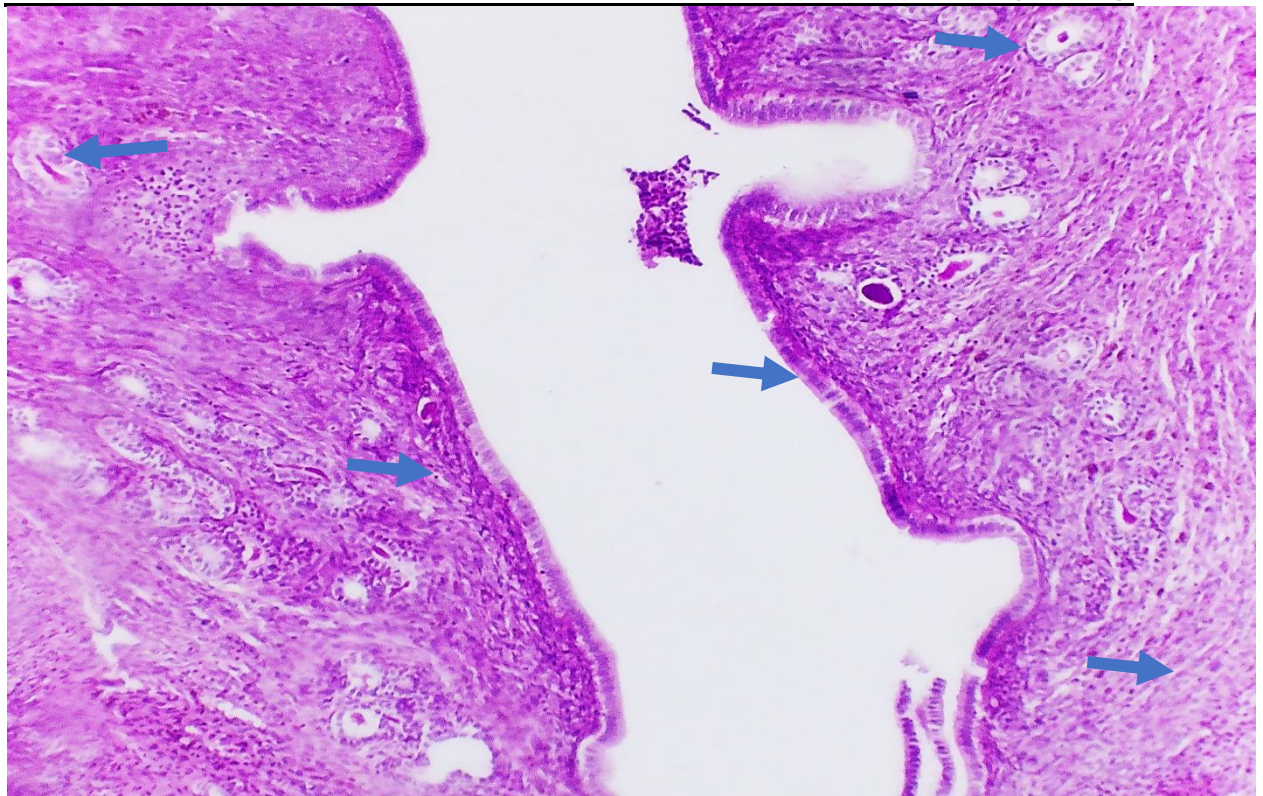


Figure 2 Morphological structure of the uterus of a white mongrel rat before prolonged consumption of energy drinks (norm) Staining with Schiff reagent and hematoxylin. The magnification is 10x20. 1- endometrium (epithelial plate); 2- own plate of the mucous membrane (stroma of the endometrium); 3- uterine glands; 4- muscle layer.

The mucous membrane forms a relief in the form of folds, which becomes even more pronounced in the neck. The endometrium is formed by two layers (plates) of the epithelial and its own. The epithelial plate is represented by a single-layered prismatic epithelium, with multi-row sections. The composition of the epithelium determines ciliated and glandular, as well as basal cells. The uterine epithelium is involved in the formation of glands (Fig. 2), which have morphological differences in the body and neck. So, in the body of the uterus, the mouths of the glands are wide and deep, little branched; in the cervix they are shorter, and the terminal sections are branched.

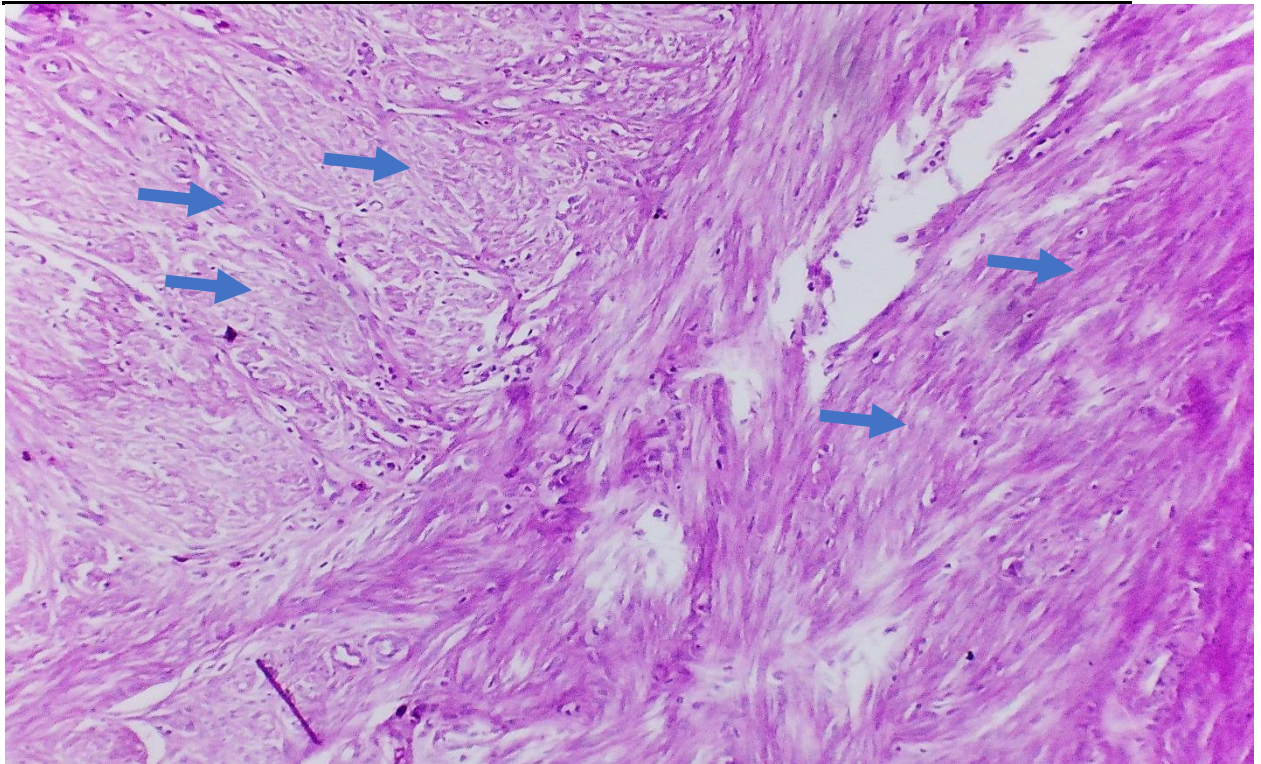


Fig. 3 Morphological structure of the uterus (myometrium) of a white mongrel rat before prolonged consumption of energy drinks (norm) Staining with Schiff reagent and hematoxylin. Magnification 10x20. 1- smooth muscles; 2- circular muscles.

The uterine myometrium of white mongrel rats, as well as in other mammals, is formed by bundles of smooth muscle tissue separated from each other by layers of loose fibrous tissue. Using an immunohistochemical study of the uterus, it was found that in the horns and body the myometrium consists of three layers: the inner (submucosal), formed by circularly oriented myocytes; the middle (vascular) with a small number of obliquely oriented smooth myocytes; the outer (supravascular) with cells of the mycoprodolar direction (Fig. 3). Many scientists have described only two layers in myometrics [Glagolev, P. A. 1977].

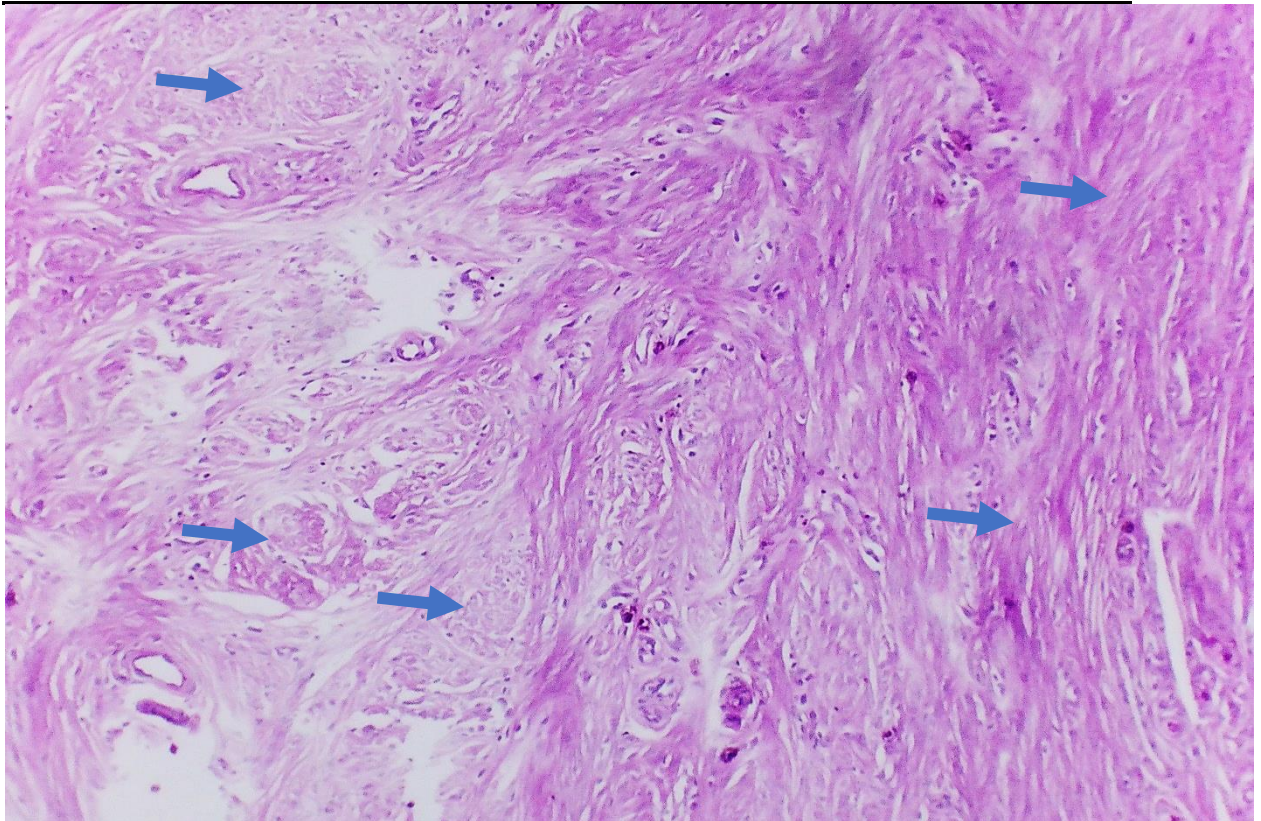


Fig. 4 Morphological structure of the uterus (myometrium) of a white mongrel rat before prolonged consumption of energy drinks (norm) Staining with Schiff reagent and hematoxylin. Magnification 10x20. 1- smooth muscles; 2- circular muscles.

In the caudal direction, the medial walls of the right and left uterine horns merge. The fusion site is characterized by the union of the outer shell of the perimetrium and part of the myometrium, namely its supravascular and vascular layers. Thus, a median septum is formed in the lower segment, separating two cavities of the indistinctly expressed body and cervix. The median septum in its structure is formed by the endometrium and myometrium. In this case, the myometrium in its structure has submucosal layers and a single vascular layer. The vascular layer is more pronounced in the body.

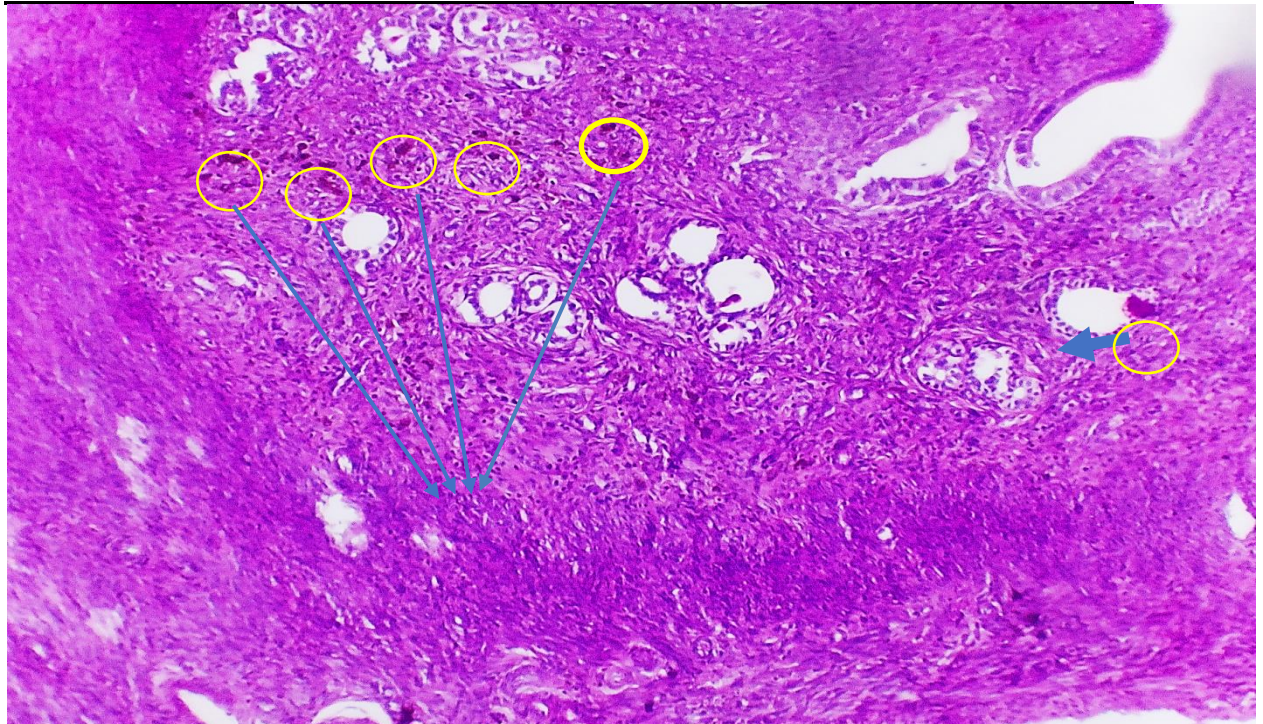


Figure 5 Morphological structure of the uterus of a white mongrel rat after prolonged consumption of energy drinks. Staining with Schiff reagent and hematoxylin. A positive CHIC reaction to the uterine tissue is an increase of 10x20. 1- proliferation and increase in the amount of type III collagen. (Reticular fibers).

In (Fig. 5), granules of mucopolysaccharides (glycosaminoglycans) that are part of collagen are outlined in yellow and give a positive reaction to CHIC.

CHIC or PAS reaction is a qualitative reaction to aldehydes.

CHIC is a non—specific reaction. Interaction with many substances is difficult due to their unique structure.

Kullingh provides a list of substances that are stained by the PAS method:

- Accumulations of actinomycetes.
- Basal membranes.
- Cartilage.
- * Cerebrosides.
- Collagen of the areolar connective tissue.
- Compound lipids.
- Glycogen.
- Kerazine (Gaucher's disease).
- Renal tubules.
- Granules of megakaryocytes.
- Mucins:
 - intestinal tract,
 - pepsin glands,
 - cervical glands,
 - salivary glands,

- conjunctiva,
- bronchial glands,
- follicles and ovarian cysts,
- secret of prostatic glands,
- amyloid corpuscles.
- Capsule of the lens of the eye.
- Pancreatic zymogenic granules.
- Granules of Paneta cells (rats, Guinea pigs, rabbits).
- Phospholipids.
- Beta cells of the pituitary gland.
- Renal hyaline casts.
- Retinal rods.
- * Taurus Roussel.
- Starch.
- Colloid of the thyroid gland.

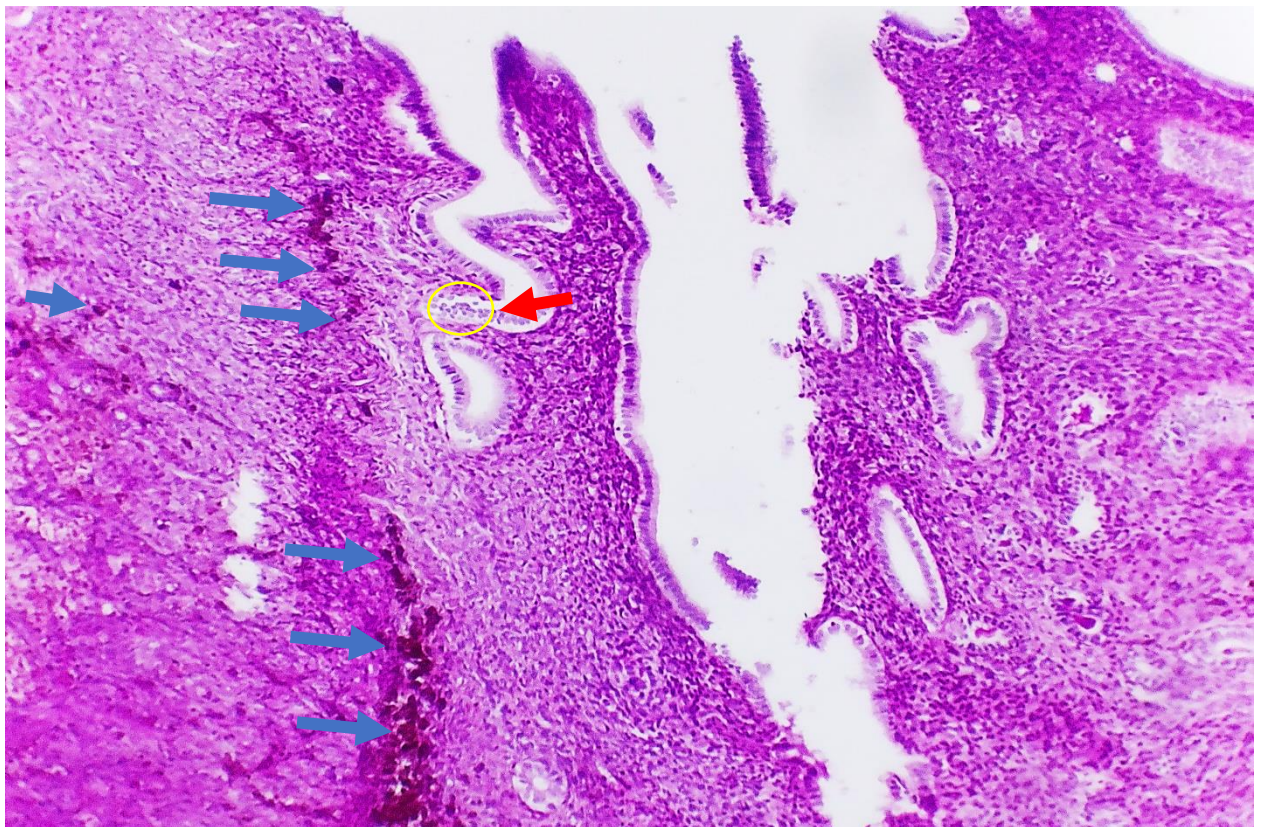


Figure 6 Morphological structure of the uterus of a white mongrel rat after prolonged consumption of energy drinks. Staining with Schiff reagent and hematoxylin. A positive CHIC reaction to the uterine tissue is an increase of 10x20. the proliferation and increase in the amount of type III collagen. (Reticular fibers); metaplasia of the epithelium of the endothelial layer.

Glycosaminoglycans (which are part of collagen) mucopolysaccharides (from Latin mucus – mucus) are the carbohydrate part of proteoglycans, polysaccharides, which

include amino sugars-hexosamines. In the body, glycosaminoglycans are covalently bound to the protein part of proteoglycans and do not occur in free form.

The middle membrane of the cervix is formed mainly by dense unformed fibrous connective tissue, where the leading functional tissue element is the intercellular substance with its fibrous part. Immunohistochemical typing of collagens of types I, II, III, IV, and VIII showed that the maximum positive expression is determined for types I, III, and IV regardless of childbearing age [Yu. In Grigoriev 2018]

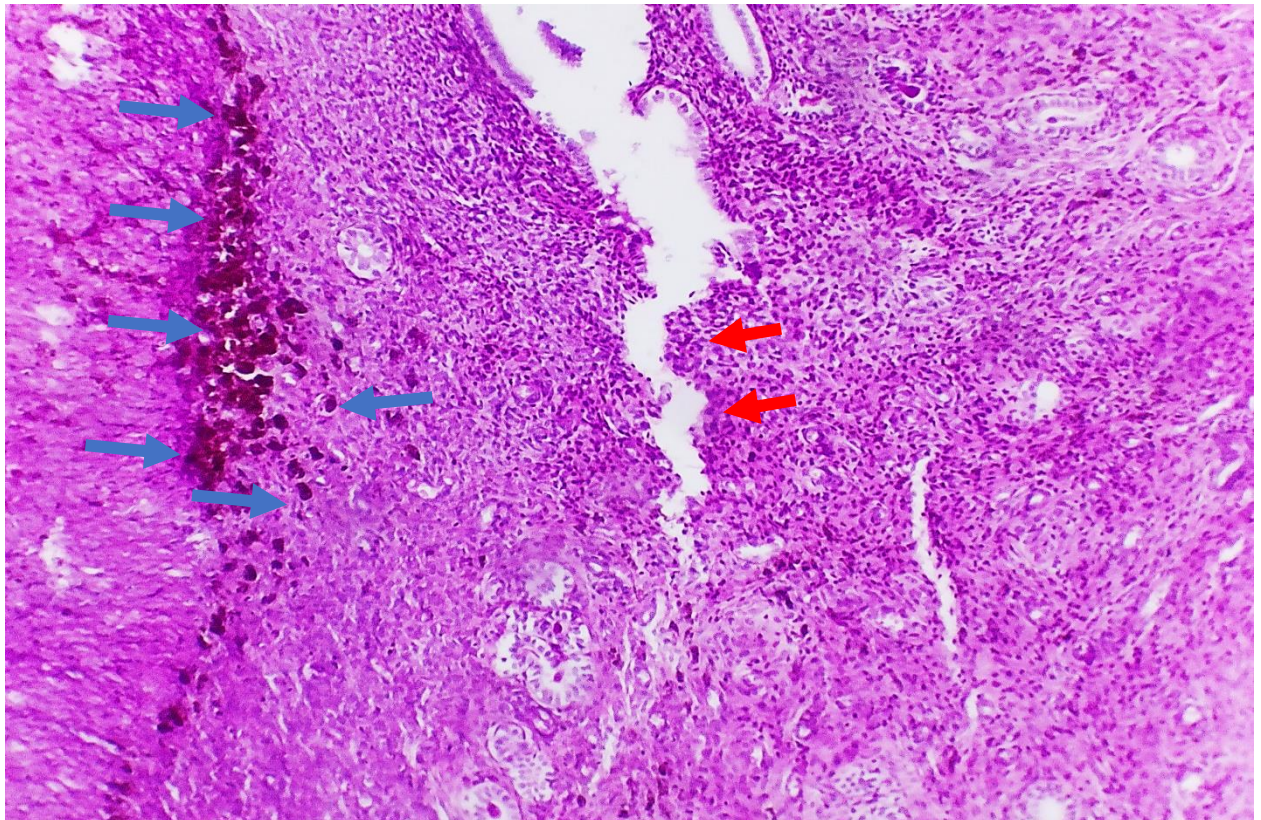


Figure 7 Morphological structure of the uterus of a white mongrel rat after prolonged consumption of energy drinks. Staining with Schiff reagent and hematoxylin. A positive CHIC reaction to the uterine tissue is an increase of 10x20. the proliferation and increase in the amount of type III collagen. (Reticular fibers). metaplasia of the epithelium of the endothelial layer.

(Fig. 7) After prolonged consumption of energy drinks, a significant change in the morphological structure of the uterus is observed in a white mongrel rat. Upon staining with Schiff reagent and hematoxylin and subsequent image magnification, a positive CHIC reaction to uterine tissue was detected. This indicates the proliferation and increase in the amount of type III collagen, which is represented by reticular fibers. Type III collagen is a key element in the structure of connective tissue and its increase may indicate the processes of fibrosis and tissue remodeling. In addition, metaplasia of the epithelium of the endothelial layer of the uterus was also detected. Metaplasia is the process by which one type of cell transforms into another type of cell. In this case, the

epithelium of the endothelial layer of the uterus has undergone changes, possibly as an adaptive response to prolonged exposure to energy drinks. These changes in the morphological structure of the uterus indicate potential pathological processes that may be associated with prolonged consumption of energy drinks and require further study and attention.

Bundles of fibers expressing types I and III collagens in the cervix have a multidirectional course. These types of collagens belong to the class of fibrillous cells that are involved in the formation of stroma of parenchymal organs experiencing constant or periodic mechanical stress [Bode, Michaela 2000]. It is during pregnancy that the cervix realizes its musculoskeletal function.

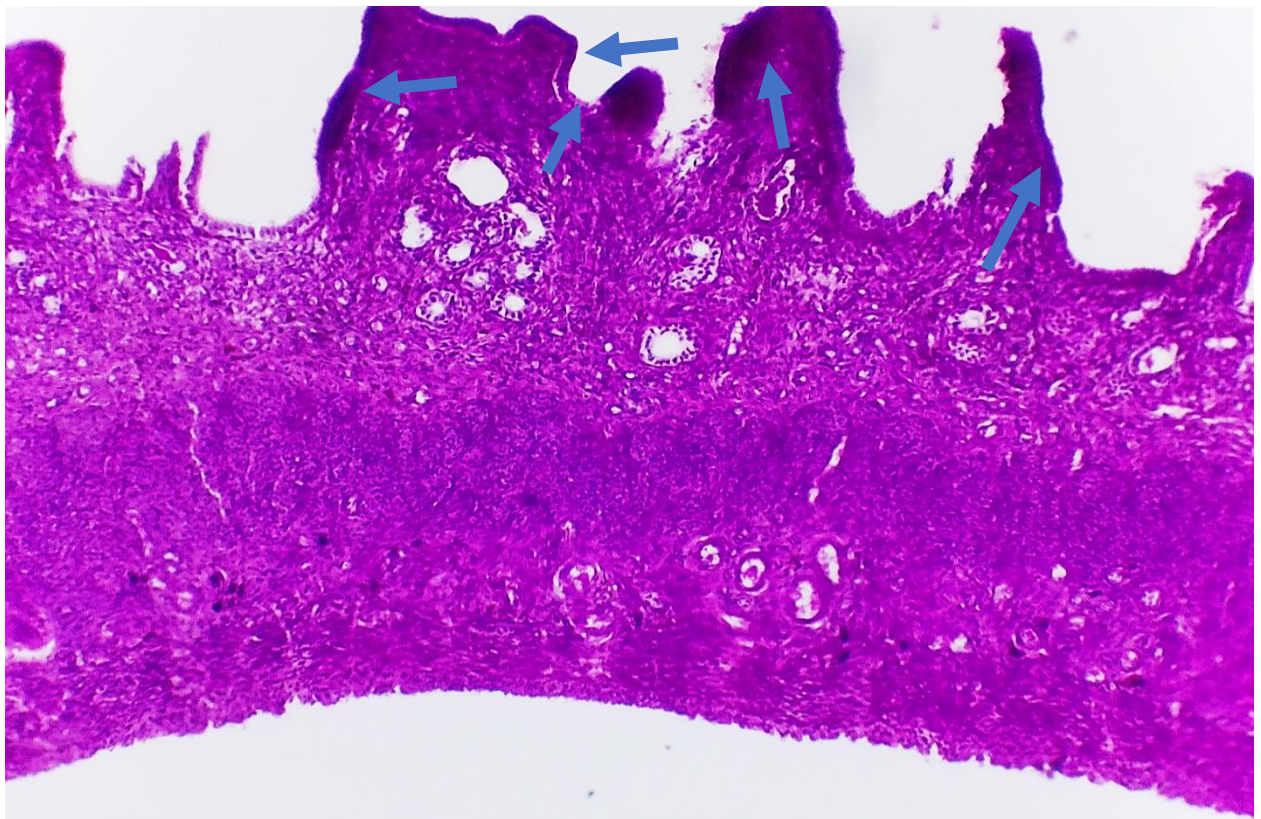


Figure 8 Morphological structure of the uterus of a white mongrel rat after prolonged consumption of energy drinks. Staining with Schiff reagent and hematoxylin. A positive CHIC reaction to the uterine tissue is an increase of 10x20. the proliferation and increase in the amount of type III collagen. (Reticular fibers).

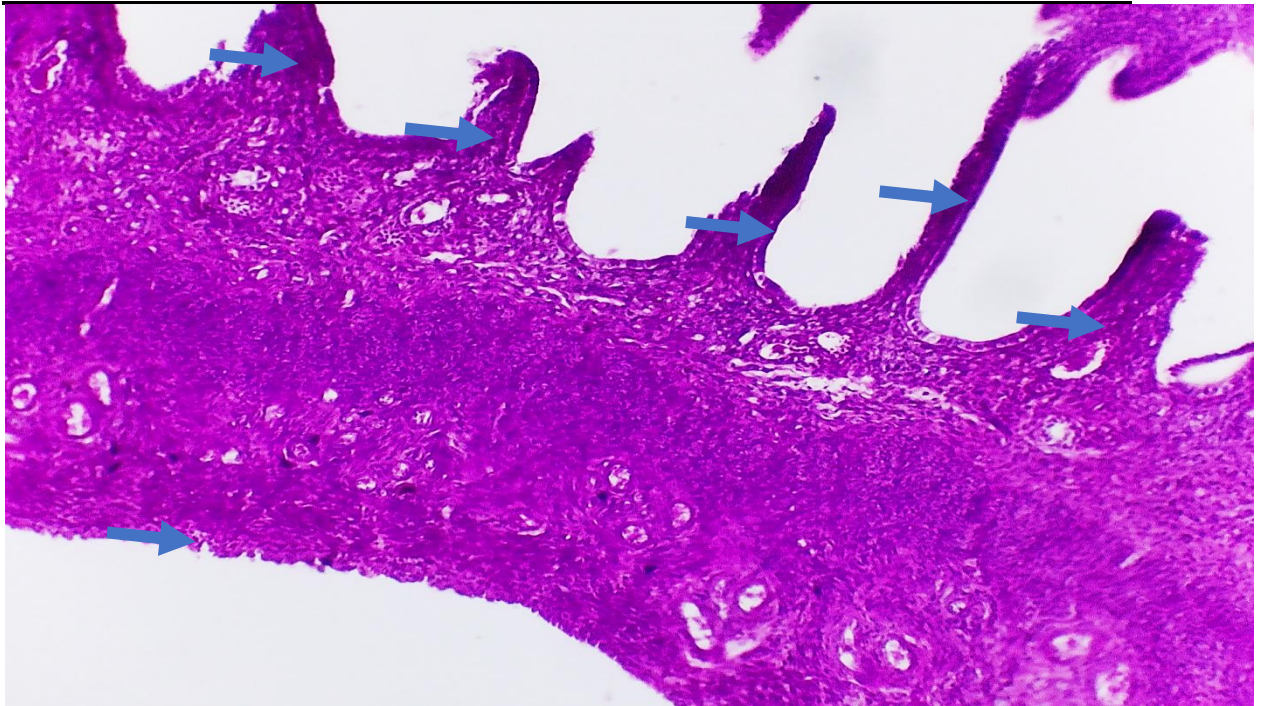


Figure 9 Morphological structure of the uterus of a white mongrel rat after prolonged consumption of energy drinks. Staining with Schiff reagent and hematoxylin. A positive CHIC reaction to the uterine tissue is an increase of 10x20. the proliferation and increase in the amount of type III collagen.

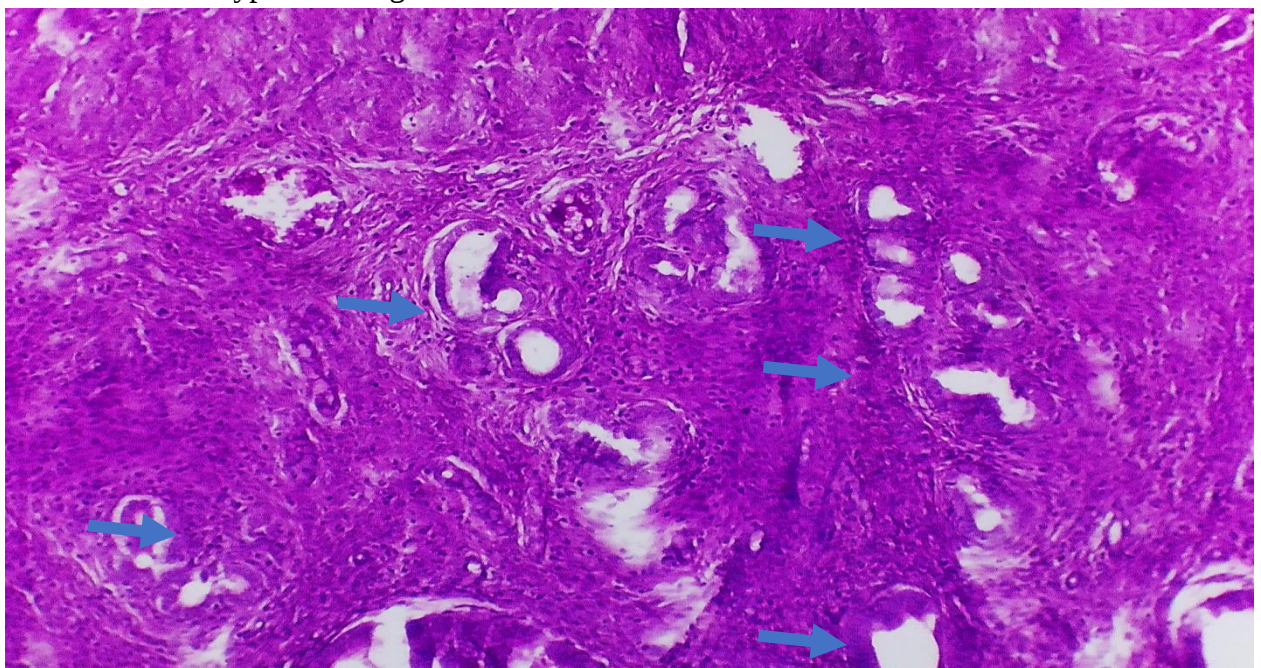


Figure 11 Morphological structure of the uterus of a white mongrel rat after prolonged consumption of energy drinks. Staining with Schiff reagent and hematoxylin. A positive CHIC reaction to the uterine tissue is an increase of 10x20. the proliferation and increase in the amount of type III collagen.

The result and discussions

The results and discussions of this study show that prolonged consumption of energy drinks in a white mongrel rat causes significant changes in the morphological structure of the uterus. The detected proliferation and increase in the amount of type III collagen, represented by reticular fibers, indicates the processes of fibrosis and tissue remodeling. This may be due to potential pathological changes in the uterus.

In addition, the detected metaplasia of the epithelium of the endothelial layer of the uterus indicates the processes in which one type of cell turns into another type of cell. In this case, it could be an adaptive response to long-term exposure to energy drinks.

The general conclusion is that prolonged consumption of energy drinks can cause pathological changes in uterine morphology, such as fibrosis and metaplasia. These results highlight the need for further study and attention to the side effects of energy drink consumption on the female reproductive system.

References:

1. Андриенко, В. С. Власюк О.В Спецвыпуск Опубликовано в Молодой учёный №27 (317) июль 2020 г.
2. Мотыгина А.С Влияние энергетических напитков на биологический материал IX Международная научная конференция г.Сочи, 7-10 ноября 2021г.
3. Григорьева, Ю. В. Особенности строения мио-метрия нижнего сегмента матки лабораторных крыс / Ю. В. Григорьева, Н. В. Ямщиков, А. В. Бормотов, К. Ф. Гарифуллина // Фундаментальные исследования. - 2012. - № 12-1. - С. 48-51.
4. Григорьева, Ю. В. Морфологическая характеристика миоцитов миометрия матки крыс при беременности и родах / Ю. В. Григорьева, Н. В. Ямщиков, Н. А. Ренц, А. В. Бормотов // Фундаментальные исследования. - 2013. - № 12-2. - С. 195-199.
5. Кладовщиков, В. Ф. Стимулировать развитие нутриеводства и кролиководства / В. Ф. Кладовщиков, В. Н. Александров // Кролиководство и нутрие-водство. - 2002. - № 3. - С. 19-20.
6. Малакшинова, Л. М. Гистологическая и гистохимическая характеристика матки крольчих / Л. М. Малакшинова // Состояние и перспективы развития агропромышленного комплекса Забайкалья: Материалы научно-практической конференции (4-6 февраля 2003 г.). - Бурятская ГСХА им. В. Р. Филиппова. - Улан-Удэ, 2003. - С. 82-86.
7. Ноздрачев, А. Д. Анатомия крысы (лабораторные животные) / А. Д. Ноздрачев, Е. Л. Поляков ; под ред. проф. А. Д. Ноздрачева. - СПб. : Лань, 2001. - 464 с.
8. Савинов, П. А. Разработка экспериментальной модели эндометриоза, адаптированной к современным хирургическим технологиям / П. А. Савинов, Д. А. Ниаури, Н. В. Ковшова // Вестник Санкт-Петербургского университета, 2006. - Сер. 11. - Вып. 3. - С. 114-119.

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9. Томитова, Е. А. Гистоструктура, гистохимические и морфологические показатели слизистой матки, шейки матки и влагалища крольчих в норме и под воздействием экзогенных половых гормонов / Е. А. Томитова // Актуальные аспекты экологической, сравнительно-видовой, возрастной и экспериментальной морфологии: Материалы международной научно-практической конференции, посвященной 100-летию профессора Вениамина Яковлевича Суетина (24-27 июня 2004 г.) / ФГОУ ВПО «Бурятская ГСХА им. В. Р. Филиппова», фак. вет. медицины. - Улан-Удэ, 2004. - С. 186-191.
10. Хрусталева, И. В. Анатомия домашних животных : учебник / И. В. Хрусталева, Н. В. Михайлов, Я. И. Шнейберг и др. - изд. 3-е, испр. - М. : Колос, 2006. -704 с.
11. Бехтерева И.А., Доросевич А.Е. Гистофизиология эпителиального и соединительнотканного компонентов влагалищной части шейки матки // Морфология. - 2009. - Т. 136. - № 5. - С. 90-96.
12. Данилова Н.В., Андреева Ю.Ю., Мальков П.Г. Дифференциальная диагностика предопухолевых и регенераторных изменений эпителия шейки матки с использованием иммуногистохимического метода // Архив патологии. - 2011. - Т. 73. - № 2. - С. 10-14.
13. Селякова М.С., Агеева Т.А. Воспалительная реакция на предопухолевые изменения в многослойном плоском эпителии шейки матки у женщин разных возрастных групп / Фундаментальные аспекты компенсаторно-приспособительных процессов молекулярно-клеточные и медико-экологические проблемы компенсации и приспособления // ФГБНУ «Научно-исследовательский институт экспериментальной и клинической медицины». Ответственный редактор: академик РАН, доктор медицинских наук, профессор В.А. Шкурупий, 2015. - С. 254-256.
14. Bode, Michaela, Characterization of type I and type III collagens in human tissues. Department of Clinical Chemistry, University of Oulu, FIN-90014 University of Oulu, Finland, 2000. P. 76. URL: <http://herkules.oulu.fi/issn03553221/>.
15. Duner P, Gongalves I, Grufman H, Edsfeldt A, To F, Nitulescu M, Nilsson J, Bengtsson E. Increased aldehyde-modification of collagen type IV in symptomatic plaques-a possible cause of endothelial dysfunction. Atherosclerosis. 2015 May;240(1):26-32. doi:10.1016/j.atherosclerosis.2015.02.043. Epub 2015 Feb 24. PubMed PMID: 25746374.
16. Koc N, Arinkan SA, Ozbay NO, Selcuk S. Colloid Carcinoma of the Uterine Cervix and Its Immunohistochemical Analysis: A Case Report. J PatholTransl Med. 2018 Jan;52(1):56-60. doi: 10.4132/jptm.2017.04.08. Epub 2018 Jan 15. PubMed PMID: 29370509; PubMed Central PMCID: PMC5784222.
17. Liu X, Wu H, Byrne M, Krane S, Jaenisch R. Type III collagen is crucial for collagen I fibrillogenesis and for normal cardiovascular development. Proceedings of the National Academy of Sciences of the United States of America. 1997;94(5):1852-1856.

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18. Pepin M.G., Schwarze U., Rice K.M. et al. Survival is affected by mutation type and molecular mechanism in vascular Ehlers-Danlos syndrome (EDS type IV). *Genet. Med.* 2014; 16: 881-888. DOI:10.1038/gim.2014.72 PMID:24922459.
19. Саноев Б. А., Ниёзова Т. Ш., ПРОЯВЛЕНИЯ Н. ЛЕЙОМИОМ МАТКИ//Новый день в медицине //Номер. – 2020. – Т. 2. – С. 526-528.
- Abdurasulovich S. B. et al. HEART DISEASES IN FORENSIC MEDICAL PRACTICE: SUDDEN CARDIAC DEATH //World Bulletin of Public Health. – 2022. – Т. 8. – С. 76-79.
20. Саноев Б. А. Морфологические И Морфометрические Характеристики Плаценты При Нормальной Беременности.« //DEVELOPMENT OF A MODERN EDUCATION SYSTEM AND CREATIVE IDEAS FOR IT, REPUBLICAN SCIENTIFIC-PRACTICAL ONLINE CONFERENCE ON"" SUGGESTIONS AND SOLUTIONS. – Т. 6. – С. 94-96.
21. Ishankulova D.X., Ilyasov A.S., Ixtiyarova G.A. Vozdeystviye energeticheskix napitkov na reproduktivnuyu sistemu cheloveka i jivotnix // Tibbiyotda yangi kun.- 2023.5(55) 341-344 s.
22. Ishankulova D.KH., Ilyasov A.S., Ikhtiyarova G.A., Ishankulova Sh.A. ANALYSIS OF CONSUMPTION OF ENERGY DRINKS AMONG TEENS IN BUKHARA REGION // International Journal of Medical Sciences And Clinical Research (ISSN – 2771-2265) VOLUME 04 ISSUE 01, 2024. PAGES: 19-24
23. Ishankulova D.KH., Ilyasov A.S., Ikhtiyarova G.A. MORPHOLOGICAL CHARACTERISTICS OF THE UTERUS OF WHITE OUTBRED RATS // American Journal Of Biomedical Science & Pharmaceutical Innovation (ISSN – 2771-2753) VOLUME 04 ISSUE 01, 2024. PAGES: 22-27.