# I. REPORTS

## IMMUNOHISTOCHEMICAL STUDY IN PATIENTS WITH TRIPLE NEGATIVE BREAST CANCER

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### ABSTRACT

**Both immunohistochemical and imaging methods and non-invasive biomarkers (microribonucleic acids, etc.) are used for the early diagnosis of breast cancer. A total of 128 women with breast cancer with an average age of 59.48±11.99 years (between 30 and 84 years), operated on between December 1, 2017 and November 30, 2020, were examined in Dr. Marko Markov Varna Oncology. The expression of estrogen and progesterone receptors in breast biopsies and operative materials was analyzed by indirect immunoperoxidase method with EnVision™ FLEX MiniKit, that of human epidermal growth factor receptor-2—with HercepTest™, and that of the proliferation index Ki-67—with the Leica Aperio Scan Scope AT2. Triple negative breast cancer was diagnosed in 15 patients (in 11.72% of cases). The average age of the patients was 56.27±12.83 years (between 32 and 78 years). Eight patients were in the age groups between 41 and 60 years, and six patients were in the age groups between 61 and 80 years. Patients with ductal invasive carcinoma predominated (seven or 46.67%), followed by those with ductal carcinoma (four or 26.67%), carcinoma not otherwise specified (three or 20.00%), and non-specific invasive carcinoma (one patient or 6.67% of cases). Seven patients each had degrees of differentiation of G2 and G3. For G2, it concerned three patients aged between 42 and 50 years, two patients—aged between 71 and 80 years and one patient each aged between 51 and 60 years and between 61 and 70 years, and for G3—two patients aged between 51 and 60 years and between 61 and 70 years, and one patient from each of the following the age groups: between 31 and 40 years, between 41 and 50 years, and between 71 and 80 years. The obtained results were of benefit in the choice of treatment for these patients.**

**Keywords:** *immunohistochemistry, triple negative cancer*

# INVESTIGATION AND SCREENING METHODS FOR EARLY DIAGNOSIS OF PATIENTS WITH GASTROINTESTINAL DISORDERS DUE TO MUSHROOM CONSUMPTION

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## ABSTRACT

**We analysed the intoxications with wild mushrooms among the population in the Northern Bulgarian Black Sea coast between 1991 and 2015. Two easily feasible methods for their diagnosis, such as Meixner's test and ELISA, were approved for the first time in Bulgaria. By means of an inquiry, the degree of awareness of the population about wild mushrooms and their usage were studied. A total of 147 patients at a mean age of 52.54±15.27 with *Amanita phalloides* poisoning were registered. The overall mortality rate amounted to 17.00%. The results from these laboratory examinations for amatoxins in 21 patients suspected of this poisoning in 2021 were negative. In the course of the study of 200 individuals, 100 males and 100 females, residing in the Region of Varna, the knowledge of the population about wild mushrooms and their usage of in everyday practice were identified. There were differences not only between male and female respondents in a single age group but also between the subjects with secondary, elementary, and higher education, and between the inhabitants of the towns and villages. The degree of respondents' awareness about wild mushrooms was satisfactory. Their knowledge of mycotherapy was scarce. Further education of the population aiming at avoiding poisoning with these mushrooms was necessary. The usage of the edible wild mushrooms was multifarious and corresponded to the manner of nutrition and lifestyle. The early diagnosis of this intoxication by using these easily feasible and reliable laboratory methods played an essential role in selecting the conservative treatment of the patients. They could be introduced into the toxicological practice in our country.**

**Keywords:** *toxicology, mashroom consumption, gastrointestinal disorders*

# QUANTITATIVE AND QUALITATIVE CHARACTERISTICS OF AUTOLOGOUS PLATELET CONCENTRATES AND THE BIOLOGICAL SIGNIFICANCE OF THEIR PROTEINS

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## ABSTRACT

**In the last decade, platelet-rich concentrates, including the so-called platelet-rich plasma (PRP), have been gaining extreme popularity and are widely used in a variety of clinical fields of medicine. The procedure is a biological therapy and is based on the observations of platelets' role in the natural process of tissue recovery of the body. Many different protocols for obtaining of autologous platelet products have been described in the literature, but there is a lack of standardization and consensus on the best preparation approach.**

**The aim of the present study is to analyze the quantitative and qualitative parameters of autologous platelet concentrates obtained by different protocols.**

**Thirty healthy participants with an average age of 42.8 years were included in the present study after their informed consent. In a sterile manner, 35 mL of venous blood was drawn from each participant, allocated for testing by four experimental methods of PRP extraction.**

**The present study presents three reliable protocols for the preparation of PRP products with an increase in platelet content of more than +337.06% compared to the baseline control value, with clearly defined cell fractions present in them. We have discussed the amount of three major proteins (PDGF-AB, TGF-b1, IL-8) in details and their correlation with blood cells in PRP products, also their dependence on activation or not were analyzed. Regarding the amount of leukocytes, protocols have been derived in which leukocyte-rich and leukocyte-poor PRP products were obtained.**

**Keywords:** *platelets, concentration, enriched plasma, growth factors, proteins*

***Acknowledgments:*** *The scientific research was carried out with the help of a small project funded by the Science Fund at the Medical University of Varna.*

# PILOT STUDY FOR DISTRIBUTION OF *TRICHOMONAS TENAX* IN PATIENTS WITH POOR ORAL HYGIENE AND PERIODONTAL DISEASES

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## ABSTRACT

**As an initial compartment of the digestive system communicating with the environment, the oral cavity is populated by a large number and diverse species of microorganisms such as bacteria, viruses, fungi, and protozoa. They adhere to various surfaces, thus forming the so-called biofilms that are included in the composition of dental plaques. Plaques are a risk factor for the development of periodontitis because of the abundance of bacteria in them. The flagellate protozoan *Trichomonas tenax* has been isolated more frequently from the oral cavity of individuals with poor oral hygiene and periodontitis. The aim of our study was to assess the distribution of *Trichomonas tenax* worldwide based on the already published scientific literature. We performed a systematic review of 50 articles abstracted in several databases in the period between 1970 and August 2022. We proved a correlation between *Trichomonas tenax* and its presence in patients with periodontal disease. Different methods for its detection were established, such as microscopy, culture, polymerase chain reaction, and loop-mediated isothermal amplification. Despite the proven correlation, further epidemiological and clinical studies are needed to focus on the virulence and pathogenicity properties of this potential paradontopathogen.**

**Keywords:** *Trichomonas tenax, periodontal diseases, oral microflora, poor oral hygiene*

# THE PLACE OF ALTERNATIVE ASSAYS IN SCREENING FOR ANTICANCER COMPOUNDS

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## ABSTRACT

**The era of chemotherapy began as a low-budget, government-supported researches. Today, cancer drug development is a multi-billion dollar industry. Although targeted therapy is promising, conventional chemotherapy is still the main therapeutic approach. Also, experimental animals are essential in the study of novel anticancer compounds, because the collected data are enabled in the prediction of the biological responses in humans. Ethical issues surrounding work with experimental animals has led to the development of a number of alternative assays. New methods, such as in vitro, are reliable and highly informative. However, they have two significant disadvantages—the price and the limited functional similarity with living organisms. Screening for anticancer compounds increasingly begins with tests with microorganisms and/or plants. For example, the *Ames* test, *Triticum* test, *Allium* test, etc. Data on antiproliferative effects observed in organisms of different species are valuable because they allow better planning of animal experiments and the preclinical stage.**

**Keywords:** *anticancer compounds, alternative assays*

***Acknowledgments:*** *This work was supported by the Bulgarian Ministry of Education and Science, grant No. RD-08-113/20.02.2023.*

# A STUDY OF THE EFFECT OF DESFERAL ON SPERMATOZOA QUALITY UNDER OXIDATIVE STRESS CONDITIONS

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## ABSTRACT

**Recently, the problem of infertility has become increasingly prevalent, with aggregated data showing that between 3–7% of all couples globally have lasting reproductive problems. According to predictions, the frequency of infertility will increase progressively over the next decade.**

**Many couples with reproductive problems resort to assisted reproductive techniques (ART). Unfortunately, success rate after ART is relatively low—about 30%. One of the reasons for both infertility and low success rates in in vitro fertilization is the oxidative stress state where the generation of active forms of oxygen exceeds the capacity of the antioxidant system of the cells to eliminate their production and harmful action.**

**While seminal plasma is rich in antioxidants, sperm contains a limited amount of cytoplasm and the antioxidants in it are insufficient. Removal of seminal plasma under treatment for ART results in increased susceptibility of spermatozoa to oxidative damage due to lack of protective systems.**

**The aim of current research is to study the possibility of preserving and improving the quality of spermatozoa used in ART by applying the iron-chelating agent desferal. The results would allow the development of science-based strategies enhancing the effectiveness of these techniques.**

**Keywords:** *desferal, oxidative stress, infertility, in vitro fertilization*

# THE CORE MARKERS FOR EVALUATION OF THE HEALTHY LIFESTYLE OF THE BULGARIAN POPULATION

**Ivayla Kuleva[1], Tsonka Dimitrova[1], Stoyan Stoyanov[1], Ivan Vachkov[1], Galina Yaneva[1], Vanya Koleva[2]**

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## ABSTRACT

**Healthy lifestyle (HL) has been an extremely popular topic in the recent decades for scientists and ordinary people. The great positive influence of HL in the treatment of a number of socially significant diseases in modern society is well known. The latest scientific developments suggest a healthy lifestyle score based on five different health behaviours: body mass index, smoking status, physical activity, alcohol consumption, and diet. There are many studies on each of these indicators among different population groups in Bulgaria. Our goal is to combine these five health behaviours and establish one healthy lifestyle score of the population of Bulgaria. A questionnaire containing 7 questions related to the 5 indicators of HL was made. In addition, we added a question regarding the daily use of herbs and 5 questions about the basic demographic characteristics of the respondents. A question about the chronic diseases of the respondents is also planned. Our expectations are to make a uniform assessment of the degree of healthy lifestyle, which is representative for the entire population of Bulgaria.**

**Keywords:** *healthy lifestyle, questionnaire, Bulgarian population*

# OXIDATIVE STRESS IN THE DIAGNOSIS OF MALE INFERTILITY

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**Aim:** **The aim of the present study was to investigate sperm parameters, sperm DNA fragmentation index (DFI), and extracellular and intracellular markers of oxidative stress and the influence of factors, centrifugation, thawing and freezing of spermatozoa, which are associated with increased oxidative stress, in relation to sperm chromatin quality and sperm parameters in men with idiopathic and unexplained infertility.**

**Materials and Methods:** **The study included 120 men who underwent a routine semen analysis according to the 2010 WHO criteria. After exclusion of underlying urological problems and endocrinopathies, patients were divided into three groups: men with idiopathic, with unexplained infertility, and fertile men. Sperm DNA integrity was evaluated by the SDI test and sperm processing was done by the direct swim up technique. Sperm freezing and thawing methods were applied and levels of glutathione (GSH), glutathione peroxidase (GPx), and catalase activity were evaluated in spermatozoa and seminal plasma by enzymatic methods. Malondialdehyde levels were measured by the thiobarbituric acid method and total antioxidant capacity (TAC) was assessed. Intracellular $H_2O_2$ and $O_2^{\bullet -}$ levels were determined by flow cytometry.**

**Results:** **The percentage of DFI of spermatozoa after processing and thawing in all three groups increased significantly.**

**Total antioxidant capacity was significantly higher in the group of fertile men (0.657) compared to samples from men with evidence of idiopathic infertility (0.293), $p < 0.001$, and men with unexplained infertility (0.360), $p<0.001$.**

**The measured intracellular levels of $H_2O_2$ in the group of fertile men and men with idiopathic infertility differed significantly. They are significantly lower in the fertile group (10.4%) compared to the infertile one (23.7%), $p=0.000$.**

**Conclusion:** **Our results suggest that men with idiopathic infertility have higher intracellular $H_2O_2$ levels and decreased TAC.**

**In these patients, an individualized approach and choice of sperm selection technique involving minimal centrifugation procedures is important, as a possible reason for the rise in DFI after thawing and their lower antioxidant capacity due to removal of seminal plasma.**

**Keywords:** *oxidative stress, infertility, hydrogen peroxide*

# VITAMIN D DEFICIENCY—THE MODERN PANDEMIC

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## ABSTRACT

**Introduction:** **Vitamin D is a valuable fat-soluble vitamin well-known for its osteoprotective, immunomodulatory, and metabolic properties. An increasing number of recent studies highlight the correlation between non-optimal vitamin D levels and unfavorable health outcomes.**

**Aim:** **The aim of this study is to determine the prevalence of vitamin D deficiency in modern society, especially in certain risk groups, and assess the importance of the diagnosis and management of hypovitaminosis D based on the already published scientific literature.**

**Methods:** **We performed a systematic review of 30 articles from several databases during the period between June 2008 and October 2022.**

**Results:** **Careful evaluation of the relevant publications proved the urgent need to address this deficiency and put into perspective the benefits of vitamin D supplementation in improving health. Despite the widespread supplementation programs that have been introduced since the last century, vitamin D deficiency (serum [25(OH)D]<20 ng/mL) and insufficiency (<50 ng/mL) still remain common medical issues in both low- and high-income countries. Currently, more than 1 billion people worldwide are considered vitamin D deficient. Age, season, northern latitudes, insufficient sun exposure, obesity, poor dietary intake, and dark skin tone all contribute to low vitamin D levels. For this reason, vitamin D screening should be performed in the prevention and treatment planning of many acute and chronic diseases.**

**Conclusion:** **Despite the information proving the high global prevalence and biological significance of low vitamin D status, there is still demand for further scientific studies to clarify the promising clinical use of vitamin D in deficient patients.**

**Keywords:** *vitamin D deficiency, fat-soluble vitamins, modern society*

# II. POSTERS

## *TOXOPLASMA GONDII* IN MARINE LIFE: A POTENTIAL RISK OF TRANSMISSION THROUGH SEAFOOD CONSUMPTION

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### ABSTRACT

***Toxoplasma gondii*** **has worldwide geographic distribution and an estimated 30% of the population is seropositive. This protozoan parasite can cause serious health issues in pregnant women and immunocompromized people. The consumption of contaminated food is the main source of infection. In 2019, the European Food Safety Authority (EFSA) report found that food-borne transmission accounts for 40–60% of *T. gondii* infections. The recent reports indicate positive samples of meat, fish, raw mollusks and shellfish, honey and potable water, and *T. gondii* was included in category III of zoonotic agents to monitor.**

**Seafood, such as oysters, mussels, and fish, has been identified as a potential source of infection due to its ability to filter and concentrate the parasite's oocysts.**

**Aquatic environments get polluted by sewage and freshwater runoffs carrying *T. gondii* oocysts. In coastal environments, oocysts can be accumulated by bivalves. This bioaccumulation has been proven experimentally and has been reported in wild and cultivated bivalves. These possibilities imply a new potential source of *T. gondii* transmission to humans, a result of the consumption of raw or undercooked contaminated seafood.**

**The aim of this study is to estimate the prevalence, parasite burden, and genotypes of** *T. gondii* **in Black Sea mussels from the Varna coastal area.**

**Keywords:** *Toxoplasma gondii, oocysts, mussels, Varna coast*

# STUDY ON THE USE OF AVIRON RAPID DURING THE COVID-19 PANDEMIC

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## ABSTRACT

**Introduction: During the pandemic of COVID-19, conventional and traditional medicine joined forces in the search for effective and safe remedies to tackle the threat looming over all humanity.**

**Aim: The aim of this article is to determine which phytoproduct was most commonly used during the COVID-19 pandemic, report the results of its use, and draw conclusions about the prospects for its future use in the fight against viral infections.**

**Materials and Methods: An online survey was used to investigate the attitudes of a random sample of inhabitants of different localities in Bulgaria regarding the use and outcome of the treatment with medicinal plants and phytoproducts administered during COVID-19.**

**Results: The majority of respondents who applied herbal treatment preferred treatment with medicinal plants—74% to treatment with ready-made herbal products—28%.**

**The surveys cite 17 phytoproducts that have been used for the prevention and treatment of various covid symptoms. The most reports are about the use of Aviron Rapid.**

**The main ingredient of Aviron Rapid that determines its antiviral effect is andrographolide, isolated from the plant *Andrographis paniculata* (Burm.f.) Wall ex Nees, family Acanthaceae.**

**The analysis of the additional curative actions of *Andrographis paniculata* (Burm.f.) Wall ex Nees showed that they were relevant to post-COVID and COVID symptoms, as well as to the indirect effects of the pandemic on the social behavior of the population.**

**Conclusion: Aviron Rapid is an extremely suitable and promising phytoproduct for the prevention of any complications that may arise from COVID-19, for both prevention and management of post-COVID symptoms.**

**Keywords:** *Andrographis paniculata, Aviron Rapid, COVID-19*

# A RETROSPECTIVE STUDY ON THE INFLUENCE OF INSULIN RESISTANCE ON ASSISTED REPRODUCTIVE TECHNOLOGIES

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## ABSTRACT

**In recent years, the relationship between two socially significant health problems, such as infertility and insulin resistance, has become of great interest. Insulin resistance mediating obesity may be one of the causes of infertility in women. Adipose tissue affects both ovarian and endometrial functions by influencing oocyte maturation and endometrial receptivity. In addition, through the development of peripheral insulin resistance, obesity creates a state of functional hyperandrogenism and hyperestrogenism, which contribute to inducing anovulation and reducing the receptivity of the endometrium and are therefore involved in causing infertility. The aim of the present article was to study insulin resistance in women with reproductive failure. We examined data on 180 women without and 60 with established and treated insulin resistance who underwent an assisted reproduction procedure. We took into account parameters such as the levels of hormones FSH, LH, E2, TSH, and progesterone as well as body mass index (BMI), number of mature eggs, number of embryo transfers, and number of children born after assisted reproduction procedure. We found a statistically significant difference in BMI ($p<0.01$) between the groups, in contrast to the rest of the studied parameters. Respectively, we assumed that treated insulin resistance does not play a major role in the outcome of an assisted reproduction procedure.**

**Keywords:** *insulin resistance, reproductive technologies, obesity*

# BETULINIC ACID AND THE NLRP3 INFLAMMASOME PATHWAY

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## ABSTRACT

**Many of the anticancer drugs exhibit relatively high toxicity to tumor cells, and to normal cells. Respectively, several current researches for novel anticancer drugs are focused on the natural compounds. An example of a natural compound with potent anticancer bioactivities is betulinic acid (BA). It has been reported that BA exhibits antitumor properties against various types of cancer cells, including breast cancers. In addition, BA combined with Taxol synergistically improves the chemosensitivity of Taxol-resistant breast cancer cell lines. Although BA has been shown to influence NF-κB, little is known about its effect on inflammasome pathways. The inflammasome complexes are responsible for the proteolytic processing of the immature pro-inflammatory cytokines as IL-1β and IL-18. Among inflammasome complexes, the NLRP3 inflammasome is most extensively studied as a promising target for immunomodulation. To date, NLRP3 inflammasome has been shown to have a critical role in promoting tumor growth, progression and metastasis in breast cancer. Although the specific mechanism of action of BA against cancer cells is still a subject of detailed research, based on the above we may hypothesize that BA is a potential modulator of the NLRP3 inflammasome pathway. Respectively, our future experiments aim to clarify the impact of BA and its derivatives on the NLRP3 inflammasome pathway and their potential as modulators of NLRP3 inflammasome-driven diseases.**

**Keywords:** *betulinic acid, anticancer drugs, NLRP3*

***Acknowledgments:*** *This research has been supported by the National Science Fund of Bulgaria, contract No. КП-06-Н69/2.*

# ASSOCIATION OF THE IL12B GENE POLYMORPHISMS RS3212227 AND RS17860508 WITH GLIOBLASTOMA RISK

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## ABSTRACT

**Glioblastoma multiforme (GBM) is one of the most aggressive types of brain cancer in adults. The rs3212227 and rs17860508 polymorphisms are functional genetic variations of the IL12B gene but little is known about their impact on glioma risk.**

**The aim of the study was to evaluate the possible impact of the functional polymorphisms, rs3212227 and rs17860508 in IL12B gene, on GBM risk in adult patients.**

**The study was conducted among 34 cases with GBM and 208 unrelated control individuals. Genotyping of rs17860508 and rs3212227 in the IL12B gene was performed by allele-specific PCR, and by restriction–fragment length polymorphism–PCR, respectively.**

**The homozygous-CC genotype of the rs3212227 polymorphism was not detected in any of the studied patients with GBMs. The variant C-allele was significantly less presented among GBM cases than in controls (15% vs. 27%; OR = 0.468; 95% CI: 0.231–0.947; p = 0.031). In the allelic and dominant genetic models, carrying of C-alleles was associated with 2-fold lower GBM risk. The genotype distribution of rs17860508 polymorphism between GBM cases and controls was significantly different (chi2 = 0.02; $p_c = 0.02$). The frequency of genotype-22 among GBM cases was higher than that in controls (41% vs. 22%; OR = 4.293; 95% CI: 1.443–12.744; p = 0.009) and may significantly be attributable to GBM susceptibility.**

**Based on the results, we could suggest that both studied IL12B polymorphisms, rs3212227, and rs17860508 can impact GBM susceptibility.**

**Keywords:** *IL-12B gene, glioblastoma multiforme, rs3212227, rs17860508*

# MAMAPRED: A NOVEL TOOL FOR RECURRENCE RISK ASSESSMENT AND MOLECULAR SUBTYPING IN EARLY-STAGE BREAST CANCER

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## ABSTRACT

**Introduction: Gene expression profiling is a recommended approach to determine the recurrence risk and guide treatment choice for invasive early-stage breast cancer patients. MamaPred constitutes an innovative tool that outperforms the prognostic value of other platforms, such as Mammaprint and OncoType, and provides improved risk categorisation and molecular subtyping.**

**Aim: The aim of this study is to determine the recurrence risk and molecular subtypes of 24 early-stage breast cancer patients.**

**Materials and Methods: The expression levels of 2560 cancer-related mRNAs were evaluated using the Oncology Biomarker Panel (OBP) and the HTG EdgeSeq System.**

**Results: A total of 24 hormone receptor positive (HR+), epidermal growth factor receptor 2 negative (HER2-) specimens were analysed. The study was conducted with 20 samples of invasive ductal carcinoma, 3 samples of invasive lobular carcinoma, and 1 sample of invasive mucinous carcinoma. MamaPred analysis showed that 20 samples had a high risk of recurrence, whereas 4 samples were determined as low-risk. Molecular stratification identified two breast cancer subtypes: luminal A and luminal B. Gene expression analysis identified several pathways, including PI3K/Akt/mTOR, that were altered in early-stage breast cancer.**

**Conclusion: MamaPred is a novel and promising tool in recurrence risk assessment and molecular subtyping in HR+/HER2- early-stage breast cancer patients with high risk of distant relapse. It outperforms the prognostic value of other platforms suggesting a more precise risk categorisation.**

**Keywords:** *MamaPred, recurrence, risk, assessment, molecular subtypes*

# AGOMELATINE AFFECTS THE AB METABOLISM BY ADJUSTING THE CONTENT OF SECRETASE ENZYMES IN ICV AB-INDUCED RAT MODEL OF ALZHEIMER'S DISEASE

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## ABSTRACT

**The cause of Alzheimer's disease (AD) is still an enigma amongst researchers. Plaques formed by the Aβ protein in the brain are distinctive mark for the disease. Their formation is related to the activity of three secretase enzymes—α, β, and γ. Enhancing the α processing could possibly diminish the severity of AD.**

**In the present study, we aimed to explore the effect of agomelatine (Ago) (40 mg/kg, i.p. for 30 days) on beta-amyloid (Aβ) metabolism in icvAβ1-42 rat model of AD. Tested animals were devided into two groups – one treated with Ago simultaneously with Aβ1-42 injection (AβAgo1) and the other—30 days later (AβAgo2). Concentration of the target molecules was measured in the frontal core and the hippocampus. Administered during the early and the late stage of AD, Ago corrected to control level the elevated Aβ1-42 in the frontal cortex (FC) and the hippocampus. The concentration of α-secretase was enhanced by AβAgo1 compared to the sham- and Aβ-veh groups in the hippocampus. No changes in the concentration of β-secretase in the FC and the hippocampus as well as of γ-secretase in the FC were observed among groups. Both the AβAgo1 and AβAgo2 attenuated to control level the Aβ-induced increased concentration of γ-secretase in the hippocampus. As our findings are still insufficient we suggest it is important to understand the mechanisms that calibrate synthesis, transport, and degradation of A-beta in order to confirm biomarkers for early diagnosis of AD.**

**Keywords:** *Alzheimer's disease, amyloid β, Aβ-induced rat model of Alzheimer's disease, secretase enzymes*

# STUDY OF NOVEL ARYL-SUBSTITUTED BENZO[A] QUINOLIZIDINES AS POTENTIAL DPP-IV INHIBITORS

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## ABSTRACT

**Dipeptidyl peptidase IV (DPP-IV) is a highly specific serine protease enzyme whose inhibition has been widely explored for the potential to treat chronic metabolic type 2 diabetes mellitus. Recent studies have outlined the benzo[a]quinolizidine ring system as an important heterocyclic framework in the structure of potential DPP-IV inhibitors. Molecular docking was used to determine the binding potential of the prepared compounds to the active site of a human dipeptidyl peptidase IV enzyme.**

**Based on this, inhibitory activity of the newly synthesized compounds against human DPP-IV was measured using DPP-IV inhibitor screening assay kit. The percentage of inhibition was calculated using sitagliptin as a positive control. Several of the investigated compounds have shown to possess inhibitory activity in the micromolar range. This result could provide a route to investigate other unexplored heterocycles with potential inhibitory activity.**

**In conclusion, future studies of heteropolycycles as potential inhibitors would be of great significance to both organic and medicinal chemistry.**

**Keywords:** *DPP-IV, DPP-IV inhibitors, aryl-substituted benzo[a]quinolizidines*

# HOW SYSTEMIC LUPUS REDUCES FERTILITY—TWO MOUSE-BASED MODELS

**Stefka Delimitreva[1], Gabriela Boneva[2], Irina Chakarova[1], Ralitsa Zhivkova[1], Maya Markova[1], Venera Nikolova[1], Anton Kolarov[1], Nikola Mladenov[1], Ana Blagoeva[1], Silviya Bradyanova[2], Nikolina Mihaylova[2], Andrey Tchorbanov[2]**

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## ABSTRACT

**Introduction:** **Systemic lupus affects predominantly women and reduces the pregnancy rate.**

**Aim:** **Two mouse lupus models were investigated: Balb/c mice with experimentally provoked lupus (by intraperitoneal injection of pristane hydrocarbon oil); MRL/lpr mouse strain which develops lupus spontaneously.**

**Methods:** **The cytoskeletal and chromatin structures were analyzed in the oocytes of mice at different stages of disease progression.**

**Results:** **Prolonged autoimmune reactions are related to reduced proportion of normally matured oocytes. The oocyte quality (both for virgin and postpartum mice) was better than for young mice. On the other hand, it is known that MRL/lpr mice are unable to get pregnant a second time. The combination of these facts suggests that the older SLE mice manage to develop some compensatory mechanisms to preserve oogenesis and produce some normally looking oocytes.**

**Conclusions:** **The reproductive failure after the first pregnancy of MRL/lpr mice is not caused by total inability to produce normal oocytes, but most probably is a result of a general inflammatory effect that impairs the functions of all systems, including ovarian function.**

**Keywords:** *systemic lupus, fertility, oocytes*

# ASSOCIATION BETWEEN ANTISPERM ANTIBODIES AND SECRETION OF IL-10 AND IL-12 IN INFERTILE PATIENTS

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## ABSTRACT

**Introduction: Antisperm antibodies (ASAs) in infertile patients indicate humoral immune response against sperm cells, but cellular immune response in infertility is insufficiently studied. Cytokines involved in it can influence gamete interaction, early embryogenesis, and implantation.**

**Aim: The aim of this article is to study humoral and cellular immunity in infertile patients.**

**Materials and Methods: Sera from patients were tested for ASA by ELISA and for IL-10 and IL-12 secretion by sandwich ELISA.**

**Results: The applied tests revealed different groups of positive reactive patients: (1) for one of the cytokines and ASA, (2) for both cytokines and ASA, and (3) for one or both cytokines but not ASA. Controls were negative.**

**Conclusion: The results showed association between the examined factors and infertility, and heterogeneity of infertile couples. Secretion of IL-10 and IL-12 could contribute to infertility, and might have diagnostic importance.**

**Keywords:** *IL-10, IL-12, infertility, antisperm antibodies*

***Acknowledgments:*** *This work was supported by Grant 2022 of MU-Sofia, under Contract No. D137/14.06.2022.*

# EFFECT OF TWO DIFFERENT VARIANTS OF FSH TREATMENT ON THE METAPHASE PLATE FORMATION IN MOUSE OOCYTES WITH EXPERIMENTAL OSTEOARTHRITIS

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## ABSTRACT

**Aim: This article studies metaphase chromatin alignment in oocytes from mice with collagenase-induced osteoarthritis (CIOA) treated by different regimens of follicle-stimulating hormone (FSH).**

**Materials and Methods: A total number of 639 mouse oocytes were subdivided into three groups as follows: 214 oocytes from 10-day FSH-treated females, FSH controls and from mice with CIOA; 139 oocytes after parallel FSH treatment (group FSH-CIOA-30), and 286 oocytes after FSH treatment prior to CIOA (group FSH-CIOA-40). All of the females were subjected to hormonal stimulation and ovulation. The oocytes were collected and their chromatin was revealed by Hoechst 33258.**

**Results: The group FSH-CIOA-30 showed higher number of aligned metaphase plates and lower rate of degeneration of the chromatin compared to both FSH controls and FSH-CIOA-40. The lowest quality of oocyte chromatin and chromosome alignment was found in the FSH-CIOA-40 group (especially in metaphase II oocytes).**

**Conclusion: Our study showed a milder effect on the quality of metaphase plates when FSH treatment was parallel with CIOA. The effect of FSH alone and prior to CIOA was related to a higher rate of misaligned and degenerative chromosomes.**

**Keywords:** *FSH, osteoarthritis, oocytes*

***Acknowledgments:*** *This work was supported by Grant 2022 of MU-Sofia, under Contract No. D-137/14.06.2022 and by the Bulgarian Ministry of Education and Science under the Young Scientists and Postdoctoral Students National Program for Research.*

# ANTI-SPERM ANTIBODIES: REACTING WITH THE SURFACE VS. REACTING WITH THE DEPTH

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## ABSTRACT

**Introduction: Various methods are used as tests for anti-sperm antibodies, each of them detecting a particular subset of these antibodies and, hence, revealing a particular aspect of anti-sperm auto- and isoimmunity.**

**Aim: The aim of the article is to investigate the possible correlation between the results of different methods of testing.**

**Materials and Methods: We applied three methods: tray agglutination test (TAT), sperm immobilization test (SIT), and ELISA.**

**Results and Conclusion: We found significant correlation between TAT and SIT but not between TAT and ELISA, or SIT and ELISA. A common feature of TAT and SIT is that both detect antibodies to surface antigens, while ELISA detects antibodies to antigens from all parts of the cell. Our results imply distinct responses to surface and intracellular antigens in different infertile patients, which may reflect differences in the causes of infertility and the role of detected antibodies.**

**Keywords:** *anti-sperm antibodies, tray agglutination test, sperm immobilization test, ELISA*

***Acknowledgment:*** *This work was supported by Grant 2022 of MU-Sofia, under Contract No. D-137/14.06.2022 and by the Bulgarian Ministry of Education and Science under the Young Scientists and Postdoctoral Students National Program for Research.*

# EFFECTS OF PROSTAGLANDIN F2 ALPHA ON IN VITRO MEIOTIC MATURATION OF MOUSE OOCYTES

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## ABSTRACT

**Introduction:** **Prostaglandin F2 alpha has been reported to reduce the developmental potential of bovine oocytes when applied to them during maturation, but the mechanism is unknown. Our previous research has shown that in ovulated oocytes, this mediator of inflammation causes degenerative changes in the cytoskeleton.**

**Aim:** **The aim of this article is to assess the effects of prostaglandin F2 alpha on oocyte morphology and meiotic progression.**

**Materials and Methods:** **We applied prostaglandin F2 alpha to mouse oocytes subjected to in vitro maturation.**

**Results and Conclusion:** **Compared to controls, prostaglandin-treated oocytes had a lower maturation rate and a thinned meiotic spindle. Most of the immature cells were in metaphase I. Based on these results, we hypothesize that prostaglandin F2 alpha interferes with the assembly and/or maintenance of the meiotic spindle in maturing oocytes, which activates the spindle checkpoint in some of them and leads to metaphase I arrest.**

**Keywords:** *prostaglandin F2, meiotic spindle, oocytes*

***Acknowledgment:*** *This work was supported by Grant 2022 of MU-Sofia, under Contract No. D-137/14.06.2022 and by the Bulgarian Ministry of Education and Science under the Young Scientists and Postdoctoral Students National Program for Research.*

# LUPUS PROGRESSION AFFECTS OOGENESIS IN A MOUSE MODEL

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## ABSTRACT

**Introduction:** **Systemic lupus is an autoimmune disease related to reproductive complications, including reduced birth rate.**

**Aim:** **The aim of this article is to investigate the relationship between the development of lupus symptoms and the quality of oogenesis.**

**Methods:** **Lupus-prone mice (MRL/lpr) were used in this study. The immune response was monitored by flow cytometry, ELISpot, ELISA, and histology. Oocytes were analyzed by fluorescence microscopy of chromatin, tubulin and actin structures.**

**Results:** **In mice with lupus symptoms, the number and quality of oocytes were affected, and the pregnancy rate was limited to 60%. In a substantial proportion of the oocytes, abnormal chromatin and cytoskeletal structures were observed.**

**Conclusion:** **Alterations in immune proocesses affect the oocyte quality; that was observed even in young asymptomatic individuals. The compromised oocyte quality can be explained by changes in the microenvironment of the relevant organs and close connection between ovulation and inflammatory processes.**

**Keywords:** *systemic lupus, oogenesis, oocyte quality*

# DOES QUININE AFFECT THE CELL CYCLE OF RENAL AND HEPATIC CELLS, TREATED WITH METFORMIN?

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## ABSTRACT

**Metformin (Glucophage) a medication for treatment of type 2 diabetes. Usually the initial dose is 500–850 mg for patients with a normal renal function and, depending on the renal function, the dosages could reach 1,000 mg. The most common adverse effects of metformin include gastrointestinal irritation (diarrhea, nausea, vomiting) and serious potential for lactic acidosis. Administration of metformin therapy to patients with chronic hypoxemic conditions, which could lead to lactic acidosis (i.e., renal and hepatic diseases), is contraindicated. Quinine is an ingredient in tonic water and also a medication used to treat malaria and piroplasmosis. For parasitic disease treatment, it is administered at a conventional dose of 25 to 30 mg/kg/d. Side effects of administration include: tinnitus and vertigo, blood and cardiovascular reactions (low platelet count and hemolytic-uremic syndrome/thrombotic thrombocytopenic purpura, long QT syndrome, disseminated intravascular coagulation, leukopenia, and neutropenia). Also, quinine is not recommended for patients with severe liver dysfunction.**

**As tonic water is a common drink and Glucophage is widely used as a drug of first choice for both type 2 diabetes, and insulin resistance, we investigated the effect of the simultaneous administration of quinine and metformin on the cells. The aim of this study is to describe the impact of quinine and metformin on the distribution of cell cycle phases of renal and hepatic cells.**

**Keywords:** *quinine, renal cells, hepatic cells, metformin*

***Acknowledgment:*** *This work was supported by Grant 2022 of MU-Sofia, under Contract No. D-137/14.06.2022 and by the Bulgarian Ministry of Education and Science under the Young Scientists and Postdoctoral Students National Program for Research.*

# EFFECT OF IFNγ ON THE IMMUNOMODULATORY PROPERTIES OF HUMAN UMBILICAL CORD MESENCHYMAL STEM CELLS

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## ABSTRACT

**Introduction: Mesenchymal stem cells (MSC) can contribute to both innate and adaptive immune responses. The immunosuppressive activity of MSC is triggered by pro-inflammatory factors, like IFNγ, TNFα, and IL-1β.**

**Aim: By examining the surface expression of molecules on T cells and the secretion of cytokines, the study aimed to explore the immunomodulatory effect of IFNγ-treated Wharton's jelly MSC (WJ-MSCγ).**

**Materials and Methods: Human WJ-MSC were isolated, expanded, characterized, and treated with IFNγ. Mononuclear cells from peripheral blood (PBMC) were also isolated. Flow cytometry of PBMC co-cultured with MSC in the presence of IFNγ was performed for CTLA-4, PD-1, and 158k. ELISA was used to determine the secretion levels of IL-10 and TGFβ.**

**Results: The amount of IL-10 secreted by WJ-MSC decreased when IFNγ was present. Our results showed that under the effect of WJ-MSC, the intensity of CTLA-4 expressed by T cells significantly decreased, while WJ-MSCγ reversely up-regulated CTLA-4 expression. PD-1 expression by T lymphocytes decreased regardless of whether WJ-MSC were primed with IFNγ or not. The concentration of IL-10 increased when PBMC were co-cultured with WJ-MSC. While IL-10 levels were lower when WJ-MSCγ were added, they were still considerably higher than in conditioned media from PBMC alone.**

**Conclusion: IFNγ affects the immunomodulatory properties of MSC, influencing the expression of surface markers on T cells and the cytokine secretion of WJ-MSC.**

**Keywords:** *IFNγ, mesenchymal stem cells, IL-10, TGFβ*

# BIOLOGICAL ACTIVITY OF NEWLY SYNTHESIZED CU(II) AND CO(II) METAL COMPLEXES WITH SCHIFF BASES ON HUMAN BREAST CANCER CELLS

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## ABSTRACT

**Cancer is the second leading cause of death globally, and was responsible for nearly 10 million deaths in 2020. Globally, about 1 in 6 deaths is because of cancer. Modern treatment of cancer includes surgery, drug therapy, and/or radiation therapy, administered alone or most often in combination. Two groups of newly synthesized metal [Cu(II), Co(II)] complexes with Schiff bases have been investigated for potential anticancer properties.**

**The aim of this study was to investigate the cytotoxicity properties of these complexes on MCF-7 and MDA-MB-231 human breast cancer cell lines. Non-cancer cell human Lep-3 cells were used as a control. The concentration applied was from 10 to 400 μg/mL and was assessed with cytotoxicity tests such as: MTT test, neutral red uptake assay, cystal violet staining. 3D colony forming method in semi-fluid medium was also performed. Morphological and cytopathological changes were observed using double staining with acridine orange and propidium iodide, immunocytochemical assays.**

**The results that were obtained revealed that the investigated metal complexes reduce cell viability and proliferation activity in a time-concentration dependence. The complexes with Cu and serine amino acid expressed higher cytotoxic profile than those with Co. Additional research is needed to better specify their mechanisms of action.**

**Keywords:** *metals, cell lines, cancer, Schiff bases, cytotoxicity*

***Aknowledgements:*** *The study was supported by the National Science Fund, Ministry of Education and Science in Bulgaria (Grant № ДКОСТ 01/10 from 22.10.2018), and a bilateral project between the Bulgarian Academy of Sciences and the Romanian Academy.*

# MEIOTIC SPINDLE AND ACTIN CAP PECULIARITIES IN OOCYTES FROM MOUSE MODEL OF OSTEOARTHRITIS AFTER FSH TREATMENT

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## ABSTRACT

**Aim:** **The aim of this study is to investigate the effect of stimulating hormone (FSH) treatment combined with collagenase-induced osteoarthritis (CIOA) on mammalian oogenesis.**

**Materials and Methods:** **Three mouse groups were subjected to FSH treatment: FSH for 10 days only (FSH controls), mice with CIOA and parallel FSH for the first 10 days after the induction (FSH-CIOA-30 group), and a group with FSH for 10 days with consequent induction of CIOA (FSH-CIOA-40 group). The oocytes were collected after standard hormonal ovarian stimulation. The cytoskeletal structures were analyzed after fluorescent labeling of meiotic spindle tubulin and actin cap.**

**Results:** **We found a higher rate of spindle abnormalities in both FSH-CIOA-30 and FSH-CIOA-40 groups compared to FSH controls. Spindle defects showed a higher rate of detached fibers (FSH-CIOA-30), and an increased number of degenerated oocytes in FSH-CIOA-40. Other spindle abnormalities (spindle pole and ooplasmic asters) showed a specific distribution among the groups—present in FSH-CIOA-30, but absent in FSH-CIOA-40. The actin cap was of similar size in all three groups, but smaller caps were more frequent in both FSH-CIOA groups compared to the FSH controls.**

**Conclusion:** **These peculiarities of the cytoskeletal structures in the oocytes combining FSH treatment with CIOA revealed the significant role of CIOA-related factors in mammalian oogenesis combined with FSH-specific effects if compared to oocytes of untreated (pure control) mice.**

**Keywords:** *meiotic spindle, FSH treatment, osteoarthritis*

***Acknowledgment:*** *This work was supported by Grant 2022 of MU-Sofia, under Contract No. D-137/14.06.2022 and by the Bulgarian Ministry of Education and Science under the Young Scientists and Postdoctoral Students National Program for Research.*

# COLORIMETRIC DETECTION OF L-ASPARAGINASE ACTIVITY FOR THERAPY MONITORING IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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## ABSTRACT

**Introduction:** **L-asparaginase is an enzyme used to treat childhood acute lymphoblastic leukemia (cALL). The therapy carries risks because the enzyme is a highly immunogenic bacterial product. Repeated applications produce antibodies that can mediate anaphylactic reactions or inactivate the enzyme.**

**Aim:** **The aim of this article is approbation and validation of two colorimetric tests for measuring enzyme activity for the purpose of monitoring L-asparaginase therapy in children with cALL.**

**Materials and Methods:** **The following tests were developed and validated: Nessler's method, which measures the amount of ammonia released during the deamination of asparagine by the enzyme, and a method with L-aspartic β-hydroxamate (ANA), which is a substrate for L-asparaginase. In parallel, an amino acid analysis of the serum was carried out. The methods were applied to 5 samples of children with cALL.**

**Results and Discussion:** **All patients showed L-asparaginase activity > 0.1 IU/mL. A strong reduction of the asparagine and glutamine amino acids was detected, which confirmed the presence of enzyme activity.**

**Conclusion:** **The developed methods for monitoring an L-asparaginase therapy provide an opportunity to optimize the treatment and to reduce adverse drug reactions.**

**Keywords:** *acute lymphoblastic leukemia, L-asparaginase, children*

***Acknowledgments:*** *Funding from MU-Plovdiv under project DPDP 04/21.*

# EXPRESSION OF LAMP PROTEINS IN COLORECTAL CARCINOMA

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## ABSTRACT

**Introduction: Colorectal carcinoma (CRC) is among the leading neoplastic diseases with high mortality worldwide. As yet, none of the classical markers applied has an accurate diagnostic and prognostic value. The discovery of precise and informative biomarkers in the initial stages of CRC may reduce mortality.**

**Aim: The aim of this article is immunohistochemical evaluation of LAMP protein expression in CRC patient tissues.**

**Materials and Methods: Thirty-one patient samples were used: tumor CRC tissue, peritumoral and healthy tissue. The intensity of the reaction was scored with a semi-quantitative scale. The expression of LAMP1 and LAMP2 proteins was examined immunohistochemically on paraffin sections. The findings were documented by photomicrographs.**

**Results and Discussion: Five regions were examined in each paraffin section—tumor parenchyma, stroma, front, as well as parenchyma and stroma in normal tissue. We found significantly higher expression of LAMP2 proteins compared to LAMP1 in the tumor stroma, parenchyma and tumor front, presented as granular staining. We recorded a low level of expression in healthy tissue.**

**Conclusion: Application of non-invasive biomarkers such as LAMP1 and LAMP2 and their association with autophagy involved in tumorigenesis would facilitate diagnosis, monitoring and prognosis in CRC.**

**Keywords:** *colorectal carcinoma, LAMP, mortality*

***Acknowledgments:*** *The study was financed by the National Institute of Scientific Research under project KP-06 ПН63/7-2022, BG 175467353-2022-04-0056, by the European Union—NextGenerationEU, through the National Plan for Recovery and Sustainability of the Republic of Bulgaria, project No. BG-RRP-2.004-0007-C01 and the National Program for Young Scientists and Postdoctoral Students NP „MUPD-2“.*

# STUDY OF THE EFFECT OF TREATMENT IN PATIENTS WITH RHEUMATOID ARTHRITIS ON KEY INDICATORS OF MITOCHONDRIAL FUNCTION

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## ABSTRACT

**Introduction:** **Rheumatoid arthritis (RA) is a severe inflammatory joint disease with systemic manifestations. New studies report an imbalance in cellular and energy metabolism and the presence of mitochondrial dysfunction in RA patients.**

**Aim:** **The aim of this article is to evaluate the treatment effect in RA patients on two therapeutic protocols by examining cellular metabolism.**

**Materials and Methods:** **Sixteen newly diagnosed RA patients subjected to two types of treatment, methotrexate and JAK inhibitors, were included in the study. Peripheral blood mononuclear cells (PBMCs) and plasma were isolated before and 6 months after therapy. Mitochondrial function and cellular metabolism assays were performed on PBMCs.**

**Results and Discussion:** **The obtained results show a significant decrease in proton leakage after therapy, which is probably due to the restoration of the mitochondrial membrane and the complexes of the electron transport chain. Another indicator that complements and supports the improvement of patients at the cellular level after applied therapy with JAK inhibitors is the elevated production of ATP. The increase after therapy may be related to the catalytic action of JAK inhibitors on oxidative phosphorylation and a switch to oxidative metabolism. This corresponds to a decrease in proton leakage.**

**Conclusion:** **Mitochondrial dysfunction was demonstrated in isolated PBMCs from RA patients, which improved to varying degree after therapy.**

**Keywords:** *rheumatoid arthritis, methotrexate, JAK inhibitors, mitochondrial function*

***Acknowledgments:*** *The study was financed under project No. 01/2022 of MU-Plovdiv and NUKBPI-BBMRI.BG project, Agreement D01-278/14.12.2022 within the framework of the National Road Map of Scientific Infrastructures.*

# YKL-40—A POTENTIAL PROGNOSTIC MARKER IN SEVERE TRAUMATIC BRAIN INJURY

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## ABSTRACT

**Introduction:** **Severe traumatic brain injury (TBI) is the third leading cause of death and disability worldwide. The study of biomarkers to track the dynamics of pathological changes in TBI will support clinical practice.**

**Aim:** **The aim of this article is to study YKL-40 glycoprotein levels in parallel with markers of inflammation (TNF-α and CRP), autophagy (LAMP-2), and neuronal injury (NSE), and to seek correlation with clinical scales to assess the severity of the trauma.**

**Materials and Methods:** **Thirty-one patients with isolated TBI were included. Severity of trauma was determined by GCS, Marshal CT, and APACHE III. The levels of YKL-40, TNF-α, CRP, LAMP-2, and NSE in plasma at 24 and 96 hours after TBI were examined by ELISA.**

**Results and Discussion**: **A significant negative correlation was found between plasma levels of YKL-40 and those of TNF-α, CRP, and NSE. A statistically significant positive correlation was found between YKL-40 and LAMP-2 values in plasma, as well as between YKL-40 concentration and GCS score. ROC analysis showed that plasma YKL-40 levels can serve as a marker for prognostication of survival after TBI.**

**Conclusion:** **The combination of the studied biomarkers reflects the mechanisms of autophagy, neuronal inflammation, and damage in TBI. The multifunctional YKL-40 glycoprotein probably exhibits a neuroprotective and anti-inflammatory role, and its increased plasma concentrations are related to better clinical outcome.**

**Keywords:** *YKL-40, brain injury, prognostic marker*

***Acknowledgment:*** *The funding of the study is under the NUKBPI-BBMRI.BG project, Agreement D01-278/14.12.2022. within the framework of the National Road Map of Scientific Infrastructures.*

# HISTOCHEMICAL CHANGES IN THE CARP (CYPRINUS CARPIO LINNAEUS, 1758) BIOINDICATOR SPECIES UNDER THE INFLUENCE OF PESTICIDES

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## ABSTRACT

**Introduction:** **Pollution of aquatic ecosystems with chemical substances is due to pesticides used in agriculture. A large number of characteristics make fish excellent models for toxicological studies and monitoring the toxicity of xenobiotics.**

**Aim:** **The aim of this article is to determine the negative effects of three pesticides on carp liver.**

**Materials and Methods:** **The experiment was conducted for 96 hours in laboratory conditions. Seven aquaria were used, and six of them were supplemented with: pirimiphos-methyl (10 µg/L and 60 µg/L), propamocarb hydrochloride (40 µg/L and 80 µg/L), and 2,4-D (50 µg /L and 100 µg/L), and the seventh was used for the control group. Objects of the study were one-year-old carp, 10 in each aquarium. The dissection was performed in accordance with animal welfare requirements. Liver cryosections were prepared and stained with Sudan Black B and PAS-reaction.**

**Results and Discussion:** **The results of the present study showed an increase in the amount of glycogen and lipids in the liver of the bioindicator species compared to the control group of individuals. Histochemical reactions showed a similar degree of expression in the three exposures with the pesticide concentrations used.**

**Conclusion:** **Histochemical analysis can serve as a tool for monitoring the health of bioindicator species. The established histochemical changes in the liver can be successfully applied as biomarkers for the toxic effect of various pesticides.**

**Keywords:** *pesticides, xenobiotics, liver*

# POST-TRANSCRIPTIONAL REGULATION OF YKL-40 IN SYSTEMIC SCLEROSIS AND ASSOCIATION WITH LUNG AND JOINT INVOLVEMENT

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## ABSTRACT

**Introduction:** **Systemic sclerosis (SSc) is a rare connective tissue disease with heterogeneous clinical manifestations. YKL-40 is a secreted glycoprotein with pleiotropic functions that is involved in acute and chronic inflammation.**

**Aim:** **The aim of this article is to determine the role of YKL-40 as a biomarker in joint and lung involvement in patients with SSc and the mechanisms of its post-transcriptional regulation.**

**Materials and Methods:** **Seventy-one patients with SSc and 23 healthy subjects were tested for serum levels of YKL-40 by ELISA. Clinical and high-frequency ultrasound (US10SSc) evaluation of joint and lung involvement was performed. Seven miRNAs and 3 long non-coding RNAs with a potential role in YKL-40 regulation were selected. qPCR and TaqMan analysis to detect their expression are included.**

**Results and Discussion:** **A significantly increased serum concentration of YKL-40 was found in the patients compared to the control group. The rate in patients with diffuse SSc (dcSSc) was significantly higher compared to limited SSc (lcSSc). Correlations between YKL-40, pulmonary fibrosis, and US10SSc were statistically significant, allowing high serum YKL-40 levels to be used to distinguish both SSc patients from healthy individuals and dcSSc from lcSSc.**

**Conclusion:** **The expression of miR-30e-5p and miR-30a-5p were downregulated in WBCs and plasma of patients, and the expression of large non-coding RNAs was upregulated. We propose a new lncRNAs/miR-30e/YKL-40 regulatory axis model in SSc.**

**Keywords:** *YKL-40, systemic sclerosis, pulmonary fibrosis*

***Acknowledgments:*** *This research was funded by the European Union - NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project No. BG-RRP-2.004-0007-C01 and the Bulgarian Association for Musculoskeletal Ultrasound (BAMSU).*

# CYTOTOXIC EFFECT OF DAUNORUBICIN-LOADED CASEIN NANOPARTICLES ON ACUTE LYMPHOCYTIC LEUKEMIA—AN IN VITRO STUDY

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## ABSTRACT

**Introduction:** **Advances in nanotechnology open new perspectives in cancer therapy through the development of innovative targeted therapy systems. They can deliver chemotherapeutics specifically to tumor cells without affecting healthy tissues, for which they must possess a certain structure, size, biocompatibility, toxicity, immunogenicity, etc. Casein is a natural biopolymer capable of self-assembly and forming micelles, which is why it is used to construct nanoscale drug delivery systems.**

**Aim:** **The aim of this article is to determine the cytotoxic potential of daunorubicin-loaded casein model nanoparticles (NPs) on the REH cell line.**

**Materials and Methods:** **REH cells were treated for 24, 48, and 72 hours with daunorubicin-loaded NP models. Tests for cytotoxicity and mitotoxicity (MTT and Seahorse) were performed. Cell penetration was determined on a fluorescence microscope.**

**Results and Discussion:** **The MTT test showed that with the extension of the treatment time, the cell viability decreased the most in the Cas1-DRB5 model, in which the strongest reduction in mitochondrial indicators was also observed. In the fluorescence analysis, presence in the cells of all investigated models was demonstrated compared to pure casein NPs and pure daunorubicin.**

**Conclusion:** **The engineered NPs show toxic potential in vitro and facilitate penetration by optimizing cellular uptake. This is a prerequisite for developing their modifications with specific antitumor effects.**

**Keywords:** *acute lymphocytic leukemia, daunorubicin, cytotoxic effect*

***Acknowledgments:*** *The research was financed under project FNI KP-06-PN 63/24 to FNI and NUKBPI-BBMRI.BG project, Agreement D01-278/14.12.2022. within the framework of the National Road Map of Scientific Infrastructures.*

# ALTERATION IN MIRNA EXPRESSION IN ORAL CARCINOMA AND ITS CLINICAL SIGNIFICANCE

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## ABSTRACT

**Introduction:** **Squamous cell carcinoma of the oral cavity (oral carcinoma (OC)) is characterized by frequent recurrences and poor survival. The process of carcinogenesis is associated with changes in microRNA (miRNA) expression.**

**Aim:** **The aim of this article is to trace the expression of a panel of miRNAs in OC.**

**Materials and Methods:** **Bioinformatic methods were used to create a panel of miRNAs with possible clinical relevance in OC. Their expression levels were measured by RT-qPCR in tumor and adjacent healthy tissues (n = 32).**

**Results and Discussion:** **Increased expression of miRNA-21-5p ($p < 0.0001$), miRNA-93-5p ($p < 0.0197$), miRNA-146b-5p ($p < 0.0012$), miRNA-155-5p ($p < 0.0001$), miRNA-182-5p ($p < 0.0001$), and miRNA-133b ($p < 0.05$) showed decreased expression. Subsequent validation in cohorts of OC patients from the Regina Elena National Cancer Institute (n = 74) and from the Cancer Genome Atlas Data Portal (n = 354) confirmed our regulation pattern. miRNA-133b ($p < 0.0004$) was more strongly expressed in T1/T2 tumor stages compared to T3/T4. While miRNA-93-5p ($p < 0.0003$), miRNA-133b ($p < 0.0017$), and miRNA-155-5p ($p < 0.0004$) were identified as markers for HPV-containing tumors. The bioinformatic analysis performed showed a correlation between the protein-coding genes regulated by these miRNAs and the survival of OC patients.**

**Conclusion:** **Our results indicate that the panel of studied miRNAs can be used as a reliable tool with diagnostic and prognostic significance in OC.**

**Keywords:** *oral carcinoma, miRNA*

***Acknowledgments:*** *The study was funded through the National Plan for Recovery and Sustainability of the Republic of Bulgaria, project No. BG-RRP-2.004-0007-C01.*

# DECREASED MIRNA-125B AND LET-7 LEVELS IN PATIENTS WITH CHRONIC INSOMNIA—A PILOT STUDY

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## ABSTRACT

**Introduction:** **Chronic insomnia (ChI) is a difficult pathology to diagnose with marked heterogeneity between patients regarding the type of disorder and sleep duration. miRNAs are short, non-protein-coding RNA molecules whose expression is altered in a number of pathological conditions, therefore they may have diagnostic value.**

**Aim:** **The present study aimed to determine specific miRNA expression in plasma and white blood cells (WBC) of patients with ChI.**

**Materials and Methods:** **In order to rule out an underlying sleep disorder in patients (n = 21) and healthy controls (HC) (n = 11), polysomnography (PSG) was performed. Patients were divided into those with shortened (SS) and normal sleep duration (NS). A panel of 10 miRNAs analyzed by TaqMan qPCR assay was created based on literature data.**

**Results and Discussion:** **Our results showed decreased expression of let-7 ($p = 0.034$) in WBC and of miRNA-125b ($p = 0.035$) in plasma of patients. In addition, for let-7, differential expression was found in WBC between SS and NS ($p = 0.035$), with a trend for stronger expression in WBC for siRNA-182 ($p = 0.052$), siRNA-30c ($p = 0.063$) and miRNA-126 ($p = 0.052$) in SS compared to NS patients.**

**Conclusion:** **For the first time in the scientific literature, a decrease in the transcriptional levels of let-7 and miRNA-125b was found in patients with ChI, raising the question of their role in the pathogenesis of the disease.**

**Keywords:** *insomnia, miRNA-125b, let-7*

***Acknowledgments:*** *The study was funded through the National Plan for Recovery and Sustainability of the Republic of Bulgaria, project No. BG-RRP-2.004-0007-C01.*

# MITOCHONDRIAL DYSFUNCTION AND CHRONIC INFLAMMATION IN PATIENTS WITH AUTISM SPECTRUM DISORDER

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## ABSTRACT

**Introduction:** **The autism spectrum disorder (ASD) encompasses a heterogeneous group of disorders in childhood development with complex pathogenesis and unknown etiology. Neuroanatomical and biochemical features result from chronic inflammatory processes, mitochondrial dysfunction, and oxidative stress. The action of various stressors leads to physiological imbalance and induction of the COX-2 protein triggered by activated immune cells. Then follows a positive feedback loop through which the transition from acute to chronic inflammation occurs and the mitochondrial function is damaged.**

**Aim:** **The aim of this article is identification of variations in mitochondrial function and COX-2 levels in children with ASD.**

**Materials and Methods:** **Twenty patients with ASD and 8 healthy controls were examined for mitochondrial activity in peripheral mononuclear cells (PBMCs) by Seahorse XFp (Agilent) and COX-2 gene expression by qPCR.**

**Results and Discussion:** **Increased respiratory reserve capacity, maximal respiration, and COX-2 expression levels were found. There was a negative correlation between respiratory reserve capacity, maximal respiration, and patient communication, as well as between COX-2 and the ADOS scale.**

**Conclusion:** **The study provides new data on changes in bioenergetic indicators and COX-2 levels, confirming the potential role of mitochondrial dysfunction in the pathogenesis of ASD.**

**Keywords:** *autism, COX-2 protein, mitochondrial dysfunction*

***Acknowledgments:*** *The study was supported by the National Institute of Scientific Research under projects No. KP-06-H33/ 6-2019, the European Union - NextGenerationEU, through the National Plan for Recovery and Sustainability of the Republic of Bulgaria, project No. BG-RRP-2.004-0007-C01, NUCBAR-BBMRI.BG, No. D01-285 / 17.12.2019.*

# HIGH YKL-40 EXPRESSION AT THE TUMOR FRONT IN COLORECTAL CARCINOMA

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## ABSTRACT

**Introduction: Colorectal carcinoma (CRC) is the third most common neoplasia and leading cause of death world-while. At present, it is still unclear how genetic alterations in CRC modulate the biology of cancer cells and how they affect their interactions with the tumor microenvironment. Reliable prognostic biomarkers for good stratification and for predicting treatment response are lacking.**

**Aim: The aim of this article is to investigate and evaluate the relationship between YKL-40 expression, tumor budding, and clinicopathological parameters in CRC patient tissues.**

**Materials and Methods: Thirty-one patient samples were studied: tumoral CRC tissue, peritumoral and healthy tissue. YKL-40 expression was assessed immunohistochemically. Tumor differentiation, lymphatic and vascular invasion, and budding were statistically evaluated.**

**Results and Discussion: YKL-40 was quantified in five different areas in each paraffin section—tumor parenchyma, stroma, front, and in normal parenchyma and stroma. Moderate to strong expression of YKL-40 was found in the tumor stroma and at the tumor front in CRC. Despite the apparently higher levels of this chitinase, there was no correlation between expression, tumor stage, and tumor budding.**

**Conclusion: YKL-40 expression and tumor budding in CRC suggest a possible role of the glycoprotein in tumor invasion and address its prognostic significance as a potential biomarker for diagnosis, stratification, and treatment of CRC.**

**Keywords:** *YKL-40, colorectal carcinoma, tumor budding*

***Acknowledgments:*** *The study was financed by the National Science Fund under project KP-06 ПН63/7 -2022, and by the National Plan for Recovery and Sustainability of the Republic of Bulgaria, project No. BG- RRP-2.004-0007-C01.*

# 3D BIOPRINTING OF COLORECTAL CARCINOMA— ASSESSMENT AT THE CELLULAR AND RNA LEVEL

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## ABSTRACT

**Introduction: Experimental model systems for preclinical drug research have serious limitations and only 0.1% of drugs reach clinical application. The introduction of in vitro models that are as close as possible to carcinomas in vivo and have higher predictive properties offer opportunities for individualizing therapy and successful development of new drugs.**

**Aim: The aim of this article is to develop and validate an accessible, modifiable, and reproducible 3D bioprinted model of colorectal carcinoma (CRC).**

**Materials and Methods: Caco-2 cell line, primary CRC samples, Cellink RGD bioink and BioX bioprinter (Cellink, Sweden), fluorescence assessment of cell viability with Calcein AM/PI, histological assessment with H&E staining, chemotherapeutics 5-fluorouracil, oxaliplatin and irinotecan, MTT proliferation assays, RNA-sequencing were used.**

**Results and Discussion: Histological evaluation of Caco-2 cells in 3D bioprints showed formation of glandular structures resembling tumors in vivo. RNA sequencing found increased levels of genes involved in cell adhesion, hypoxia, and in the EGFR/KRAS signaling pathway, and decreased expression of cell cycle-related genes. Testing three of the most commonly used CRC chemotherapeutics revealed increased resistance in 3D bioprinted cells versus 2D cell cultures. Our workflow has been successfully extended to primary CRC samples.**

**Conclusion: We describe the development and validation of a novel, affordable CRC modeling and drug testing platform that represents an innovative opportunity for personalized therapeutic screening.**

**Keywords:** *RNA, colorectal carcinoma, 3D bioprinting*

***Acknowledgments:*** *The study was funded through the National Plan for Recovery and Sustainability of the Republic of Bulgaria, project No. BG-RRP-2.004-0007-C01.*

# IN VITRO DIFFERENTIATION OF CHONDROCYTES FROM ADIPOSE TISSUE

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## ABSTRACT

**Introduction: Cartilage repair and replacement has long been a focus of personalized medicine. The growing frequency of articular cartilage damage necessitates the ex vivo creation of replacement cartilage tissue for the needs of regenerative and aesthetic-reconstructive medicine.**

**Aim: The aim of this article is to standardize a protocol for the production of chondrocytes based on adipose tissue mesenchymal stem cells for in vitro 3D biofabrication of cartilage tissue.**

**Materials and Methods: Patient adipose tissue obtained after liposuction was dissociated into single cells, which were cultured in 12- and 96-well plates in nutrient medium and growth factors for differentiation. Differentiating chondrocytes were examined for morphology, specific staining, and RNA expression.**

**Results and Discussion: After induction of chondrocyte differentiation for 3 weeks, intense alcian blue staining and increased gene expression of the aggrecan and Sox9 chondrocyte markers were observed. The expressed factors provide functionality and vitality to the cells, allowing their proliferation and the production of significant cell mass *in vitro*.**

**Conclusion: The possibility of in vitro differentiation of chondrocytes from autologous adipose tissue is proven, which creates the possibility of accumulating chondrocytes for subsequent 3D bioprinting.**

**Keywords:** *adipose tissue, chondrocytes, in vitro*

***Acknowledgments:*** *The study was funded by the MU-Plovdiv under project NO-04/2021 and by the National Plan for Recovery and Sustainability of the Republic of Bulgaria, project No. BG-RRP-2.004-0007-C01.*

# GENE EXPRESSION OF YKL-40 AND LAMPS IN CENTRAL NERVOUS SYSTEM INFECTIONS

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## ABSTRACT

**Introduction:** **Central nervous system (CNS) infections are associated with high mortality and tremendous social significance owing to the possible permanent neurological sequelae. YKL-40, LAMP-1 and LAMP-2 (LAMPs) are glycoproteins that have shown promise as biomarkers involved in inflammation and autophagic cell death.**

**Aim:** **The aim of our study is to determine the gene expression levels of YKL-40 and LAMPs in patients with neuroinfections.**

**Material and Methods:** **Sixteen patients with CNS infections with undefined etiology, admitted at St. George University Hospital, and 16 healthy subjects as control group, were included in the current study. Blood samples were obtained and total RNA was extracted from white blood cells (WBCs). Following cDNA synthesis, gene expression of YKL-40, LAMP-1, and LAMP-2 was determined by qPCR.**

**Results:** **Our results showed that mRNA levels of YKL-40 were significantly downregulated in WBCs of patients with CNS infections ($p < 0.005$) compared to controls. Decrease in LAMPs expression was also observed. Positive correlation between LAMP-1 and LAMP-2 FC values was detected ($p < 0.05$).**

**Conclusion:** **Decreased mRNA expression of YKL-40 and LAMPs in patients could reflect dysregulation of inflammation and autophagy in the course of CNS infections.**

**Keywords:** *YKL-40, LAMP, central nervous system infections*

***Acknowledgments:*** *The study was financed by MU-Plovdiv under project № 02/2022 and National Recovery and Resilience Plan of the Republic of Bulgaria, Project №BG-RRP-2.004-0007-C01 „Strategic Research and Innovation Program for the Development of MU–PLOVDIV – (SRIPD-MUP)".*