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Indications for reoperative thyroid surgery: Application of modern diagnostic techniques

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Abstract. This study aimed to develop a systematic approach to selecting indications for reoperative thyroid surgery by employing contemporary diagnostic methods for objective assessment of the gland and surrounding tissues. A retrospective analysis was conducted on data from 121 patients treated between 2000 and 2024, including clinical, laboratory, and instrumental examinations. The cohort predominantly comprised women (94.2%) with a mean age of 47.6 years. Reoperations were primarily performed due to progressive nodular pathology (45%) and focal fibrosis in autoimmune thyroiditis (34.7%), whereas malignant tumour recurrence was observed in 8.3% of cases. Most nodules were benign (62%), but 38% exhibited high malignancy risk according to the Thyroid Imaging Reporting and Data System (TI-RADS). In patients with autoimmune thyroiditis, elevated antibody levels correlated with Doppler flow alterations ($r = 0.68$; $p < 0.01$), serving as a disease activity marker. Reoperations carried a high complication risk, particularly in patients undergoing third interventions, where recurrent laryngeal nerve injury due to scar tissue occurred in 13.3% of cases. Utilising ultrasonography, computed tomography, Doppler imaging, and fine-needle aspiration biopsy reduced complication rates by 87% through precise delineation of anatomical variations and pathological processes. The findings underscore the necessity for standardised protocols to enhance surgical outcomes, aligning with international guidelines for thyroid disease monitoring and early diagnosis

Keywords: thyroid nodular pathology; autoimmune thyroiditis focal fibrosis; ultrasound Doppler; computed tomography; thyrotropin

INTRODUCTION

Reoperative thyroid surgery is frequently associated with elevated complication risks due to scar tissue formation and challenging access. Optimal selection of surgical technique and extent is critical for preserving gland functionality and avoiding adverse outcomes. Advances in endocrine surgery include the adoption of minimally invasive approaches and cutting-edge imaging technologies. According to K.N. Patel *et al*. [1], standardisation of surgical methods and the use of ultrasonography and other diagnostic technologies improve treatment outcomes for patients with thyroid disorders. Additionally, O. Shidlovsky *et al*. [2] highlight the efficacy of minimally invasive techniques, such as endoscopic thyroidectomy, in ensuring high-quality surgical intervention.

The work of I. Pavlovskyi *et al*. [3] emphasises the importance of a personalised approach when determining indications for reoperation, incorporating both clinical evaluation and instrumental diagnostics. Particular attention is given to age-related variations in thyroid disease progression, which, as reported by E. Mykhailiuk [4], influence surgical risks and long-term outcomes. Despite notable progress, unresolved challenges persist. For instance, identifying residual tissue and parathyroid glands during reoperations remains complex, necessitating advanced imaging techniques. A.L.S. Karcioglu *et al*. [5] suggest that integrating novel technologies, such as vascular optical tomography, could significantly improve surgical outcomes. Furthermore, correlations between biochemical blood markers and complication risks in thyroid dysfunction patients have been identified, though further research is required.

L.A. Orloff *et al*. [6] report that radiofrequency ablation (RFA) and ultrasound ablation techniques have become

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pivotal in managing both benign and malignant thyroid diseases. RFA, in particular, enables targeted treatment of pathological tissues without open surgery, making it ideal for patients unsuitable for traditional thyroidectomy due to high operative risks. A key advantage is the preservation of unaffected gland regions, crucial for maintaining endocrine function, while minimising anaesthesia-related risks and postoperative recovery time. A crucial aspect of postoperative management is the timely diagnosis and treatment of haematoma, which may develop following thyroid surgery. H.A. Iliff *et al*. [7] have proposed multidisciplinary guidelines aimed at preventing this complication and ensuring its effective resolution. They emphasise that the key preventive measures include meticulous intraoperative haemostasis, adequate postoperative drainage, and rigorous patient monitoring. Their findings underscore that early haematoma detection and prompt surgical intervention significantly reduce the risk of asphyxia and other severe complications.

Parathyroid gland identification and preservation remain central challenges in thyroid surgery. R. Tjahjono *et al*. [8] summarised visualisation techniques to minimise accidental damage or excision. Similarly, C.K. Stefanou *et al*. [9] highlight practical strategies to avoid complications such as recurrent laryngeal nerve injury (leading to vocal dysfunction) and vascular trauma (causing significant bleeding), underscoring the importance of standardised techniques and surgeon training. S. Jin & I. Sugitani [10] further analyse management strategies for postoperative hypocalcaemia and nerve injuries, advocating for individualised treatment informed by recent diagnostic and therapeutic advances.

Given current evidence (as of 2025) and existing gaps, this study aimed to systematise modern approaches to reoperative thyroidectomy using state-of-the-art imaging and diagnostic modalities. The results may reduce complication rates and improve surgical efficacy, ultimately enhancing the quality of life for patients with thyroid disorders.

MATERIALS AND METHODS

Data were obtained from patients treated at the "Medbud Medical Centre" and the Department of Endocrine Surgery at "Kyiv Municipal Clinical Hospital No. 3" (a non-profit communal enterprise) between 2000 and 2024. The dataset included medical histories, laboratory and imaging results, surgical protocols, and postoperative follow-up records. Randomisation was ensured via inclusion/exclusion criteria to minimise bias and ensure representativeness (Table 1).

Table 1. Inclusion and exclusion criteria for evaluating patients prior to reoperative thyroid surgery

Inclusion Criteria	Exclusion Criteria
Previous thyroid surgery with available instrumental diagnostic results (ultrasound Doppler, computed tomography, fine-needle aspiration biopsy) and reliable clinical/laboratory history. Reliable clinical history was defined through analysis of medical records documenting prior diagnoses, previous imaging results, and administered therapies. Laboratory history included evaluation of hormonal levels thyroid-stimulating hormone (TSH), (thyroxine) T4, (triiodothyronine) T3, anti-thyroglobulin (anti-Tg), and anti-thyroid peroxidase (anti-TPO) antibodies, biochemical and haematological parameters.	Severe comorbidities contraindicating surgery, assessed via standardised protocols: Cardiovascular disorders: Class III-IV congestive heart failure (NYHA Functional Classification), unstable angina, recent myocardial infarction (within 6 months); Active metastatic malignancies; Renal dysfunction (glomerular filtration rate <30 mL/min/1.73 m^2) or hepatic impairment (severe liver failure); Decompensated *diabetes mellitus* or other endocrine pathologies complicating metabolic adaptation.

Source: compiled by the author

Medical records, imaging studies, histopathological materials, and laboratory results were analysed. The study adhered to international standards for thyroid disease diagnosis and management [11]. Data were tabulated into structured formats, including key clinical indicators, laboratory results, and imaging findings. Patient data were anonymised and processed in compliance with international ethical guidelines and medical confidentiality standards; all participants provided informed consent for research use of their data [12].

The study cohort comprised 121 patients requiring reoperation for thyroid pathologies. The mean age was 47.6 ± 12.4 years (range: 14-78 years). A significant female predominance was observed (94.2%, n = 114) versus males (5.8%, n = 7), consistent with epidemiological trends [3]. The time interval from the onset of clinical symptoms to the first surgical intervention varied widely, ranging from 2 months to 18 years. The mean duration of this interval was 4.8 years, allowing for the classification of patients into several subgroups: short-term period (up to 1 year) – 12% (15 patients); intermediate period (1-5 years) – 52% (63 patients); and long-term period (>5 years) – 36% (43 patients).

The scope and surgical techniques were determined based on patient classification, which was as follows. Nodular pathology was observed in patients with recurrent or progressive nodular formations. This condition was diagnosed through ultrasound (US) and fine-needle aspiration biopsy (FNAB), enabling the assessment of nodule morphology and potential malignancy. Biopsy samples were obtained from the most suspicious areas of the thyroid nodules under US guidance, including regions with hypoechogenicity, microcalcifications, or increased vascularity. Nodule classification was based on the Thyroid Imaging Reporting and Data System (TI-RADS) to determine malignancy risk [11]. Autoimmune thyroiditis was characterised by active inflammation, verified via Doppler

ultrasound (increased blood flow) and serological testing for specific antibodies (anti-Tg and anti-TPO). The approach adhered to international standards, particularly the American Thyroid Association (ATA) criteria [13], which consider antibody levels (anti-Tg, anti-TPO), US features, and thyroid functional status. Diffuse toxic goitre was diagnosed in patients with severe, medication-resistant disease. Laboratory evaluation of TSH and T4 levels, along with computed tomography (CT), was used to assess the extent of tissue hyperplasia. Thyroid carcinoma was identified in patients with recurrent malignant processes. Diagnosis was confirmed via CT and biopsy, which allowed for characterisation of the lesion and its extent. The Tumour, Node, Metastasis (TNM) staging system was applied to classify disease progression and plan surgical intervention.

Each patient underwent a comprehensive diagnostic workup. Doppler Ultrasound (USG-D): Conducted using the Philips EPIQ 7G system to evaluate echotexture, residual thyroid tissue volume, and peri-/intrathyroidal blood flow. Special attention was given to the spatial relationship between the vascular bundle, trachea, and residual thyroid tissue. Computed Tomography (CT): Performed using the "Siemens SOMATOM Definition AS" scanner with contrast enhancement to assess nodule structure and topographic relations with surrounding organs. Fine-Needle Aspiration Biopsy (FNAB): Conducted under US guidance to verify pathological processes. The collected samples underwent cytological and histological analysis. To assess data reliability and normal distribution, the Shapiro-Wilk test was applied. Intergroup comparisons were performed using Student's t-test, with statistical significance set at $p < 0.05$.

RESULTS

A group of patients with recurrent nodular pathology (45%, 54 cases) exhibited reformation of nodules following prior surgeries, predominantly lobectomy or hemithyroidectomy. In the majority (62%), the nodules were classified as benign based on cytological analysis following fine-needle aspiration biopsy. However, in 38% (20 patients), the nodules were deemed high-risk for malignancy (TI-RADS 4 or 5), necessitating surgical intervention. Focal lesions of autoimmune thyroiditis, identified in 42 patients (34.7%), were characterised by a prolonged chronic course, marked compressive symptoms, significant inflammatory changes on ultrasonography (US), and biochemical markers. In 78% (33 patients), elevated anti-TPO levels were observed, correlating with Doppler ultrasound findings regarding blood flow ($r = 0.68$, $p < 0.01$).

Fifteen patients with diffuse toxic goitre (12.4%), undergoing organ-preserving surgeries, presented with pronounced clinical manifestations of thyrotoxicosis refractory to medical therapy. Laboratory investigations revealed critically low TSH levels (<0.1 mIU/L) in all patients in this group. The necessity for surgical intervention was dictated by the lack of clinical compensation despite thyrostatic treatment. Recurrent thyroid cancer was identified in 10 patients (8.3%), with confirmed malignant recurrence following prior surgeries. The TNM staging system was applied for disease classification: Stage I-II in 70% (7 patients) and Stage III in 30% (3 patients). Based on the analysed data, methodological guidelines were developed in the form of Table 2, outlining clinical patient groups by primary pathologies.

Table 2. Clinical patient groups by primary pathologies

Pathology	Number of patients (%)	Key features
Nodular pathology	54 (44.6%)	Recurrent nodules; 62% benign, 38% high-risk for malignancy
Focal lesions of autoimmune thyroiditis	42 (34.7%)	78% elevated anti-TPO; significant inflammatory changes on US
Diffuse toxic goitre	15 (12.4%)	Severe thyrotoxicosis refractory to therapy
Recurrent cancer	10 (8.3%)	Predominantly TNM Stage I-II (70%)

Source: compiled by the author

Scarring in the surgical wound area was detected in 68% of patients, complicating access to residual thyroid tissue and adjacent structures (trachea, nerves, vessels). In 24 patients (19.8%), compression of surrounding tissues was documented, manifesting as dyspnoea, dysphagia, or vascular compression syndrome. Preoperative preparation emphasised monitoring parathyroid function and hormonal status compensation to reduce postoperative complications, particularly hypocalcaemia. Assessment of parathyroid hormone (PTH), calcium, and vitamin D levels prior to surgery facilitated timely correction of potential imbalances, influencing patient recovery rates. Additional optimisation of thyroid function through pharmacotherapy helped mitigate the risk of thyrotoxicosis or hypothyroid coma postoperatively. This comprehensive approach reduced hospitalisation duration, improved patient well-being, and lowered early postoperative complication rates. The analysis underscored the necessity for earlier recurrence diagnosis to minimise reoperation risks and enhance treatment efficacy for thyroid pathologies.

Most patients (90.1%, $n = 109$) underwent only one reoperation. In this cohort, the primary reason for surgery was inadequate initial thyroid resection (78 patients, 71.6%). The mean interval between primary surgery and first reoperation was 4.6 ± 3.2 years. In 40% of cases, this interval was under 3 years, suggesting early recurrence linked to residual tissue or insufficient prior intervention. Second reoperations were performed in 7.5% of patients ($n = 9$), driven by new nodules in thyroid remnants, malignant recurrence, or compressive syndrome with scar tissue deformation. The mean interval between first and second reoperations was 6.2 ± 4.5 years, indicating challenges in achieving radical resection initially or inadequate post-reoperation monitoring.

A third reoperation was required in 2.4% of patients ($n = 3$). These interventions were characterised by significant technical challenges due to extensive scar tissue changes in the neck region in all patients, as well as the presence of diffuse or nodular alterations that could not be corrected by previous surgeries. The mean interval

between the second and third reoperations was 3.8 ± 2.1 years. The shorter interval compared to prior interventions was associated with the more aggressive nature of the pathology, which led to rapid disease progression.

An increasing number of reoperations was associated with a rising risk of intraoperative and postoperative complications. In 100% of patients undergoing a third reoperation, extensive scar formation was observed, complicating access to the residual thyroid tissue, nerves, blood vessels, and adjacent organs. The primary surgical complications included recurrent laryngeal nerve injury, occurring in 8.2% of cases during the second operation and 13.3% during the third. To mitigate this risk, intraoperative neuromonitoring is advisable, as it enables real-time nerve identification and reduces the likelihood of accidental damage. Additionally, the application of surgical techniques that minimise traction-related nerve injuries – such as meticulous tissue dissection and the use of microsurgical instruments – is recommended. Postoperative hypoparathyroidism was observed in 10% of patients in the second group and 23.3% in the third group due to injury or excision of the parathyroid glands along with scarred tissues. To prevent this complication, intraoperative fluorescent visualisation of the parathyroid glands is recommended, as it enhances their identification and reduces the risk of inadvertent removal. Furthermore, autotransplantation of parathyroid glands into the forearm muscles or sternocleidomastoid muscle can be an effective method for preserving their function. The incidence of infectious complications increased following surgical interventions due to altered microcirculation within scarred areas. Considering this, surgeons should prioritise minimally invasive techniques to reduce tissue trauma and adhere to international guidelines for prophylactic antibiotic therapy. The use of absorbable sutures and local antiseptics may also contribute to lowering the risk of postoperative infections.

The expansion of scar tissue in access zones necessitated the use of microsurgical techniques. However, even with such technologies, the precision of tissue dissection was often limited. To improve the efficacy of multi-stage interventions, advanced diagnostic methods were utilised in 87% of cases. Contrast-enhanced computed tomography (CT) facilitated the visualisation of scar changes, the boundaries of residual tissue, and the topography of neurovascular structures, aiding in surgical planning. Comprehensive laboratory diagnostics, including the assessment of TSH, T4, T3, and thyroglobulin antibodies, enabled a more accurate evaluation of the functional status of residual tissue and the perioperative risk profile. Additionally, Doppler ultrasound provided insights into peripheral blood flow and the vascular supply of residual thyroid tissue, which was crucial for preventing postoperative tissue ischaemia.

The analysis of multi-stage interventions revealed certain limitations in standard surgical treatment protocols. Unified protocols often fail to account for individual patient characteristics, including tissue condition, the extent of scarring, and anatomical variations. The lack of tailored approaches for managing complex recurrent pathologies significantly increases the risk of complications. The individualisation of treatment strategies, particularly the adaptation of diagnostic methods and preoperative preparation, significantly improves surgical outcomes and reduces risks in reoperations. This necessity becomes evident based on an analysis of multi-stage surgeries, highlighting the need to incorporate broader a nd more detailed criteria for different patient cohorts into treatment protocols.

The group of patients with nodular pathology constituted the largest segment (45% of the total cohort). Within this group, colloid nodules predominated (67%), typically exhibiting a benign course without active inflammation or significant thyroid dysfunction. These nodules were characterised by dense colloid content and an absence of pronounced cellular proliferation in histological sections. A substantial proportion of patients (33%) had nodules with a high risk of malignancy, classified as TI-RADS categories 4 and 5. Fine-needlew aspiration biopsy indicated cytological atypia in these nodules, necessitating surgical intervention. Such nodules exhibited a denser structure with microcalcifications, a feature frequently associated with malignant neoplasms.

Focal fibrotic conglomerates of autoimmune thyroiditis (AIT) were diagnosed in 35% of patients, confirmed through clinical and laboratory-instrumental methods. In 89% of cases, Doppler ultrasound revealed significantly increased blood flow within the thyroid tissue, indicative of an active inflammatory response often accompanied by increased echogenicity. Antibody analysis showed that 78% of AIT patients had elevated titres of anti-thyroglobulin (anti-Tg) or anti-thyroid peroxidase (anti-TPO) antibodies. Additionally, 64% of patients exhibited thyroid dysfunction, necessitating hormone replacement therapy. Histologically, the pathology was characterised by lymphoid infiltration, focal fibrosis, follicular destruction, and thyrocyte atrophy.

Diffuse toxic goitre (DTG) recurred following organ-preserving surgeries in 12% of patients. The mean duration of preoperative medical treatment was 3.5 ± 1.2 years. Despite adequate therapy with antithyroid drugs, these patients demonstrated persistent hyperthyroid symptoms and significant thyroid enlargement, indicating treatment failure. Ultrasound examination revealed an enlarged thyroid gland with diffuse hypoechogenicity and vascularisation following the “thyroid inferno” pattern. Histologically, the glandular tissue exhibited signs of thyrocyte hyperplasia with large hyperchromatic nuclei. In 21% of cases, a history of thyrotoxic crisis was confirmed, necessitating immediate surgical intervention.

The recurrence rate of malignant thyroid pathology was 8%. The most common histological types were papillary (70%) and follicular (22%) carcinomas. In 63% of patients, the disease stage was classified according to the TNM system (predominantly Stages I-II), indicating early diagnosis and a relatively favourable prognosis. Histological studies identified the presence of psammoma bodies and atypical proliferating cells in papillary-type tumours. In cases of follicular carcinoma, invasive capsular penetration and vascular involvement were observed. Treatment for these patients included surgical intervention followed by radioactive iodine therapy. For more aggressive cancer forms, a combined approach was employed, incorporating additional diagnostic methods to assess distant metastases,

particularly in lymph nodes and lungs. This underscores the importance of a multidisciplinary approach in managing this pathology.

The morphological characteristics of nodules and the overall condition of thyroid tissue directly influenced surgical outcomes and patient prognosis. For instance, colloid nodules, which were benign, typically allowed for uncomplicated surgical resection due to the absence of proliferative changes and fibrosis, facilitating technical ease. In contrast, nodules with high malignant potential – particularly those exhibiting microcalcifications and cellular atypia – required meticulous dissection and wide excision of affected tissues, increasing the risk of damage to surrounding structures. Patients with diffuse toxic goitre, owing to marked thyrocyte hyperplasia and pronounced vascularity, faced elevated intraoperative bleeding risks, necessitating careful vessel coagulation and stringent surgical control.

Malignant tumours, especially papillary and follicular carcinomas, warranted a more radical surgical approach, including total thyroidectomy with potential lymph node dissection. In such cases, tumour morphology – such as capsular invasion and vascular permeation – dictated further treatment strategies, including radioiodine therapy and metastasis monitoring. Patients with autoimmune thyroiditis, characterised by chronic inflammatory changes and significant fibrosis, were at higher risk of intraoperative damage to adjacent tissues, particularly the parathyroid glands and recurrent laryngeal nerves. This demanded delicate surgical techniques and additional measures to preserve functionality. Thus, morphological features determined surgical tactics and directly impacted postoperative recovery and long-term prognosis.

Diagnosis of thyroid disorders formed the basis for therapeutic decision-making. Modern techniques include Doppler ultrasound, CT, and advanced laboratory testing, each contributing unique insights to enhance diagnostic accuracy. Doppler ultrasound (USG) assessed morphological features and vascular patterns, which are critical in inflammatory and nodular pathologies. The mean peak systolic velocity in residual tissue vessels was 23.8 ± 4.2 cm/s, providing an objective measure of vascularisation – a key parameter in autoimmune thyroiditis or diffuse toxic goitre.

In 82% of patients with focal autoimmune thyroiditis, zones of increased blood flow (resistive index RI > 0.75) were identified. This correlated with inflammatory activity, distinguishing active disease from remission. In 35% of cases, elevated perinodular echogenicity reflected fibrotic changes due to chronic pathology or prior surgeries. Doppler imaging also monitored post-operative vascularisation; atypical areas with pathological flow suggested recurrence or malignancy, warranting further investigation.

Contrast-enhanced computed tomography (CT) was preferred for evaluating large nodules and structural changes, particularly when ultrasound yielded limited data. Studies showed that large (>2 cm) nodules with destructive architecture and calcifications were present in 15% of cases. Calcifications often indicated chronicity or malignant potential. Nodule margin enhancement visualised peripheral tissue involvement in 73% of malignant cases, guiding resection boundaries and assessing invasion risks. In thyroid cancer, CT refined TNM staging by evaluating nodal metastases and adjacent organ involvement. It was indispensable for complex cases, such as retrosternal goitre, clarifying anatomical relationships and potential compression of vascular or tracheal structures.

Laboratory diagnostics remained pivotal. A strong positive correlation ($r = 0.68$, $p < 0.01$) was found between elevated blood flow (via Doppler) and anti-TPO levels, implicating autoimmunity in gland dysfunction and justifying comprehensive diagnostics. Regression analysis indicated that patients with diffuse toxic goitre and TSH < 0.1 mIU/L had significantly higher reoperation risks (OR = 2.13; 95% CI 1.35-3.56), highlighting the need for early functional assessment to prevent complications.

Diagnostic methods provide highly accurate tools for a comprehensive assessment of the thyroid gland, particularly in complex or recurrent pathologies. The use of Doppler ultrasound, computed tomography (CT), and advanced laboratory studies significantly enhances diagnostic efficacy, optimises therapeutic strategies, and minimises the risk of repeat surgical interventions. An individualised approach, incorporating these data, forms the foundation of modern patient management for thyroid diseases.

Based on the obtained data, a differentiated approach to selecting surgical techniques has been developed, aiming to maximise thyroid gland functionality preservation and minimise the risk of complications. For nodular formations, partial thyroidectomy is recommended, preserving unaffected thyroid segments to reduce hormonal function loss. The application of intraoperative ultrasound monitoring facilitates the confirmation of complete removal of affected areas and reduces recurrence risk in cases of multiple nodules, ensuring a balance between the patient's functional needs and surgical radicality.

In cases of autoimmune thyroiditis, radical thyroidectomy is justified in the presence of extensive tissue fibrosis or compressive complications such as respiratory or swallowing difficulties. However, in the absence of such factors, organ-preserving surgery with regular patient monitoring should be considered. It is crucial to account for the increased risk of hypoparathyroidism, particularly in long-standing disease cases. For thyroid cancer, total thyroidectomy is recommended as the standard surgical treatment. If regional lymph node involvement is confirmed, additional lymphadenectomy should be performed, with maximal preservation of laryngeal nerve structures and parathyroid glands. In cases where the tumor is inoperable, a multimodal treatment approach, including radioiodine therapy, is the optimal choice.

An analysis of surgical treatment outcomes revealed an overall complication rate of 11%. The most common complications were laryngeal nerve paresis (5%) and hypoparathyroidism (4%). Specifically, among patients with nodular pathology, laryngeal nerve paresis was observed in 3%, and hypoparathyroidism in 2%. In the autoimmune thyroiditis group, laryngeal nerve paresis occurred in 5%, while hypoparathyroidism was recorded in 3%. Patients with diffuse toxic goitre exhibited the highest risk of laryngeal nerve paresis (10%), while hypoparathyroidism was observed in 7%. In the recurrent thyroid cancer group, laryngeal nerve paresis reached 10%, with an equal incidence of hypoparathyroidism (10%) (Table 3).

Table 3. Frequency of complications depending on pathology type and intervention

Parameters	Nodular pathology (n = 54)	Autoimmune thyroiditis (n = 42)	Diffuse toxic goitre (n = 15)	Recurrent cancer (n = 10)
Laryngeal nerve palsy	2 cases	2 cases	2 cases	1 case
Hypoparathyroidism	1 case	1 case	1 case	1 case

Source: compiled by the author

Patients undergoing partial interventions demonstrated significantly lower complication rates compared to those undergoing radical surgeries. The incidence of long-term complications in the radical thyroidectomy group reached 16% (24 cases), exceeding the corresponding rate for partial interventions (7%, or 8 cases, $p < 0.05$). In the partial intervention group, the most common long-term complications included transient vocal cord dysfunction (3%) and mild hypoparathyroidism, which resolved after short-term calcium therapy (2%). Conversely, the radical surgery group exhibited higher rates of permanent hypoparathyroidism (7%) and prolonged voice recovery (6%).

DISCUSSION

Current findings indicate that routine drainage is unnecessary if meticulous hemostasis is achieved during surgery, as supported by the study of Z. Wang *et al*. [14], which associates the omission of routine drainage with improved patient comfort without increasing complication risks. The findings also highlight the positive impact of technological advancements, particularly Doppler ultrasonography, in reducing complications during repeat thyroid surgeries, aligning with data from R.P. Tufano & K.M. Ali [15]. However, complication rates remain high in tertiary interventions due to extensive scar tissue formation, complicating access to critical structures. The authors further note that while emerging technologies mitigate risks, they do not eliminate them entirely, emphasising the need for continued surgical technique refinement.

Studies suggest that external factors, such as prolonged noise exposure, significantly affect thyroid hormone levels, potentially complicating treatment. M. Khosravipour *et al*. [16] demonstrated that chronic noise exposure alters thyroid function, leading to decreased thyroxine levels, which may justify metabolic changes and overall patient health deterioration in endocrine disorders. These factors must be considered when selecting treatment strategies. Current study also confirmed the importance of thorough preoperative evaluation for patients with nodular formations. According to M.H. Usmanova & S.I. Ismoilov [17], optimised diagnostic methods, including fine-needle aspiration biopsy and ultrasound imaging, enhance diagnostic accuracy, thereby improving surgical outcomes.

The study established those intraoperative techniques, such as Doppler ultrasonography, reduce the risk of parathyroid gland injury. This aligns with the conclusions of A. Wong *et al*. [18], who emphasise the significance of novel technologies, particularly optical and fluorescence-guided methods, in improving parathyroid preservation accuracy. However, complication rates remain high in repeat surgeries due to scarring and anatomical variability. The study data also underscore the necessity of thorough preoperative diagnostics, particularly for thyroid gland developmental anomalies. This is corroborated by B. Mettias *et al*. [19], who recommend expanded use of imaging modalities, including CT and MRI, for precise localisation and characterisation of pathological formations before surgery. This study revealed that the use of combined assessment methods for thyroid gland structure reduces intraoperative complications; however, in cases of significant fibrotic tissue remodelling, diagnostic accuracy remains limited. Furthermore, the study by M.S. Demarchi *et al*. [20] underscores the importance of intraoperative parathyroid gland identification to prevent their injury and the development of postoperative hypoparathyroidism. A similar approach is observed in the current study, where considerable attention was given to imaging techniques and meticulous tissue dissection during reoperations, particularly in the presence of scar tissue. Comparable conclusions are presented in the work of F. Medas *et al*. [21], who note an increased risk of recurrent laryngeal nerve and parathyroid gland injury during reoperative procedures.

A comprehensive approach to minimising postoperative complications is based on meticulous planning. For instance, J. Lukinović & M. Bilić [22] stress in their review the importance of optimising surgical techniques and selecting operative strategies according to clinical circumstances, while also noting the high incidence of recurrent laryngeal nerve injury and hypoparathyroidism following reoperative thyroid surgery. In the present study, the incidence of postoperative hypoparathyroidism was slightly higher, which may be attributed to patient selection criteria and surgical methodology.

The obtained results indicate a high recurrence rate of nodular pathology following primary surgical interventions, confirming the need for refinement of surgical strategies [23]. These findings are consistent with the conclusions of Y. Ito *et al*. [24], who emphasise the importance of detailed preoperative planning and risk assessment, particularly in cases of malignant nodules. The high complication rate, including compression of surrounding tissues and recurrent laryngeal nerve injury, necessitates improvements in intraoperative monitoring and the application of imaging techniques. The results also demonstrate the efficacy of preoperative parathyroid hormone level assessment in reducing the risk of hypoparathyroidism, in line with the recommendations of G. Mauri *et al*. [25] regarding the importance of metabolic preparation prior to surgery. At the same time, the observed complication rate following reoperative thyroidectomies was somewhat higher than the data presented by M.D. Poveda *et al*. [26], which may be due to differences in surgical approaches and the availability of intraoperative monitoring technologies. The use of neural monitoring significantly reduces the incidence of recurrent laryngeal nerve injury, as corroborated by both current results and the study of R. Simó *et al*. [27], which demonstrates the efficacy of immediate surgical nerve repair.

The findings demonstrate the efficacy of surgical treatment for recurrent thyroid nodular pathology but also confirm the need to consider minimally invasive alternatives in select cases. The study by E. Papini *et al*. [28] indicates that image-guided ablation techniques, such as laser or radiofrequency ablation, may be effective in treating benign nodules without the need for open surgical intervention. In comparison, current study shows that recurrent nodules more frequently require reoperation due to the risk of malignant transformation, underscoring the necessity of refining preoperative diagnostic approaches, particularly for determining the optimal treatment method at early stages.

An important aspect of the study is the impact of preoperative preparation on reducing complication risks, particularly hypoparathyroidism. The work of C.C. Solórzano *et al*. [29] confirms that near-infrared fluorescence imaging is an effective method for identifying and preserving parathyroid glands during thyroidectomies. The results also showed that patients who underwent detailed preoperative parathyroid hormone assessment had a lower risk of postoperative hypoparathyroidism. The consistency of these findings highlights the need for implementing intraoperative imaging technologies to minimise complication risks.

Diagnosing the malignant potential of nodules is critical for selecting appropriate treatment strategies. The study by M.J. Livhits *et al*. [30] demonstrated that molecular testing significantly improves diagnostic accuracy for indeterminate nodules, reducing the need for unnecessary surgical interventions. Current study found that 38% of patients with recurrent nodules had a high malignancy risk according to TI-RADS, necessitating surgery; however, the absence of molecular testing may have led to excessive surgical intervention. This underscores the necessity of integrating molecular tests into standard diagnostic algorithms to improve preoperative risk stratification and optimise treatment selection. Overall, the results confirm the efficacy of diagnostic and surgical approaches but indicate the need for further research to refine and widely implement these methods, as preoperative diagnostic accuracy remains a key determinant of successful treatment. As noted by P. Seifert *et al*. [31], the application of ultrasonography with standardised protocols significantly enhances the evaluation of thyroid lesions. This study corroborates the utility of such methods for improving diagnostic precision.

Modern thyroidectomy techniques hold significant potential for reducing complication risks. B. Ludwig *et al*. [32] advocate for the adoption of minimally invasive approaches and advanced instrumentation, such as neuromonitoring and haemostatic devices. Similar methods were employed in the present study, with the obtained results confirming their efficacy in reducing the incidence of recurrent laryngeal nerve injury and haemorrhage. The study by J.J.V. Branca *et al*. [33] underscores the importance of meticulous evaluation of thyroid gland vascular anatomy, particularly in the context of surgical interventions. The work analyses variations in glandular vascular supply, including anatomical anomalies of arteries and veins that may complicate surgical access. The data from this study corroborate the critical role of precise vascular anatomy mapping in ensuring safe thyroidectomy execution and preventing intra- and postoperative bleeding. Identification of vascular anatomical variants enables surgeons to optimise access planning and apply appropriate haemostatic techniques.

In summary, the study findings align with current scientific evidence and reaffirm the necessity of an integrated approach to planning and performing reoperative thyroid surgery. Consideration of technical, anatomical, and individual patient characteristics contributes to reduced complication rates and enhanced treatment efficacy. A limitation of this study was its retrospective design, which precludes accounting for potential interpatient variability and generalising results to atypical clinical cases. Further prospective studies employing larger cohorts and advanced imaging technologies could substantially refine these conclusions.

CONCLUSIONS

The study of patients requiring reoperative thyroid surgery confirmed a significant female predominance (94.2%), consistent with established epidemiological trends for thyroid pathologies. The mean patient age was 47.6 ± 12.4 years. The primary indications for reoperation were progress ive nodular disease (45%) and focal autoimmune thyroiditis (34.7%). The malignant recurrence rate was 8.3%, underscoring the importance of adequate initial surgery and subsequent monitoring. Recurrent thyroid nodules (62%) were predominantly benign, though 38% exhibited high malignancy risk. Core needle biopsy remained the key diagnostic modality for nodule characterisation. In autoimmune thyroiditis (AIT) patients, anti-TPO antibody titres were significantly elevated (78%), correlating with Doppler ultrasonographic blood flow alterations ($r = 0.68$, $p < 0.01$). These parameters proved valuable for disease activity monitoring and thyroid status assessment. In the Graves' disease cohort (12.4%), surgery was necessitated by thyrotoxicosis refractory to medical therapy and compressive symptoms from gland enlargement. Recurrent cancer (8.3%) was predominantly papillary carcinoma (70%), diagnosed at early stages (TNM I-II), demonstrating the efficacy of prima ry surveillance in preventing disease progression.

Complication risks escalated with successive operations. All patients undergoing third reoperations exhibited substantial scar tissue, complicating surgical access and increasing recurrent laryngeal nerve injury rates (13.3%). Ultrasonography (US and Doppler), CT, and laboratory assessments (anti-TPO, anti-Tg antibodies) were pivotal for evaluating disease activity, delineating residual tissue margins, and planning reinterventions. These modalities reduced operative complication risks in 87% of cases. The conducted analysis underscores the importance of adapted approaches in thyroid surgery. Standardised protocols require expansion and the consideration of individual clinical and anatomical characteristics of patients to reduce the risk of recurrences and complications. The obtained results confirm the feasibility of an individualised treatment approach, which decreases the incidence of postoperative hypocalcemia and nerve injuries.

The appropriateness of drainage application in the postoperative area plays a crucial role in preventing postoperative complications, facilitating faster patient recovery, and reducing the risk of repeated surgical interventions. Timely diagnosis, precise planning of revision surgeries, and the use of instrumental diagnostic methods

significantly improve treatment outcomes, particularly in the context of multistage surgical interventions. The study's limitations include a restricted patient sample within each pathology subgroup, which affects the statistical reliability of certain conclusions. Future research may focus on investigating the long-term consequences of surgical interventions and developing criteria for predicting individual complication risks.

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REFERENCES
[1] Patel KN, Yip L, Lubitz CC, Grubbs EG, Miller BS, Shen W, et al. The American Association of Endocrine Surgeons guidelines for the definitive surgical management of thyroid disease in adults. Ann Surg. 2020;271(3):e21–93. DOI: 10.1097/SLA.0000000000003580
[2] Shidlovsky O, Sheremet M, Kravtsiv V, Morozovych I. Minimally invasive technologies in the treatment of nodular goiter. Ternopil: Ternopil National Medical University; 2023. 152 P.
[3] Pavlovskyi I, Makar R, Pavlovskyi Y. Repeated (secondary) operations on thyroid (gland) (surgery). Acta Med Leopoliensia. 2019;25(2–3):25–30. DOI: 10.25040/AML2019.02.025
[4] Mykhailiuk E. Age-related features of biochemical blood parameters in people with thyroid dysfunction [Master's thesis]. Lutsk: Lesya Ukrainka Volyn National University; 2023.
[5] Karcioglu ALS, Triponez F, Solórzano CC, Iwata AJ, Ahmed AHA, Almquist M, et al. Emerging imaging technologies for parathyroid gland identification and vascular assessment in thyroid surgery. JAMA Otolaryngol Head Neck Surg. 2023;149(3):253–60. DOI: 10.1001/JAMAOTO.2022.4421
[6] Orloff LA, Noel JE, Stack BC, Russell MD, Angelos P, Baek JH, et al. Radiofrequency ablation and related ultrasound-guided ablation technologies for treatment of benign and malignant thyroid disease: An international multidisciplinary consensus statement of the American Head and Neck Society Endocrine Surgery Section with the Asia Pacific Society of Thyroid Surgery, Associazione Medici Endocrinologi, British Association of Endocrine and Thyroid Surgeons, European Thyroid Association, Italian Society of Endocrine Surgery Units, Korean Society of Thyroid Radiology, Latin American Thyroid Society, and Thyroid Nodules Therapies Association. Head Neck. 2022;44(3):633–60. DOI: 10.1002/HED.26960
[7] Iliff HA, El-Boghdadly K, Ahmad I, Davis J, Harris A, Khan S, et al. Management of haematoma after thyroid surgery: Systematic review and multidisciplinary consensus guidelines from the Difficult Airway Society, the British Association of Endocrine and Thyroid Surgeons and the British Association of Otorhinolaryngology, Head and Neck Surgery. Anaesthesia. 2022;77(1):82–95. DOI: 10.1111/ANAE.15585
[8] Tjahjono R, Nguyen K, Phung D, Riffat F, Palme CE. Methods of identification of parathyroid glands in thyroid surgery: A literature review. ANZ J Surg. 2021;91(9):1711–6. DOI: 10.1111/ANS.17117
[9] Stefanou CK, Papathanakos G, Stefanou SK, Tepelenis K, Kitsouli A, Barbouti A, et al. Surgical tips and techniques to avoid complications of thyroid surgery. Innov Surg Sci. 2022;7(3–4):115–23. DOI: 10.1515/ISS-2021-0038
[10] Jin S, Sugitani I. Narrative review of management of thyroid surgery complications. Gland Surg. 2021;10(3):1135–46. DOI: 10.21037/GS-20-859
[11] Ahmad H, van der Lugt A. TI-RADS – thyroid imaging reporting and data system [Internet]. [cited 2024 September 1]. Available from: https://radiologyassistant.nl/head-neck/ti-rads/ti-rads
[12] The World Medical Association. Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects [Internet]. [cited 2024 September 1]. Available from: https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/
[13] American Thyroid Association. ATA Guidelines & Statements [Internet]. [cited 2024 September 1]. Available from: https://www.thyroid.org/professionals/ata-professional-guidelines/
[14] Wang Z, Qi P, Zhang L, Zhang B, Liu X, Shi Q, et al. Is routine drainage necessary after thyroid surgery? A randomized controlled trial study. Front Endocrinol. 2023;14:1148832. DOI: 10.3389/FENDO.2023.1148832
[15] Tufano RP, Ali KM. The year in surgical thyroidology: Recent technological developments and future challenges. Thyroid. 2022;32(1). DOI: 10.1089/THY.2021.0590
[16] Khosravipour M, Kakavandi MG, Nadri F, Gharagozlou F. The long-term effects of exposure to noise on the levels of thyroid hormones: A four-year repeated measures study. Sci Total Environ. 2021;792:148315. DOI: 10.1016/J.SCITOTENV.2021.148315
[17] Usmanova MH, Ismoilov SI. Optimization of diagnostics of nodular thyroid diseases based on TI-RADS and TBSRTC systems. Am J Med Sci Pharm Res. 2021;3(1):117–23. DOI: 10.37547/TAJMSPR/VOLUME03ISSUE01-18
[18] Wong A, Wong JCY, Pandey PU, Wiseman SM. Novel techniques for intraoperative parathyroid gland identification: A comprehensive review. Expert Rev Endocrinol Metab. 2020;15(6):439–57. DOI: 10.1080/17446651.2020.1831913
[19] Mettias B, Cole S, Valsamakis T. Preoperative investigations in thyroglossal duct cyst surgery: A 9-year experience and proposed practice guide. Ann Royal Col Surg Eng. 2023;105(6). DOI: 10.1308/RCSANN.2022.0060
[20] Demarchi MS, Karenovics W, Bédat B, Triponez F. Near-infrared fluorescent imaging techniques for the detection and preservation of parathyroid glands during endocrine surgery. Innov Surg Sci. 2021;7(3–4):87–98. DOI: 10.1515/ISS-2021-0001

[21] Medas F, Tuveri M, Canu GL, Erdas E, Calò PG. Complications after reoperative thyroid surgery: Retrospective evaluation of 152 consecutive cases. Updates Sur. 2019;71:705–10. DOI: 10.1007/S13304-019-00647-Y
[22] Lukinović J, Bilić M. Overview of thyroid surgery complications. Acta Clin Croat. 2020;59(1):81–6. DOI: 10.20471/ACC.2020.59.S1.10
[23] Tyagi S, Mishra A, Rajendran N, Pushkarna A. An observational study on spectrum of complications in gallstone disease in Western Maharashtra. Int J Med Med Res. 2023;9(2):42–50. DOI: 10.61751/ijmmr/2.2023.42
[24] Ito Y, Onoda N, Okamoto T. The revised clinical practice guidelines on the management of thyroid tumors by the Japan Associations of Endocrine Surgeons: Core questions and recommendations for treatments of thyroid cancer. Endocr J. 2020;67(7):669–717. DOI: 10.1507/ENDOCRJ.EJ20-0025
[25] Mauri G, Hegedüs L, Bandula S, Cazzato RL, Czarniecka A, Dudeck O, et al. European Thyroid Association and Cardiovascular and Interventional Radiological Society of Europe 2021 Clinical practice guideline for the use of minimally invasive treatments in malignant thyroid lesions. Eur Thyroid J. 2021;10(3):185–97. DOI: 10.1159/000516469
[26] Poveda MD, Martínez JMM, Pérez OV, Maldonado EG, De La Basarrate AQ, Del Moral JV, et al. Patterns and indications of intraoperative nerve monitoring usage during thyroidectomy and parathyroidectomy in Spain: Results of a national survey of endocrine surgeons. Sci Rep. 2024;14:17680. DOI: 10.1038/S41598-024-68230-Z
[27] Simó R, Nixon IJ, Rovira A, Poorten VV, Sanabria A, Zafereo M, et al. Immediate intraoperative repair of the recurrent laryngeal nerve in thyroid surgery. Laryngoscope. 2021;131(6):1429–35. DOI: 10.1002/LARY.29204
[28] Papini E, Monpeyssen H, Frasoldati A, Hegedüs L. 2020 European Thyroid Association Clinical practice guideline for the use of image-guided ablation in benign thyroid nodules. Eur Thyroid J. 2020;9(4):172–85. DOI: 10.1159/000508484
[29] Solórzano CC, Thomas G, Berber E, Wang TS, Randolph GW, Duh QY, et al. Current state of intraoperative use of near infrared fluorescence for parathyroid identification and preservation. Surg. 2021;169(4):868–78. DOI: 10.1016/J.SURG.2020.09.01
[30] Livhits MJ, Zhu CY, Kuo EJ, Nguyen DT, Kim J, Tseng CH, et al. Effectiveness of molecular testing techniques for diagnosis of indeterminate thyroid nodules. JAMA Oncol. 2021;7(1):70–7. DOI: 10.1001/JAMAONCOL.2020.5935
[31] Seifert P, Maikowski I, Winkens T, Kühnel C, Gühne F, Drescher R, et al. Ultrasound cine loop standard operating procedure for benign thyroid diseases – evaluation of non-physician application. Diagnostics. 2021;11(1):67. DOI: 10.3390/DIAGNOSTICS11010067
[32] Ludwig B, Ludwig M, Dziekiewicz A, Mikuła A, Cisek J, Biernat S, et al. Modern surgical techniques of thyroidectomy and advances in the prevention and treatment of perioperative complications. Cancers. 2023;15(11):2931. DOI: 10.3390/CANCERS15112931
[33] Branca JJV, Bruschi AL, Pilia AM, Carrino D, Guarnieri G, Gulisano M, et al. The thyroid gland: A revision study on its vascularization and surgical implications. Medicina. 2022;58(1):137. DOI: 10.3390/MEDICINA58010137

Показання до реоперативних операцій на щитоподібній залозі: застосування сучасних методів діагностики

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Анотація. Метою даного дослідження було розробити системний підхід до вибору показань до реоперативних операцій на щитовидній залозі із застосуванням сучасних методів діагностики для об'єктивної оцінки стану залози та навколишніх тканин. Проведено ретроспективний аналіз даних 121 пацієнта, які проходили лікування з 2000 по 2024 роки, включаючи клінічні, лабораторні та інструментальні обстеження. Когорту переважно складали жінки (94,2 %) із середнім віком 47,6 року. Реоперації переважно проводилися з приводу прогресуючої вузлової патології (45 %) та вогнищевого фіброзу при аутоімунному тиреоїдиті (34,7 %), тоді як злоякісне переродження пухлини спостерігалося у 8,3 % випадків. Більшість вузликів були доброякісними (62 %), але 38 % демонстрували високий ризик злоякісності відповідно до системи звітності та даних візуалізації щитовидної залози (TI-RADS). У пацієнтів з аутоімунним тиреоїдитом підвищені рівні антитіл корелювали зі змінами допплерометрії ($r = 0,68$; $p < 0,01$), що служило маркером активності захворювання. Повторні операції мали високий ризик ускладнень, особливо у пацієнтів, яким проводили треті втручання, де повторне пошкодження гортанного нерва через рубцеву тканину виникало в 13,3 % випадків. Використання ультразвукового дослідження, комп'ютерної томографії, допплерографії та тонкоголкової аспіраційної біопсії знизило частоту ускладнень на 87 % завдяки точному визначенню анатомічних змін і патологічних процесів. Результати підкреслюють необхідність стандартизованих протоколів для покращення хірургічних результатів, які відповідають міжнародним рекомендаціям щодо моніторингу захворювань щитовидної залози та ранньої діагностики

Ключові слова: вузлова патологія щитоподібної залози; вогнищеві ущільнення аутоімунного тиреоїдиту; ультразвукова доплерографія; комп'ютерна томографія; тиреотропний гормон

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Innovative approach to laparoscopic hernioplasty (TAPP) without mesh fixation

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Abstract. The purpose of this study was to compare hernioplasty techniques with and without mesh prosthesis fixation. The study included 187 patients with primary inguinal hernias who were treated at the Municipal Non-Profit Enterprise "Kyiv City Clinical Hospital No. 1" from 2020 to 2024, divided into 2 groups: Group 1 (control group) – 92 patients, and Group 2 (experimental group) – 95 patients. The duration of surgery for lateral hernias was statistically significantly longer ($p < 0.01$) than for medial and femoral hernias. In both groups, a significant ($p < 0.01$) reduction in pain was noted within 1 month after surgery. The level of pain in the control group was greater ($p < 0.01$) compared to the group that underwent hernioplasty without fixation of the mesh endoprosthesis. In surgical intervention without mesh fixation, the incidence of complications was substantially reduced: seroma formation decreased from 10.9 ± 0.3% in the control group to 2.1 ± 0.1% in the main group ($p = 0.031$); oedema was observed in 28 patients (30.4 ± 0.5%) from the control group, and in 12 patients (12.6 ± 0.3%) from the main group ($p = 0.005$). Based on the analysed data obtained in this study, hernioplasty without fixation of the mesh endoprosthesis has considerable advantages, according to all criteria, compared to surgical intervention using a herniostapler. Hernioplasty without mesh fixation is a safe and effective technique that reduces pain, complications, and the cost of surgery, while maintaining the success of hernia repair. Proper patient selection, sufficient mesh overlap, and careful peritoneal closure are key to the success of the surgical intervention

Keywords: surgical intervention; herniostapler; inguinal hernia; hernioplasty technique; allohernioplasty

INTRODUCTION

The transabdominal preperitoneal access (TAPP) is a widely used laparoscopic technique for inguinal hernia repair. Conventionally, mesh fixation during TAPP has been used to prevent relapse. However, this practice has been associated with complications such as chronic pain due to nerve or vascular damage from fixation devices. Surgeons should carefully consider factors such as defect size, patient anatomy, and potential risks and benefits when deciding whether to use mesh during TAPP. Continued research in this area is essential to optimise patient outcomes and improve surgical techniques. Depending on the clinical situation, a surgeon can choose the type of surgery that will lead to the least number of complications. As of 2025, many scientific papers have proven that laparoscopic methods of hernioplasty have considerable advantages over open surgical interventions.

K. Doden *et al*. [1] showed that fixing the mesh on the lateral and dorsal sides of the urethral canal is dangerous due to the possibility of clamping the urethral nerve and blood vessels. In this case, the use of a self-absorbing mesh during TAPP is a rational solution in terms of avoiding fixation or suturing around the obturator canal while maintaining stable mesh fixation to prevent relapse. The researchers concluded that laparoscopic TAPP repair

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with self-retractable mesh is a rational treatment option that reduces the risk of damage to the phrenic nerve while maintaining secure mesh fixation to prevent relapse and chronic pain.

A. Elhadidi *et al*. [2] concluded that postoperative complications and chronic groin pain were reported for each technique. An equal number of participants were present in the stapler and suture groups, with the majority having one point of American Society of Anaesthesiologists (ASA) score. The mean age was 42.50 ± 13.86 years, and the body mass index (BMI) was 27.47 ± 5.88. The average duration of surgery in the stapler group was shorter than in the suture group. However, the stapler group had a considerably greater mean VAS score than the suture group. Most participants in the suture group (89.2%) had a 1-day LOS, while the 2-day LOS was considerably greater in the stapler group (12.3%) than in the suture group (9.2%). No patient reported mesh erosion, conversion, relapse, testicular atrophy, or mesh infection. Early postoperative pain was greater in the stapler group, along with prolonged hospital stay, but both were nonsignificant. The results for chronic postoperative pain and relapse rates during the 3-year follow-up were also comparable. The rate of re-hospitalisation was minimal, and no significant complications occurred. That is, the study confirmed the advantages of the method of surgery without mesh fixation by all criteria except for the incidence of complications.

The study analysed the research conducted by C. Zhang *et al*. [3], which included 6 randomised trials involving 679 patients who underwent TAPP without mesh fixation and 964 patients with mesh fixation. No significant difference in relapse was observed between the two groups. The use of self-absorbing mesh may not affect the effectiveness of TAPP. It does not increase the relapse rate and may lead to a reduction in postoperative pain in inguinal hernias with a small hernia defect (less than 3 cm). This study showed that this technique is effective for a small defect, which is a significant indication for the use of TAPP, because in case of a large hernia defect, another surgical technique should be chosen.

V.C. Nikolian *et al*. [4] conducted a fairly complete analysis of the advantages and disadvantages of hernioplasty using mesh. Mesh placement resulted in shorter hospital stays and a return to daily activities. A hernioplasty without mesh is unlikely to lead to seroma development, which is why this method is preferred in low-income countries due to the low cost and lesser availability of mesh materials. G. Caruso *et al*. [5] confirmed that TAPP is associated with shorter postoperative rehabilitation, less chronic pain and numbness in the surgical area, no risk of mesh prosthesis infection, and a faster return to daily activities. The study also noted that the disadvantages of laparoscopic interventions include a longer training period for the surgeon and higher direct costs; these costs can be offset by reducing indirect costs: shorter hospital stays and a quicker return to work.

The technique of TAPP is constantly being improved. For example, M. Furtado *et al*. [6] confirmed the effectiveness of TAPP without mesh fixation based on clearly defined anatomical landmarks describing the "inverted Y" concept. The study used the identification of five triangles and three zones of dissection for laparoscopic inguinal hernia repair. A new anatomical concept was introduced to understand the overall anatomy of the inguinal region from a laparoscopic approach and to achieve a critical view of the "danger zones". The new anatomical concepts enabled surgeons to expedite and facilitate hernia sac release and prevent intraoperative complications.

In all analysed studies, TAPP without mesh fixation was shown to reduce postoperative pain, shorten the patients' recovery after surgery, but did not affect the relapse rate. However, surgeons are still debating how best to place the mesh endoprosthesis and which surgical technique to choose [7-9]. Thus, the purpose of this study was to improve the effectiveness of surgical treatment of inguinal hernias by comparing laparoscopic hernioplasty methods, with and without mesh prosthesis fixation, and to substantiate the choice of allohernioplasty method.

MATERIALS AND METHODS

The study included 187 patients with primary inguinal hernias who were treated at the Municipal Non-Profit Enterprise "Kyiv City Clinical Hospital No. 1" from 2020 to 2024. Inclusion criteria: patients with inguinal hernias (diagnosed according to the recommendations of the European Association of Herniology and the intraoperative classification of hernia types by L.M. Nyhus [10]), men and women, over 18 years of age, for women of reproductive age – a negative pregnancy test result [11]. Exclusion criteria: a history of patients with a BMI above 40 kg/m^2, relapsed, strangulated, and inguinal hernias, decompensated cardiovascular, respiratory, renal, or hepatic failure; pregnancy and lactation; alcohol or drug abuse; logistical problems (patients' failure to attend the examination on time), non-compliance with the recommended diagnostic and treatment plan; conversion of surgery to open access.

All patients were admitted to the department as planned after undergoing a standard preoperative package of laboratory and instrumental tests (general blood test, liver and kidney complexes, electrocardiogram, abdominal ultrasound). All patients were informed about the existing methods of surgical treatment of inguinal hernias, possible complications or changes in the scope of surgery, the course of the postoperative period, and the required regimen. All patients who took part in the clinical trial gave their written voluntary informed consent following the provisions of the Declaration of Helsinki of the World Medical Association "Ethical Principles for Medical Research Involving Human Subjects Human Subjects for Medical Research" [12].

The selection of patients and their allocation to the control and experimental groups was random. The operations were performed in the absence of contraindications to anaesthesia, carboxyperitoneum, and the patients' willingness to be operated on in the way proposed based on the group assignment. Thus, 95 patients were included in the experimental group. All patients in this group underwent TAPP – inguinal hernia repair without fixation of a mesh endoprosthesis. The control group included 92 patients who underwent classical TAPP – inguinal hernia repair with a mesh endoprosthesis with fixation of the latter using the Protack 5.0 hernia stapler with titanium clips.

All patients included in the study underwent hernioplasty using a laparoscopic approach with preperitoneal placement of the mesh endoprosthesis. In patients of both study groups, only polypropylene mesh endoprostheses were used for the purity of the experiment. In the first group (control), polypropylene mesh was used. The

second group (main group) used 3D mesh. The study included 187 patients divided into 2 groups: Group 1 (control group) – 92 patients, and Group 2 (main group) – 95 patients. The groups were comparable in terms of age and gender (88.04% of men in the control group, 88.42% in the main group, and 11.96 and 11.58% of women, respectively). The average age of the patients was 59.4 years in the control group and 58.6 years in the main group. The physical status of patients before surgery was assessed according to the ASA classification [13].

Hernioplasties in the control and intervention groups were performed under general anaesthesia with muscle relaxation and artificial lung ventilation using the MAQUET FLOW-i apparatus and the OLYMPUS VISERA ELITE III video endoscopic stand. Endoscopic equipment included OLYMPUS OEV321UH endoscopic monitor, 4K, 32 inches; OLYMPUS VISERA ELITE III OTV-S700 endoscopic camera, 4K HDR; OLYMPUS VISERA ELITE III CLL-S7004 endoscopic illuminator; OLYMPUS UHI-4 CO_2 insufflator; OLYMPUS endoscopic instrument stand, OLYMPUS ESG-410 surgical diathermocoagulator; aspirator-irrigator. Statistical processing was performed using Statistical software EZR v. 1.54 (graphical user interface for R statistical software version 4.0.3, R Foundation for Statistical Computing, Vienna, Austria), MedStat v. 5.2, and Excel (Microsoft Office 2010, 2013) in the Windows 10 operating system. The Chi-square test was employed to compare the frequency of postoperative complications.

RESULTS

The analysis of all the collected data helped to identify crucial advantages of TAPP over hernioplasty with mesh prosthesis fixation for both patients and doctors. The findings of the study were evaluated based on the analysis of the results of 187 patients who underwent laparoscopic TAPP hernioplasty and the analysis of the medical records of the operated patients. All the above results, i.e., pain relief and reduced complication rates, were achieved thanks to the innovative TAPP technique without mesh fixation. After a thorough examination of the working area and determination of the type of hernia (lateral or medial), peritoneal dissection was performed. The peritoneal dissection was based on a new anatomical concept – the "inverted Y", the definition of five triangles and three dissection zones, which was introduced by Brazilian surgeons in 2018.

The new anatomical concept was introduced to understand the overall anatomy of the inguinal region from a laparoscopic approach and to achieve a critical view of the "danger zones". The new anatomical insights have enabled surgeons to expedite and facilitate hernia sac release, prevent intraoperative complications (vascular and nerve damage), and reduce postoperative complications such as early and chronic postoperative pain. This concept is based on the visual representation of an "inverted Y", the definition of five triangles, and the division of the inguinal fossa into three zones of peritoneal dissection. The visual representation of the "inverted Y" when examining the inguinal fossa from the laparoscopic approach allows recognising the anatomical structures of the inguinal area with the following elements: the lower epigastric vessels, the vas deferens, and the vessels of the spermatic cord. When analysing the data on the duration of surgical intervention in both groups (control and experimental), the duration of surgical intervention was found to depend on the type of hernia defect (lateral, medial, femoral).

Thus, the duration of TAPP can vary depending on the type of hernia defect. Lateral (indirect) hernia – usually takes longer than medial hernia due to the need for a large incision, especially in cases where a large sac extends into the inguinal canal. Medial (direct) hernia – usually faster to repair because the defect is within the Hesselbach's triangle, which often requires less incision. Femoral hernia – may require additional time due to its deeper location and the need for careful placement of the mesh to cover the femoral ring without harming the adjacent structures. Other factors that affect the duration of the surgery include the patient's anatomy, the presence of bilateral or relapsing hernias, the surgeon's experience, and any intraoperative complications. The surgical technique depends on the type of hernia that is troubling the patient. Table 1 shows the distribution of patients included in the study by hernia type.

Table 1. Results of the distribution of patients by type of hernia

	Control group					Experimental group					Total
	0	1	2	3	x	0	1	2	3	x	
PL	8	15	20	5	–	6	21	12	4	–	91
PM	10	13	15	3	–	5	19	20	3	–	88
PF	2	1	–	–	–	3	2	–	–	–	8
Total	20	29	35	8	–	14	42	32	7	–	187
	92					95					

Notes: PL – primary lateral hernia; PM – primary medial hernia; PF – primary femoral hernia
Source: compiled by the authors

Among the studied patients, 91 patients (48.66%) had primary lateral hernias, 88 patients (47.06%) had primary medial hernias, and 8 patients (4.28%) had primary femoral hernias. In the control group, 48 patients (52.17%) had lateral hernias, 41 patients (44.57%) had medial hernias, and 3 patients (3.26%) had femoral hernias. According to the size of the hernia defect in this group, patients were divided as follows: hernia that was not identified during the study – 20 patients (21.74%); under 1.5 cm – 29 patients (31.52%); 1.5-3 cm – 35 patients (38.04%), over 3 cm – 8 patients (8.70%). In the experimental group, patients with medial hernias were most common – 47 patients (49.47%), lateral hernias accounted for 43 patients (45.26%), and femoral hernias – 5 patients (5.26%). The patients were divided according to the size of the hernia defect as follows: the defect was not identified – 14 patients (14.74%), under 1.5 cm – 42 patients (44.21%); 1.5-3 cm – 32 patients (33.68%), over 3 cm – 7 patients (7.37%).

Table 2 shows the average duration of surgery in minutes depending on the type of hernia defect. The longest duration of surgical intervention was recorded in the control group for lateral hernias (74.5 ± 0.8 min). Surgical intervention for lateral hernias in the experimental group lasted almost half as long (47.7 ± 0.4 min), i.e., the absence of mesh fixation significantly reduces the duration of the operation. The surgical approach and technique of the operation can greatly save time. Thus, with the modified technique without fixation of the mesh, the operation time is considerably saved. In both groups of patients, the duration of surgery for lateral hernias was found to be statistically significantly longer ($p < 0.01$) than for medial and femoral hernias. There was no statistically significant difference in the duration of surgery for medial and femoral hernias ($p > 0.05$), which is conditioned by the identical anatomical area.

Table 2. Estimation of the average duration of surgical intervention in the control and intervention groups

Study group	Type of hernia defect	Number of patients	Mean value of surgical intervention duration (in min), X ± m
Control group	Lateral	48	74.5 ± 0.8
	Medial	41	65.6 ± 0.5
	Femoral	3	67.7 ± 1.5
Experimental group	Lateral	43	47.7 ± 0.4
	Medial	47	38.3 ± 0.6
	Femoral	5	32.4 ± 1.1

Notes: to present a point estimate, the table shows the mean values of the duration of the surgical intervention (X) and the standard errors of the mean (m)
Source: compiled by the authors of this study

When comparing the duration of surgical intervention in the control and experimental groups, divided by the type of hernia defect, the duration of surgical intervention in the control group was found to be statistically significantly ($p < 0.001$) longer than in the experimental group. Notably, the systematic algorithm of parietal peritoneal dissection influenced the speed of the operation. According to the present study, the key factors in reducing the duration of surgery included the surgeon's experience, preoperative planning, minimal adhesions, effective mesh fixation, and proper peritoneal closure.

On the first day after surgery, as soon as the patient regained consciousness and agreed to the assessment of pain, they were asked to indicate the level of their subjective pain on a visual analogue pain scale (VAS). Thus, in the control group, the scores ranged from 2 to 6 points, with an average of 4 ± 0.1 points, and in the experimental group – from 2 to 5 points, with an average of 3.8 ± 0.09 points. Most patients complained of pain in the area of trocar insertion and discomfort in the area of surgery. The first day after surgery, patients were recommended bed rest and avoidance of knee and hip flexion on the side of surgery (Table 3).

Table 3. Estimation of the average value of pain in patients in the control group

Term of pain syndrome assessment	Patients	Mean value of patients' pain (in points), Me ± m
Day 1	n = 92	4 ± 0.1
Day 3		3 ± 0.1
Day 7		2 ± 0.1
1 month		1 ± 0.1

Source: compiled by the authors

When assessing pain on day 3 after surgery, those patients who stayed in the hospital were also asked to assess pain by marking it on a visual analogue pain scale, while the patients discharged from the hospital were interviewed by telephone and were asked to rate their pain syndrome from 0 to 10 points, analogous to the VAS scale. On day 3, the pain scores ranged from 1 to 5 points in the control group, with an average of 3 ± 0.1 points, and from 1 to 4 points in the experimental group, with an average of 2.3 ± 0.06 points. On the second day, all patients were raised from bed and offered to walk around the hospital, with those who felt well being discharged from the hospital. 7 days after the surgery, patients operated on with mesh fixation with a herniostapler had pain scores ranging from 0 to 4, with an average of 2 ± 0.1 points. In the group where the mesh endoprosthesis was not fixed, the pain syndrome varied from 0 to 1 point, which averaged 0.5 ± 0.52 points (Table 4). When assessing pain one month after surgery in the control group, the scores ranged from 0 to 3 points (average 1 ± 0.1 points), in the main group from 0 to 1 points, respectively, and the average was 0.22 ± 0.04 points (Fig. 1).

Table 4. Estimation of the average value of pain sensations of patients in the experimental group

Term of pain syndrome assessment	Patients	Mean value of patients' pain (in points), Me ± m
Day 1	n = 95	3.8 ± 0.09
Day 3		2.3 ± 0.06
Day 7		0.5 ± 0.52
1 month		0.22 ± 0.04

Source: compiled by the authors

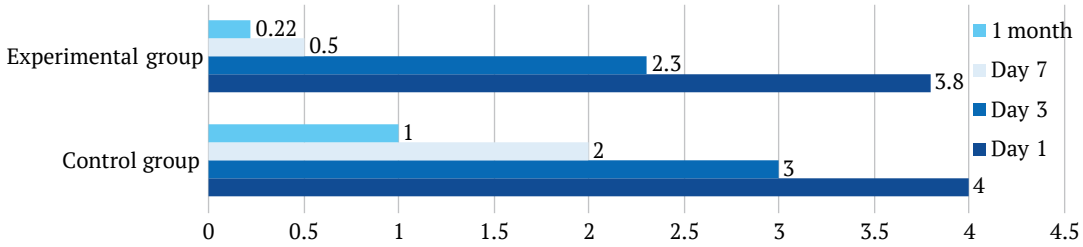

Figure 1. Comparative assessment of the average value of the pain syndrome according to the VAS
Source: compiled by the authors

Thus, in both groups, a statistically significant ($p < 0.01$) decrease in pain was observed within 1 month after hernioplasty. The level of pain in the control group compared to the group that was offered TAPP plastic surgery without fixation of the mesh endoprosthesis was noted to be statistically significantly higher at $p < 0.01$. Reducing pain during TAPP hernia repair without mesh fixation is an essential point for improving patient recovery. Such results were achieved by the correct choice and placement of the mesh endoprosthesis. The mesh was placed in the pre-abdominal space, where intra-abdominal pressure helps to keep it in place. During the surgery, adequate overlap (3-5 cm) outside the defect was ensured to prevent relapse without the need for fixation. Correct dissection created a sufficiently wide preperitoneal space for the mesh to lie flat without wrinkles. In the patients of the experimental group, the mesh was glued by closing the peritoneal flap, because proper suturing or attachment of the peritoneum to the mesh helps to fix it naturally. During the surgery, it was critical to avoid damage to the ilioinguinal, iliohypochondriac, and genito-femoral nerves, which prevented neuropathic pain. The surgeon avoided placing the mesh too medially or using excessive dissection near the pain triangle (lateral to the spermatic vessels), where the main nerves pass. During the surgery, the CO_2 insufflation pressure was minimised, and a lower intra-abdominal pressure (10-12 mm Hg) was used, which reduced postoperative pain. During the operation, the surgeon ensured that the peritoneum was closed without tension to prevent mesh migration or contact with the intestinal loops. The use of these techniques resulted in a reduction in postoperative pain, minimisation of chronic discomfort, and a low relapse rate.

Table 4 shows the incidence of postoperative complications in the two study groups: the incidence of complications (%) and the standard error of the incidence (± m%). The study analysed the incidence of the principal complications of hernioplasty, such as haematoma, seroma, and oedema. The most common complication of surgery among patients in both groups was oedema, while seroma was the least common complication in the operated patients. In the control group, the incidence of complications was statistically significantly greater compared to the experimental group where the surgical intervention was performed using an innovative method. The analysis revealed a decrease ($p = 0.027$ by the Chi-square criterion with Yates' correction) in the incidence of haematomas from 16.3 ± 0.4% (for patients operated on using the conventional method) to 5.3 ± 0.2% (for patients operated on using the TAPP method without fixation of the mesh endoprosthesis). The incidence of seroma decreased from 10.9 ± 0.3% in the control group to 2.1 ± 0.1% in the experimental group, at $p = 0.031$. Oedema was observed in 28 patients (30.4 ± 0.5%) from the control group and 12 (12.6 ± 0.3%) patients from the main group, which is statistically significant ($p = 0.005$) less (Table 5).

Table 5. Frequency of postoperative complications in the study groups

Postoperative complications	Control group (n = 92)		Experimental group (n = 95)		Significance level of differences between groups, p
	Abs. Number	% ± m%	Abs. Number	% ± m%	
Haematoma	15	16.3 ± 0.4	5	5.3 ± 0.2	p = 0.027
Seroma	10	10.9 ± 0.3	2	2.1 ± 0.1	p = 0.031
Oedema	28	30.4 ± 0.5	12	12.6 ± 0.3	p = 0.005
Increased body temperature	33	35.9 ± 0.5	14	14.7 ± 0.4	p = 0.001

Source: compiled by the authors

The increase in body temperature in patients ranged within 37.2-38.3°C on the first day after surgery, who were prescribed antipyretics. The next day, as a rule, no fever was observed. In the experimental group, 14.7 ± 0.4% had a fever, which was statistically significantly less ($p = 0.001$) than in the control group (35.9 ± 0.5%). The presence or absence of inguinal hernia relapse was assessed by palpation of the superficial inguinal ring and determination of the "coughing impulse" symptom and ultrasound diagnostics of the inguinal area, particularly the area of the endoprosthesis. Palpation was performed the day after surgery. Ultrasound of the inguinal area was performed on the day of suture removal. Usually, sutures were removed on day 7 after hernioplasty. The subjects were invited for a repeated examination and ultrasound of the inguinal area and the area of the placed endoprosthesis 1 and 6 months after the surgery. Early relapse after hernioplasty was assessed within 6 months after surgery using the classical and non-fixation methods. When analysing the data, relapse was detected in 8 patients (8.7 ± 0.3%) of the control group and 4 patients (4.2 ± 0.2%) of the experimental group, the difference was not statistically significant at $p = 0.341$ (Fig. 2). The statistically insignificant difference in the relapse rate requires further investigation with a larger number of patients.

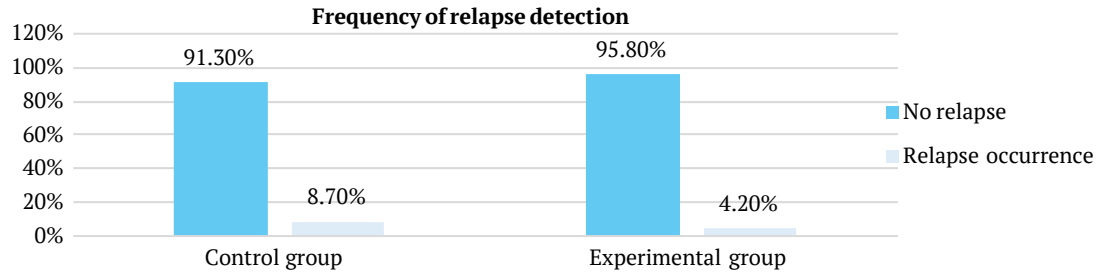

Figure 2. Presence of relapses in the postoperative period in the examined patients
Source: compiled by the authors

Minimisation of complications and relapses was achieved by proper selection of patients for TAPP, careful surgical technique, and adherence to postoperative care recommendations. The findings of the present study confirmed the significant benefits of TAPP without mesh fixation, which greatly reduced the risk of complications associated with fixation. Less postoperative pain and discomfort can enable patients to return to their daily activities and work more quickly. Not using a stapler eliminates the risk of related complications such as vascular damage, bleeding, or tissue damage. It also reduces costs by eliminating the use of fixation devices.

DISCUSSION

TAPP hernioplasty without mesh fixation is becoming an increasingly popular technique for inguinal hernia repair. This approach eliminates the need for buttons, staples, or glue, relying on adequate mesh overlap and intra-abdominal pressure to maintain its position [13, 14]. Fixing the mesh with staples or buttons can cause nerve irritation or injury, leading to postoperative pain and chronic groin pain. Avoiding fixation greatly reduces the risk of postoperative neuralgia and foreign body sensation in the groin. Patients often experience less discomfort during movement, which enables them to return to their daily activities more quickly.

Chronic pain (lasting more than 3 months) is a well-documented complication of hernia surgery, often associated with nerve impingement or tissue inflammation caused by fixation. Studies show that non-fixation techniques reduce the incidence of chronic pain, increasing overall patient satisfaction [15, 16]. A carefully selected and positioned mesh with sufficient overlap (at least 3-5 cm) ensures low relapse rates even without fixation [17]. That is why the present study used high-quality mesh prostheses and tailored to the size of the defect. In the first group (control), polypropylene mesh was used. In the second group (experimental), a 3D mesh was used (photo of the mesh, size, characteristics). To minimise the relapse rate, patients were strictly selected according to the inclusion criteria, because in case of a large defect or relapsing hernia, the surgeon may choose a mesh-fixed technique or even open access to reduce further complications. Therefore, the choice of surgery technique should be made individually, according to the characteristics of each patient.

Several clinical trials show no substantial difference in relapse rates between fixation and non-fixation techniques when performed by experienced surgeons. For small and medium-sized hernias (especially those under 3 cm), mesh fixation may not be necessary, as the correct positioning of the mesh in the preperitoneal space provides adequate stabilisation. However, in case of large hernias or weak tissues, the risk of relapse may be slightly higher without fixation [18, 19].

Thus, in the study by K.A. Riemenschneider *et al*. [20], after analysing seven prospective randomised controlled trials of 1,732 patients, 737 procedures were performed without fixation and 995 procedures with mesh fixation, it was concluded that the current evidence is very uncertain and mesh fixation may have a small or no effect on hernia relapse and chronic postoperative inguinal pain in patients undergoing TAPP inguinal hernia repair. The findings of the study by K.A. Riemenschneider *et al*. are consistent with the data of the present study that TAPP without mesh fixation has advantages over laparoscopic hernioplasty with mesh fixation.

The risk of mesh migration is minimal if the peritoneum is properly closed, and anatomical landmarks are observed [21-23]. Less postoperative pain facilitates early ambulation, reducing the risk of complications such as deep vein thrombosis and pulmonary complications. Many patients resume normal activities and return to work earlier than those who underwent fixation [24]. Devices for fixing the mesh (buttons, staples, glue) increase the cost of the procedure. Avoiding fixation reduces operating costs without compromising clinical outcomes.

Risk of mesh removal: proper dissection and placement are essential to prevent folds or migration that can lead to relapse. Learning curve for surgeons: this technique requires experience to ensure proper mesh placement and secure peritoneal closure [25]. Patient selection is essential: it is best suited for uncomplicated primary hernias, whereas large, complex, or relapsing hernias may benefit from fixation [10]. Thus, only patients with uncomplicated hernias were included in the current study, and hernia relapse is a contraindication to TAPP without mesh fixation, in which case the doctor chose another technique for the operation.

The study by A.K. Nahid *et al*. [26] reflected analogous results to the present study. Thus, it was confirmed that pain and the time required to return to normal life and work after surgery were significantly less in the non-fixation group. The absence of mesh fixation was cost-effective, with less postoperative pain and a lower relapse rate [27]. In the current study, patients who underwent TAPP without mesh fixation also returned to life faster and experienced less discomfort in the postoperative period than patients who underwent hernioplasty with mesh fixation.

R.F. Ali *et al*. [28] reached the same conclusion as in the present study. Thus, in this study, the researchers were found that the pain score 7 days after surgery, during the first month, and after three months were significantly higher in the group with mesh fixation ($p < 0.001$). The group with mesh fixation had a longer operative time than the group without fixation (69.34 ± 13.55 vs. 60.92 ± 10.18). The relapse rates did not differ between the groups, as in the current study. The absence of fixation of the mesh endoprosthesis for TAPP is effective with minimal postoperative pain and minimal risk of relapse. In the current study, analogous data were obtained regarding the duration of pain and the time of surgery.

The findings of other colleagues' studies are also fully comparable to the data obtained in the present study. T.A.A.M. Habeeb *et al*. [29] confirmed that fixation of a mesh endoprosthesis with a stapler increases the risk of chronic groin pain. Laparoscopic TAPP inguinal hernia repair without mesh fixation was significantly safer and reduced the relapse rate. Furthermore, mesh fixation increased the risk of postoperative complications and patient costs.

S. Qureshi *et al*. [16] studied the long-term results after hernioplasty one year later. In the study, the difference in pain scale scores at the time of discharge and the average number of days of hospital discharge were statistically significant ($p < 0.005$), analogous to the results of the current study. After 1 year of follow-up, the relapse rate was comparable and low in both groups. At one year of follow-up, there was no significant difference in relapse and chronic pain between the mesh-fixed group and the non-mesh-fixed group. In their studies, surgeons almost unanimously confirmed that laparoscopic TAPP hernioplasty without mesh fixation is much more effective and practical than surgical interventions using a stapler. Therefore, in patients without contraindications, when choosing a surgical intervention, TAPP plastic surgery without the use of a mesh endoprosthesis should be preferred.

TAPP without mesh fixation provides considerable benefits in the postoperative period, including pain reduction, faster recovery, and lower rates of chronic pain, with relapse rates comparable to fixation methods. It is a cost-effective and patient-friendly approach, especially when performed by experienced surgeons.

CONCLUSIONS

The analysis of the data obtained revealed a considerable advantage of laparoscopic hernioplasty without mesh fixation, using the technique involving a herniostapler. The time for surgical intervention depends on the type of hernia defect. The longest time for surgical intervention among all the patients examined was recorded in patients with a lateral hernia when the hernia defect was repaired by fixing the mesh. Laparoscopic peritoneal surgery without mesh fixation significantly reduces the time of surgery. The absence of mesh fixation when repairing a hernia defect using the laparoscopic method greatly reduces pain in patients in the dynamics, compared to those in whom the mesh was fixed. The method of performing operations without fixation of the mesh endoprosthesis leads to a decrease in the incidence of postoperative haematomas. According to the analysed data, surgical intervention without the use of a herniostapler considerably reduces the incidence of postoperative seromas and oedema. The incidence of temperature reactions in patients with hernioplasty by the non-fixation method was significantly reduced. The frequency of relapse in 6 months after surgery was not high in both groups and did not depend on the surgical technique.

The analysis of the data obtained in the study showed significant advantages, according to all criteria, of hernioplasty without fixation of the mesh endoprosthesis, compared with surgical intervention using a herniostapler. TAPP without mesh fixation appears to be a safe and effective approach for appropriately selected patients, reducing postoperative pain and chronic discomfort while maintaining a comparable relapse rate for small to moderate hernias. However, careful patient selection is crucial, and in some cases, fixation may be necessary to ensure optimum long-term results. The relapse rate did not differ depending on the surgical technique, and it is probable that a greater number of patients should be included in the study to investigate the complication rate depending on the surgical technique more accurately. Further studies may compare various techniques of laparoscopic preperitoneal hernioplasty without mesh fixation to further improve the surgical technique.

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CONFLICT OF INTEREST

None.

REFERENCES

[1] Doden K, Yoshimura T, Iwaki Y, Kato H, Kawaguchi M, Watanabe T. Laparoscopic transabdominal preperitoneal obturator hernioplasty with self-gripping mesh: A case report with operative video. Int J Surg Case Rep. 2021;89:106657. DOI: 10.1016/J.IJSCR.2021.106657

[2] Elhadidi A, Negm A, Shouma A. Comparing stapler and sutured mesh fixation techniques for laparoscopic TAPP repair: A study on chronic groin pain on 3-year follow-up. Updates Surg. 2024;76(4):1467–73. DOI: 10.1007/S13304-024-01754-1

[3] Zhang CX, Li J, Suo HJ, Bai JP. Non-fixation versus fixation of mesh in laparoscopic transabdominal preperitoneal repair of inguinal hernia: A systematic review and meta-analysis of randomized controlled trials. PLoS One. 2024;19(12):e0314334. DOI: 10.1371/JOURNAL.PONE.0314334

[4] Nikolian VC, Coleman NL, Podolsky D, Novitsky YW. Robotic-assisted transabdominal preperitoneal ventral hernia repair. Surg Technol Int. 2020;36:95–7.

[5] Caruso G, Evola G, Benfatto SAM, Gangemi M. Transabdominal Preperitoneal (TAPP) inguinal hernia repair. IntechOpen. 2022. DOI: 10.5772/intechopen.101962

[6] Furtado M, Claus CMP, Cavazzola LT, Malcher F, Bakonyi-Neto A, Saad-Hossne R. Systemization of laparoscopic inguinal hernia repair (TAPP) based on a new anatomical concept: Inverted Y and Five Triangles. ABCD Arq Bras Cir Dig. 2019;32(1). DOI: 10.1590/0102-672020180001E1426
[7] Jiang T, Zhang C, Wang XL, Yue DC, Yuan XP, Wang DC. Meta-analysis of RCTs on the safety of non-fixation of mesh in TAPP inguinal hernia repair: An updated meta-analysis. BMC Surg. 2024;24(1):317. DOI: 10.1186/S12893-024-02628-4
[8] Klymenko A, Kravchenko B, Klymenko V, Kravchenko S, Polishchuk K. Assessment of chronic pain syndrome after laparoscopic transabdominal preperitoneal plastic surgery in patients with inguinal hernia. Zaporozhye Med J. 2024;26(4):296–302. DOI: 10.14739/2310-1210.2024.4.300008
[9] Miserez M, Alexandre JH, Campanelli G, Corcione F, Cuccurullo D, Pascual MH, et al. The European hernia society groin hernia classification: Simple and easy to remember. Hernia. 2007;11(2):113–6. DOI: 10.1007/S10029-007-0198-3
[10] Nyhus LM. Individualization of hernia repair: A new era. Surgery. 1993;114(1):1–2.
[11] Stabilini C, van Veenendaal N, Aasvang E, Agresta F, Aufenacker T, Berrevoet F, et al. Update of the international HerniaSurge guidelines for groin hernia management. BJS Open. 2023;7(5):zrad080. DOI: 10.1093/BJSOPEN/ZRAD080
[12] The World Medical Association. Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects [Internet]. [cited 2024 July 10]. Available from: https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/
[13] Hendrix JM, Garmon EH. American society of anesthesiologists physical status classification system. In: StatPearls. Treasure Island: StatPearls Publishing; 2025.
[14] Berney CR. Guidelines for laparoscopic (TAPP) and endoscopic (TEP) treatment of inguinal hernia. Surg Endosc. 2012;26:3350–1. DOI: 10.1007/S00464-012-2293-5
[15] Spampatti S, La Regina D, Pini R, Mongelli F, Gaffuri P, Porcellini I, et al. Three-point mesh fixation in robot-assisted transabdominal preperitoneal (R-TAPP) repair of 208 inguinal hernias: Preliminary results of a single-center consecutive series. Langenbecks Arch Surg. 2022;407(6):2555–61. DOI: 10.1007/S00423-022-02542-4
[16] Qureshi S, Ghazanfar S, Leghari AA, Zubair M, Chisti S, Memon F, et al. A comparative follow up study of transabdominal preperitoneal mesh repair in inguinal hernias with or without mesh fixation. J Pak Med Assoc. 2021;71(1(A)):28–30. DOI: 10.47391/JPMA.827
[17] Bianco FM, Dreifuss NH, Chang B, Schlottmann F, Cubisino A, Mangano A, et al. Robotic single-port surgery: Preliminary experience in general surgery. Int J Med Robot. 2022;18(6):e2453. DOI: 10.1002/RCS.2453
[18] Wieland L, Alfarawan F, Bockhorn M, El-Sourani N. Comparison of eTEP and IPOM for ventral hernia surgery in the early postoperative period: A retrospective cohort study of a tertiary university centre. Hernia. 2024;28(6):2195–206. DOI: 10.1007/S10029-024-03125-6
[19] Lockhart K, Dunn D, Teo S, Ng JY, Dhillon M, Teo E, et al. Mesh versus non-mesh for inguinal and femoral hernia repair. Cochrane Database Syst Rev. 2018;9:CD011517. DOI: 10.1002/14651858.CD011517.PUB2
[20] Riemenschneider KA, Lund H, Pommergaard HC. No evidence for fixation of mesh in laparoscopic transabdominal preperitoneal (TAPP) inguinal hernia repair: A systematic review and meta-analysis of randomized controlled trials. Surg Endosc. 2023;37(11):8291–300. DOI: 10.1007/S00464-023-10237-0
[21] Henriksen NA, Montgomery A, Kaufmann R, Berrevoet F, East B, Fischer J, et al. Guidelines for treatment of umbilical and epigastric hernias from the European Hernia Society and Americas Hernia Society. Br J Surg. 2020;107(3):171–90. DOI: 10.1002/BJS.11489
[22] Schlosser KA, Arnold MR, Otero J, Prasad T, Lincourt A, Colavita PD, et al. Deciding on optimal approach for ventral hernia repair: Laparoscopic or open. J Am Coll Surg. 2019;228(1):54–65. DOI: 10.1016/J.JAMCOLLSURG.2018.09.004
[23] Novik B, Sandblom G, Ansorge C, Thorell A. Association of mesh and fixation options with reoperation risk after laparoscopic groin hernia surgery: A Swedish hernia registry Study of 25,190 totally extraperitoneal and transabdominal preperitoneal repairs. J Am Coll Surg. 2022;234(3):311–25. DOI: 10.1097/XCS.0000000000000060
[24] Bosley ME, Felix Z, Salgado-Garza G, Lansing S, Nikolian VC. Short-term outcomes of transabdominal preperitoneal ventral hernia repair with rectus aponeuroplasty (TAPPRA) for the management of incisional hernias. J Abdom Wall Surg. 2024;3:13195. DOI: 10.3389/JAWS.2024.13195
[25] Yaguchi Y, Inaba T, Kumata Y, Horikawa M, Kiyokawa T, Fukushima R. Two cases of early recurrence after transabdominal preperitoneal inguinal hernia repair. Asian J Endosc Surg. 2018;11(1):71–4. DOI: 10.1111/ASES.12408
[26] Nahid AK, Rahman S, Veerapatherar K, Fernandes R. Outcomes on mesh fixation vs non-fixation in laparoscopic totally extra peritoneal inguinal hernia repair: A comparative study. Turk J Surg. 2021;37(1):1–5. DOI: 10.47717/TURKJSURG.2021.4962
[27] Wu JJ, Way JA, Eslick GD, Cox MR. Transabdominal pre-peritoneal versus open repair for primary unilateral inguinal hernia: A meta-analysis. World J Surg. 2018;42(5):1304–11. DOI: 10.1007/S00268-017-4288-9
[28] Ali RF, Elhussainy RM, Aouf AM, Ismail TA, Ismail KA. Outcomes of mesh fixation versus non fixation in laparoscopic transabdominal preperitoneal inguinal hernia repair: A randomized clinical study. J Pak Med Assoc. 2023;73(4):8–12. DOI: 10.47391/JPMA.EGY-S4-2
[29] Habeeb TAAM, Mokhtar MM, Sieda B, Osman G, Ibrahim A, Metwalli AEM, et al. Changing the innate consensus about mesh fixation in trans-abdominal preperitoneal laparoscopic inguinal hernioplasty in adults: Short and long term outcome. Randomized controlled clinical trial. Int J Surg. 2020;83:117–24. DOI: 10.1016/J.IJSU.2020.09.013

Новаторський підхід до лапароскопічної герніопластики (TAPP) без фіксації сітки

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Анотація. Метою даного дослідження було порівняння технік герніопластики з фіксацією сітчастого протезу та без. В дослідження було включено 187 пацієнтів із первинними пахвинними грижами, які знаходились на лікуванні в Комунальне некомерційне підприємство «Київська міська клінічна лікарня № 1» з 2020 р. по 2024 р., розділених на 2 групи: 1 група (контрольна група) – 92 пацієнта, та 2 група (експериментальна група) – 95 пацієнтів. Тривалість оперативного втручання при латеральних грижах статистично значно довша ($p < 0,01$), ніж при медіальних та стегнових грижах. В обох групах відзначалось значуще ($p < 0,01$) зниження больового синдрому протягом 1 місяця після виконання оперативного втручання. Рівень болю у контрольній групі в порівняні з групою, якій було запропоновано герніопластику без фіксації сітчастого ендопротезу був вищий ($p < 0,01$). При оперативном у втручанні без фіксації сітки суттєво знизилась частота ускладнень: утворення сером знизилась від $10,9 \pm 0,3$ % в контрольній групі, до $2,1 \pm 0,1$ % в основній групі ($p = 0,031$); набряки спостерігались у 28 хворих ($30,4 \pm 0,5$ %) з контрольної групи, та у 12 ($12,6 \pm 0,3$ %) хворих з основної групи ($p = 0,005$). На основі проаналізованих даних, отриманих у дослідженні, можна зробити висновок, що герніопластика без фіксації сітчастого ендопротезу має значні переваги, за всіма критеріями, порівняно з оперативним втручанням з використанням герніостеплера. Герніопластика без фіксації сіткою – це безпечна та ефективна методика, яка зменшує біль, ускладнення і вартість операції, зберігаючи при цьому успішність відновлення грижі. Правильний відбір пацієнтів, достатнє перекриття сітки та ретельне закриття очеревини є ключем до успіху оперативного втручання

Ключові слова: оперативне втручання; герніостеплер; пахові грижі; техніка герніопластики; алогерніопластики

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Enhancing aesthetic and functional outcomes in mastopexy: A modified Pitanguy-Ribeiro technique

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Abstract. One of the unresolved issues in aesthetic mammoplasty remains the prevention of gravitational ptosis in the long-term postoperative period. The aim of this study was to improve the outcomes of mastoptosis treatment by refining classical surgical techniques to help prevent recurrent ptosis in the long term. The main group consisted of 50 patients diagnosed with mastoptosis. These patients underwent surgery using a modified Pitanguy-Ribeiro method, which included fixation to reduce cranial displacement and anchoring to the fascia of the pectoralis major muscle. The study results highlight the importance of individually selecting mastopexy techniques for each patient to achieve sustained aesthetic and functional outcomes. This paper presents the distribution of patients according to the mastopexy technique used, as well as a comparison of early and late postoperative ptosis indicators across each group. It was found that mastopexy performed using the Pitanguy-Ribeiro technique is effective in the short term but carries a significant risk of late postoperative ptosis. Reduction mastopexy shows a high risk of ptosis in the long-term postoperative period. In contrast, the modified Pitanguy-Ribeiro technique proves to be an effective approach, offering more stable results compared to the other techniques considered in preventing postoperative ptosis – demonstrated by the complete absence of early ptosis and the lowest incidence of late ptosis, indicating its superiority. This study identifies the modified Pitanguy-Ribeiro technique as the most effective for mastopexy correction, ensuring long-term stability, prevention of ptosis recurrence, and a high level of patient satisfaction. The proposed modification can be recommended as the optimal technique for performing mastopexy. The study findings may be used to refine surgical techniques, support the development of personalised recommendations for mastopexy method selection, and inform planning of long-term postoperative preventive measures

Keywords: breast ptosis; surgical correction; fascia fixation; aesthetic mammoplasty

INTRODUCTION

Breast ptosis is one of the most common conditions resulting from a decrease in tissue elasticity and represents a natural process in women typically after the age of 30-40. In such cases, the mammary glands decrease in size and descend, often leading to aesthetic discomfort. According to O. Khrapach [1], breast ptosis can be categorised into several types of defects: pseudoptosis, partial ptosis, and true ptosis. As described by B. Atiyeh *et al*. [2], the chosen correction methods vary depending on the specific type of ptosis and its potential association with different forms of hypoplasia. The authors classified breast ptosis into true ptosis, glandular ptosis, and abnormal parenchymal distribution (or pseudoptosis), which allowed for more consistent results and fewer complications. 85% of patients who had experienced at least one pregnancy reported changes in breast shape post-pregnancy; 35% noted a decrease in size, while 30% reported an increase. Lifetime weight fluctuations, such as significant weight loss or high body mass index, larger bra cup size, number of pregnancies, and smoking history have all been identified as significant risk factors for ptosis.

According to F. Pazhoohi *et al*. [3], ptosis can occur in women of all ages and with varying breast sizes, and is commonly associated with ageing, macromastia, weight loss, pregnancy, and hormonal changes. Obesity contributes to ptosis, as the accumulation of adipose tissue increases the volume of the mammary glands and overstretches the supporting ligaments. The shape of the breast can also

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influence the condition of the supporting structures; for example, in rounded breast shapes, the Cooper's ligaments are arranged in a way that optimally redistributes weight and surface area. During pregnancy and breastfeeding, breast volume increases substantially, and the stretched skin does not always return to its former state. If breastfeeding was predominantly from one breast, asymmetry may also develop. According to official statistics from the American Society of Plastic Surgeons (ASPS), 280,121 mastopexy procedures were performed in the United States in 2020 [4], with ptosis diagnosed in 65% of women in the post-lactation period. As noted by S.J. Lee *et al*. [5], ptosis is often linked to genetic predisposition, associated with individual variations in collagen and elastin synthesis, and is frequently observed in family history. Additionally, M.L. Mangialardi *et al*. [6] report that beyond causing psychological discomfort, ptosis may lead to physical complications such as submammary eczema and cervicodynia.

Secondary breast ptosis may occur as a complication of augmentation mammoplasty. Its development is primarily due to an initial weakness of the breast's supportive and suspensory structures, as well as technical imperfections in the surgical approach. This is often linked to the surgeon's attempt to address the mismatch between stretched skin and reduced glandular tissue volume solely through augmentation, without the mastopexy that would be necessary in such cases. According to A. Hoyos [7], the incidence of recurrent ptosis following mastopexy combined with augmentation mammoplasty ranges from 9.3 to 36%. Mesh allografts have been proposed as a means to prevent recurrent ptosis by reinforcing the sutures and the natural supporting structures of the breast. Contemporary surgical correction methods for breast ptosis include mammoplasty with augmentation and mastopexy. Mammoplasty with augmentation is frequently performed using endoprostheses, although not all women are willing to undergo implant placement. Mastopexy remains the procedure of choice, though it carries certain risks, such as the development of recurrent ptosis or hypertrophic scarring [8]. J. Gunn *et al*. [9] observed that a significant drawback of mastopexy is the unavoidable need for incisions on the chest, which may be visible depending on their placement.

L.C. Nuzzi *et al*. [10] described the four principal mastopexy techniques: periareolar, vertical, inverted T-shaped, and L-shaped scar patterns. Modern mastopexy methods employ various surgical strategies, including the fixation of displaced breast tissue to the chest's denser structures using strong sutures – a critical element of the procedure – as well as excision of excess skin in the gland's lower segment, with superior repositioning of the nipple-areolar complex. To enhance breast contour, a flap of tissue from the lower pole of the gland is often transposed upward and secured in the retromammary space to the anterior chest wall. This approach not only contributes to a fuller upper pole but also extends the longevity of the surgical outcome. Additional techniques aim to avoid scarring in the area between the gland and the sternum.

One of the persistent challenges in aesthetic mammoplasty is the prevention of gravitational ptosis in the long-term postoperative period. Plastic surgeons must carefully evaluate the benefits and limitations of each technique to effectively address breast ptosis. The objective of the present study was to improve surgical outcomes in patients with mastoptosis by advancing surgical methods aimed at preventing breast ptosis in the long-term postoperative phase.

MATERIALS AND METHODS

The study was conducted at one of the leading medical centres in the city of Kremenchuk – LLC "Medical Centre 'Doctor Droga'", specialising in plastic surgery. Surgical interventions were performed over a 24-month period, from September 2022 to September 2024. All operations were carried out under general anaesthesia in accordance with standard surgical protocols. The appropriate method for performing mastopexy depends on an individualised approach for each patient, particularly through the assessment of the degree of ptosis. In this study, ptosis grading was determined using anatomical landmarks, specifically the position of the nipple-areolar complex relative to the inframammary fold (the fold beneath the breast). According to the classification by R. Regnault [11], the degrees of ptosis in women are categorised as follows: Grade 0 – No ptosis: the nipple is located at or above the level of the inframammary fold. Grade 1 – Mild ptosis: the nipple is at the level of or slightly below the inframammary fold, but still above the most projecting part of the breast. Grade 2 – Moderate ptosis: the nipple lies below the inframammary fold, but remains above the lower contour of the breast. Grade 3 – Severe ptosis: the nipple is significantly below the inframammary fold and falls beneath the most projecting part of the breast. Pseudoptosis: the upper breast appears flat, while the nipple remains at or above the inframammary fold, but the main glandular tissue is displaced downward. Glandular ptosis: characterised by a reduction in breast tissue volume, with the nipple still positioned at or above the inframammary fold.

A total of 90 patients with a confirmed diagnosis of mastoptosis participated in the study. The average age of the patients was 36 years, ranging from 19 to 54 years. All patients underwent mastopexy for the first time. They were divided into three groups based on the surgical technique employed: Group 1: 30 patients underwent surgery using the Pitanguy-Ribeiro technique [14]; Group 2: 10 patients underwent classical reduction mastopexy (inverted T-shaped technique) [12]; Group 3: 50 patients underwent a modified Pitanguy-Ribeiro technique, which included cranial displacement shortening and fixation to the fascia of the pectoralis major muscle. Postoperative follow-up was conducted over a period ranging from 2 to 24 months, with the average follow-up duration being approximately 9.5 months. Postoperative photographs were taken at 3-month intervals. All 90 patients provided consent for photographic follow-up within 24 months after surgery, and 8 of them provided long-term follow-up images extending beyond one year. To ensure the reliability of the results, standardise surgical procedures, and minimise variations that could influence outcomes, all operations were performed by a single surgeon and his dedicated team. None of the patients had serious comorbid conditions that might have impacted the study results.

All patients involved in this study provided informed written consent for the use of their personal data and photographs for scientific purposes, in accordance with the principles of confidentiality and personal data protection.

Reports of the study were prepared with full regard for participant confidentiality, in compliance with the Declaration of Helsinki of the World Medical Association [13] and the Law of Ukraine No. 2297-VI "On the Protection of Personal Data" [14]. Adherence to ethical standards in the conduct of scientific research ensures appropriate protection of the rights and interests of all participants and upholds high standards of research practice. Data collected from all participants were processed using statistical methods to identify differences in patient condition, the frequency of postoperative complications, and levels of satisfaction with surgical outcomes. The study findings were systematised and summarised to draw conclusions regarding the effectiveness of surgical treatment for patients with mastoptosis, as well as methods for preventing breast ptosis in the long-term postoperative period.

RESULTS AND DISCUSSION

The results of the study highlight the importance of selecting an appropriate mastopexy technique to achieve optimal aesthetic and functional outcomes. Table 1 presents the distribution of patients across the groups based on the mastopexy techniques utilised, along with diagnostic indicators of early and late postoperative ptosis.

Table 1. Division of patients into groups according to the method of mastopexy and diagnosis of early and late postoperative ptosis

Patient group	Surgery technique	Early postoperative ptosis								Late postoperative ptosis	
		1 month (after surgery)		3 months (after surgery)		6 months (after surgery)		1 year (after surgery)		from 1 year	
		n	%	n	%	n	%	n	%	n	%
1	Pitanguy-Ribeiro, n = 30	-	-	-	-	1	3.33	3	10	21	70
2	Classic reduction mastopexy (inverted T-shaped), n = 10	-	-	1	10	1	10	3	30	6	60
3	Pitanquy-Ribeiro with modification, n = 50	-	-	-	-	-	-	2	4	3	6

Source: provided by the author

The analysis of patient distribution by mastopexy technique, along with indicators of early (within 1 year) and late (beyond 1 year) postoperative ptosis for each group, demonstrates the following findings: In Group 1 – patients who underwent mastopexy using the Pitanguy-Ribeiro technique – no cases of early postoperative ptosis were recorded at 1- and 3-months post-surgery. Ptosis was observed in one patient (3.33%) at 6 months, and in three patients (10%) at 1 year. However, late ptosis (occurring more than 1 year after surgery) was identified in 21 patients (70%). These findings suggest that, while the Pitanguy-Ribeiro technique is associated with a relatively low incidence of early ptosis, its long-term outcomes indicate a high risk of recurrent ptosis two years after surgery.

In Group 2 – patients who underwent classical reduction mastopexy (inverted T-shaped) – early postoperative ptosis was observed in one patient (10%) at 3 months, one patient (10%) at 6 months, and three patients (30%) at 1 year. Late postoperative ptosis was recorded in six patients (60%). These results indicate that the classical reduction mastopexy technique carries a comparatively higher risk of ptosis, particularly in the late postoperative period, suggesting the need to consider alternative techniques or modifications to mitigate this risk. In Group 3 – patients who underwent mastopexy using the modified Pitanguy-Ribeiro technique – no cases of early postoperative ptosis were recorded at 1-, 3-, or 6-months following surgery. Ptosis was observed in two patients (4%) at 1 year, while late postoperative ptosis (beyond 1 year) was noted in three patients (6%). These outcomes demonstrate that the modified technique offers superior long-term stability with minimal incidence of both early and late postoperative ptosis. Thus, mastopexy performed using the Pitanguy-Ribeiro technique demonstrates short-term effectiveness but is associated with a significant risk of developing late postoperative ptosis (70%). Reduction mastopexy also shows a high likelihood of ptosis, particularly in the late postoperative period. In contrast, mastopexy using the modified Pitanguy-Ribeiro technique yields the most favourable outcomes in both the early and late postoperative phases, indicating its superiority over the other methods. This analysis supports a reasoned selection of mastopexy technique in favour of the modified Pitanguy-Ribeiro approach for effective correction of mastoptosis and prevention of breast ptosis in the long-term postoperative period.

Patient selection for mastopexy techniques took into account the need to reduce the risk of complications, as the chosen method should minimise the likelihood of postoperative ptosis, tissue necrosis, scar formation, and asymmetry. The effectiveness of each mastopexy technique is evaluated by its ability to prevent recurrent ptosis and maintain a stable aesthetic contour of the mammary glands over time. Successful mastopexy also contributed to improvement in the patients' physical and psychological well-being. During the surgical procedure using the Pitanguy-Ribeiro technique, the operation began with deepithelialisation of the designated skin area of the mammary glands, according to the appropriate marking. This was followed by excision of the cutaneous-glandular flap. A retromammary pocket was prepared cranially, based on the Pitanguy-Ribeiro pedicle, creating a bed for repositioning and fixation of the cutaneous-glandular flap, which was secured with Prolene sutures to the pectoralis major muscle. The deep layer of the superficial fascia was then sutured and cranially anchored to the fascia of the pectoralis major muscle a few millimetres above the cutaneous-glandular flap fixation site. These ligatures were additionally tied together, providing enhanced correction of mastoptosis, improved upper pole fullness, and more secure fixation of the breast.

Figure 1 shows the postoperative appearance of the breasts in a 34-year-old patient diagnosed with stage III breast ptosis. The procedure was performed using the Pitanguy-Ribeiro technique on 06.10.2023. At the 6-month postoperative evaluation, ptosis was noted to have recurred, though to a lesser degree. The upper pole of the breast remained insufficiently filled. Figure 2 presents the postoperative result in a 19-year-old patient diagnosed with stage III breast ptosis, who underwent classical reduction mastopexy (inverted T-shaped technique) on 19.06.2024. At the 3-month postoperative follow-up, the patient exhibited recurrent ptosis of the mammary glands, with "bottoming out" deformity – manifested as elongation of the lower breast pole due to the inability of the lower pole tissues (skin-glandular flap) to withstand gravitational forces.

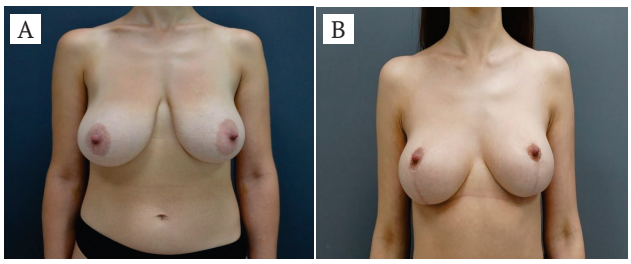

Figure 1. Appearance of the breast of a 34-year-old patient who was diagnosed with stage III breast ptosis
Notes: A) before surgery; B) 6 months after surgery
Source: author's photo

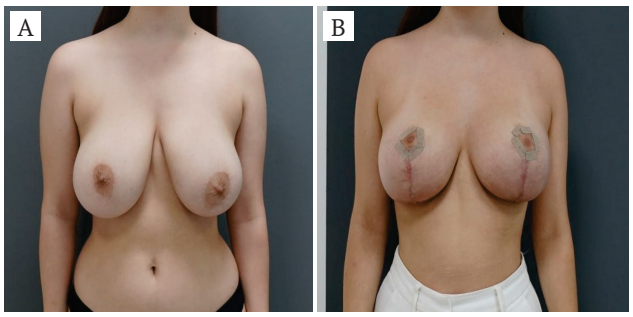

Figure 2. Appearance of the breast of a 19-year-old patient who was diagnosed with stage III breast ptosis
Notes: A) before surgery; B) 3 months after surgery, ptosis of the mammary glands developed "bottoming out"
Source: author's photo

Figure 3 shows the clinical appearance of the breasts in a 45-year-old patient diagnosed with Grade 3 breast ptosis. Surgical intervention using the modified Pitanguy-Ribeiro technique was performed on 16 October 2023. At the one-year postoperative follow-up, the development of breast ptosis was observed; however, its severity was significantly lower compared to patients in other groups who underwent surgery using alternative techniques. The reduced severity of ptosis highlights the high effectiveness of the modified technique in achieving long-term aesthetic and functional outcomes, as well as improved stability of surgical results due to the modification implemented.

Figure 4 presents the clinical outcome in a 34-year-old patient diagnosed with Grade 3 breast ptosis. The modified Pitanguy-Ribeiro technique was applied during surgical intervention in March 2023. After one year of follow-up, no signs of recurrent breast ptosis were observed. The results demonstrated stability in the aesthetic appearance of the breasts, with preservation of anatomical position and contour. No secondary complications were reported, and no further surgical intervention was required.

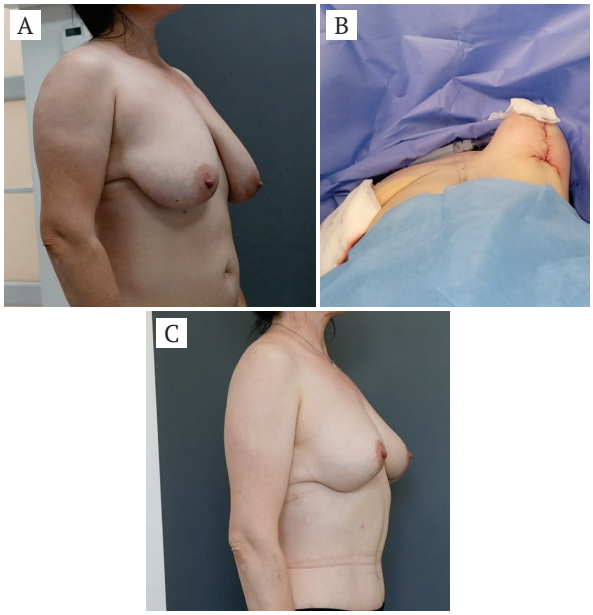

Figure 3. Appearance of the breast of a 45-year-old patient who was diagnosed with Grade 3 breast ptosis
Notes: A) before surgery; B) appearance of the breast immediately on the operating table; C) 1 year after surgery
Source: author's photo

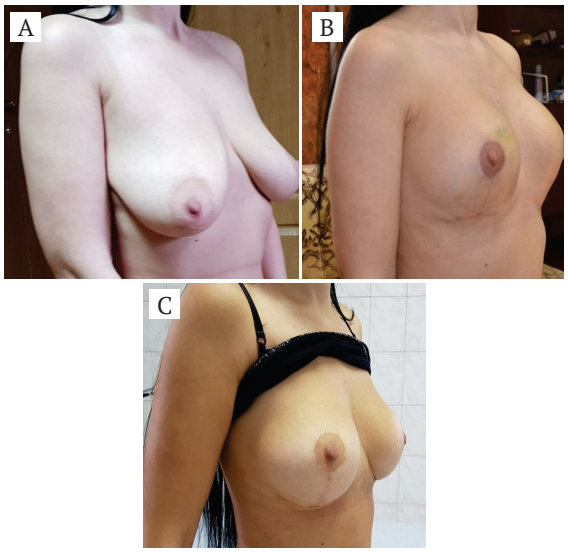

Figure 4. Appearance of the breast of a 34-year-old patient who was diagnosed with Grade 3 breast ptosis
Notes: A) before surgery; B) 1 month after surgery; C) 1 year after surgery
Source: author's photo

Thus, the modified Pitanguy-Ribeiro technique demonstrated clinical superiority, confirming its feasibility for achieving long-term aesthetic outcomes and reducing the likelihood of ptosis recurrence. No deaths, pulmonary embolism, deep vein thrombosis, or postoperative complications were recorded during the surgical interventions. Overall, the effectiveness of mastopexy techniques depends on appropriate selection based on the degree of ptosis, individual anatomical features of the mammary glands, and the presence of comorbidities. The growing application of mastopexy techniques is associated with the development and implementation of various surgical approaches employing autologous tissues and allomaterials. Among the widely recognised and relevant mastopexy techniques in plastic surgery are the methods developed by J.O. Strombeck [15] and L. Ribeiro [16].

R. Abdelkader *et al*. [17] proposed augmentation mastopexy using a five-stage standardised strategic approach. The authors presented a retrospective study involving 50 patients seeking both breast lifting and volume enhancement, requiring augmentation combined with mastopexy to achieve the desired aesthetic outcomes. The classical Pitanguy-Ribeiro technique, employed in the present study, remains one of the safest techniques for treating mastoptosis. It is applicable to women with varying breast sizes and degrees of ptosis. However, it does have certain contraindications and is not suitable for patients with gigantomastia or severe mammary ptosis [18]. H. Zavrides [19] reported a study involving 140 women with mastoptosis who underwent surgical treatment using the Pitanguy-Ribeiro technique, which by 2025 had evolved into two modified forms. The first modification, described in the works of E. Swanson [20], D.A. Hidalgo [21], and A. Kostenko *et al*. [22], involves vertical dissection of the upper breast pole down to the pectoralis major fascia. Following this, the medial flap is elevated cranially, rotated 90 degrees, and sutured, while the lateral flap is placed beneath the medial flap.

S. Wall *et al*. [23] described the combined procedure of breast augmentation and mastopexy. This approach is often considered complex, as it essentially involves two surgical interventions in one. The authors reviewed the principles and guidance for various mastopexy techniques, as well as the associated methods of breast augmentation. In cases of mastopexy with adequate breast volume, satisfactory surgical outcomes may be achieved through recombination of breast tissue and elevation of the lower pole without the use of implants, as reported by H. Jian *et al*. [24] and E.J. Moya-Rosa & Y. Moya-Corrales [25]. The circumvertical technique is typically employed for patients with moderate ptosis [26], defined by a vertical excess of 3-4 cm accompanied by significant horizontal laxity.

R. Cohen-Shohet *et al*. [27] examined the performance of mastopexy in both private clinical settings and academic institutions. They identified seven main indications for mastopexy: ptosis, postpartum atrophy, non-surgical weight loss, surgical weight loss, breast asymmetry, reconstruction, and revision of previous augmentation. The second modification of the classical Pitanguy-Ribeiro technique involves the separation of the lower flap from surrounding structures while preserving its attachment to the vascular network of the chest wall, thereby maintaining the necessary breast volume. This approach is discussed in the work of P.G. di Summa *et al*. [28]. In this technique, the upper flap of the breast is used to cover the lower flap.

The classical Pitanguy-Ribeiro technique and its two modifications offer a versatile, well-vascularised pedicle, enabling reliable repositioning of the nipple-areola complex to the desired height. The selection of a mastopexy technique should be guided by a comprehensive analysis of the patient's individual characteristics, the degree of ptosis, and any accompanying pathologies. The application of the classical Pitanguy-Ribeiro technique and its modifications confirms their high effectiveness in delivering both aesthetic and functional results. The proposed approach, which enhances tissue fixation to the pectoralis major fascia, addresses the limitations of conventional techniques by providing longer-lasting outcomes and reducing the risk of recurrence. The innovations presented in this study are aimed at maximising aesthetic results, preserving breast functionality [29], and minimising postoperative complications, making this method a promising option for widespread adoption in surgical practice. The proposed technique for counteracting postoperative mammary ptosis – through shortening, reinforcement, cranial repositioning, and fixation of the inner layer of the superficial breast fascia to the deep fascia of the pectoralis major muscle – based on the modified Pitanguy-Ribeiro method, demonstrated positive outcomes.

CONCLUSIONS

The study revealed significant differences in the outcomes of various mastopexy techniques in the correction of mastoptosis and the prevention of postoperative ptosis. The results confirm the importance of an individualised approach to the selection of surgical technique – particularly with reference to the degree of ptosis, as classified by the Pitanguy-Ribeiro scale – in order to achieve optimal aesthetic and functional outcomes. Mastopexy performed using the Pitanguy-Ribeiro technique demonstrated a low incidence of early postoperative ptosis (13.33%) but showed a high rate of late ptosis (70%), thereby limiting its long-term effectiveness. Reduction mastopexy (inverted T-shaped) was associated with a higher incidence of early ptosis (50%) but demonstrated greater long-term stability, with a lower incidence of late ptosis (6%). The most favourable results were achieved with the modified Pitanguy-Ribeiro technique. Early postoperative ptosis at one year was observed in only two patients (4%), and the incidence of late ptosis was the lowest among all groups (6%). The incorporation of a modification involving fixation of the cutaneous-glandular flap to the pectoralis major muscle, along with additional anchoring of the deep sheet of the superficial fascia, significantly improved the stability of surgical outcomes, reduced complication rates, and ensured an excellent aesthetic appearance of the mammary glands. The proposed surgical refinement – involving the application of additional ligatures to the inner sheet of the superficial fascia with cranial repositioning – enhances the projection of the upper breast contour and substantially reduces the incidence of recurrent ptosis in the late postoperative period. All patients experienced improvements in both physical and psychological well-being. No cases of serious complications, such as pulmonary embolism, deep vein

thrombosis, or tissue necrosis, were reported. Based on the analysis, it can be concluded that the modified Pitanguy-Ribeiro technique is the most effective approach for the correction of mastoptosis, providing long-term stability, preventing recurrence of ptosis, and ensuring a high level of patient satisfaction. The direction of future research should focus on further enhancing the prevention of recurrent breast ptosis in the late postoperative period.

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REFERENCES
[1] Khrapach OV. Types of postoperative aesthetic complications after breast endoprosthetics (review of the leading researches). Med Sci Ukr (MSU). 2024;20(4):126–36. DOI: 10.32345/2664-4738.4.2024.14
[2] Atiyeh B, Ghieh F, Chahine F, Oneisi A. Ptosis and bottoming out following mastopexy and reduction mammoplasty. Is synthetic mesh internal breast support the solution? A systematic review of the literature. Aesth Plast Surg. 2022;46(1):25–34. DOI: 10.1007/s00266-021-02398-x
[3] Pazhoohi F, Garza R, Kingstone A. Effects of breast size, intermammary cleft distance (cleavage) and ptosis on perceived attractiveness, health, fertility and age: Do life history, self-perceived mate value and sexism attitude play a role? Adapt Hum Behav Physiol. 2020;6:75–92. DOI: 10.1007/s40750-020-00129-1
[4] Pearlman RL, Wilkerson AH, Cobb EK, Morrissette S, Lawson FG, Mockbee CS, et al. Factors associated with likelihood to undergo cosmetic surgical procedures among young adults in the United States: A narrative review. Clin Cosmet Investig Dermatol. 2022;15:859–77. DOI: 10.2147/CCID.S358573
[5] Lee SJ, Han WY, Eom JS, Kim EK, Han HH. Analysis on the difference between the practical brassiere size and real breast volume. Plast Reconstr Surg Glob Open. 2023;11(8):e5141. DOI: 10.1097/GOX.0000000000005141
[6] Mangialardi ML, Ozil C, Lepage C. One-stage mastopexy-lipofilling after implant removal in cosmetic breast surgery. Aesth Plast Surg. 2022;46(4):1542–50. DOI: 10.1007/s00266-021-02727-0
[7] Hoyos A. Total definer: Atlas of advanced body sculpting. 1st ed. New York: Thieme Medical Pub; 2022. 514 P.
[8] Ma X, Xu B, Ouyang Y, Du X, Liu C. Preoperative three-dimensional measurement-based periareolar augmentation mastopexy: Indication and breast crown approach. Plast Reconstr Surg. 2022;150(2):310–5. DOI: 10.1097/PRS.0000000000009356
[9] Gunn J, Dortch J, TerKonda S, Schilling K, Li Z, Diehl N, et al. Comparing morbidity rates between wise pattern and standard horizontal elliptical mastectomy incisions in patients undergoing immediate breast reconstruction. Breast J. 2019;25:20–5. DOI: 10.1111/tbj.13150
[10] Nuzzi LC, Firriolo JM, Pike CM, Cerrato FE, DiVasta AD, Labow BI. The effect of surgical treatment on the quality of life of young women with breast asymmetry: A longitudinal, cohort study. Plast Reconstr Surg. 2020;146(4):400–8. DOI: 10.1097/prs.0000000000007149
[11] Regnault P. Breast ptosis: Definition and treatment. Clin Plast Surg. 1976;3(2):193–203.
[12] Khrapach VV. Basics of plastic and reconstructive surgery. Kyiv: Electronic Textbook; 2021. 148 P.
[13] The World Medical Association. Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects [Internet]. [cited 2024 August 5]. Available from: https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/
[14] Law of Ukraine No. 2297-VI. On the Protection of Personal Data [Internet]. 2010 June 1 [cited 2024 August 5]. Available from: https://www.president.gov.ua/documents/2297vi-11567
[15] Strombeck JO. Breast reconstruction. I. Reduction mammaplasty. Mod Trends Plast Surg. 1964;16:237–55.
[16] Ribeiro L. A new technique for reduction mammaplasty. Plast Reconstr Surg. 1975;55(3):330–4.
[17] Abdelkader R, Raafat S, Sakr W, Abdelaziz M, ElNoamany S. Augmentation mastopexy: A five-step standardized strategy approach. Plast Reconstr Surg Glob Open. 2022;10(6):e4349. DOI: 10.1097/GOX.0000000000004349
[18] Sá JZ, Santa Cruz Oliveira F, Santinoni WF, Freire A, de Oliveira Rodrigues AE, de Andrade Aguiar JL. Evaluation of the sensitivity of the nipple-areola complex in patients undergoing breast ptosis correction with periareolar dermis release: A randomized controlled trial. Aesth Plast Surg. 2020;44(5):1405–11. DOI: 10.1007/s00266-020-01835-7
[19] Zavrides H. The classic Pitanguy technique and its modifications in mammaplasty: Ten years of experiences. Ann Plast Surg. 2017;79(5):433–7. DOI: 10.1097/SAP.0000000000001145
[20] Swanson E. All seasons vertical augmentation mastopexy: A simple algorithm, clinical experience, and patient-reported outcomes. Plast Reconstr Surg Glob Open. 2016;4(12):e1170. DOI: 10.1097/GOX.0000000000001170
[21] Hidalgo DA. Breast augmentation: Choosing the optimal incision, implant, and pocket plane. Plast Reconstr Surg. 2000;105(6):2202–16.
[22] Kostenko AA, Galych SP, Dabizha OY, Samko KA, Borovyk DV. Current state of surgical treatment for patients with tubular breast type II (a literature review). Zaporozhye Med J. 2021;23(1):146–51. DOI: 10.14739/2310-1210.2021.1.224962
[23] Wall S, Wall H, Claiborne JR. Simultaneous short-scar mastopexy augmentation. In: Calobrace MB, Kortesis BG, Bharti G, Mays C, editors. Augmentation Mastopexy. Cham: Springer; 2020. P. 139–55. DOI: 10.1007/978-3-030-48226-8_9
[24] Jian H, Xiao Jun S, Bo T, Xiao Xia L, Xiong Yu Z. A new technique of mastopexy for mastoptosis: With the upturned glandular flap. Int J Transplant Plast Surg. 2019;3(S2):000S2–006. DOI: 10.23880/ijtps-16000S2-006

[25] Moya-Rosa EJ, Moya-Corrales Y. Treatment of the mammary ptosis utilizing the technique of Liacyr Ribeiro type I modified. Rev Arch Med Camagüey. 2019;23(1):28–36.
[26] Ikander P, Sørensen JA, Thomsen JB. Mastopexy with autologous augmentation in women after massive weight loss: A randomized clinical trial. Aesth Plast Surg. 2021;45(1):127–34. DOI: 10.1007/s00266-020-01642-0
[27] Cohen-Shohet R, Bloom J, Mast B. Mastopexy: Current trends in private practice and academic practice and the impact on resident training. Ann Plast Surg. 2020;84(6S):414–16. DOI: 10.1097/SAP.0000000000002273
[28] Di Summa PG, Oranges CM, Watfa W, Sapino G, Keller N, Tay SK, et al. Systematic review of outcomes and complications in nonimplant-based mastopexy surgery. J Plast Reconstr Aesthet Surg (JPRAS). 2019;72(2):243–72. DOI: 10.1016/j.bjps.2018.10.018
[29] Orupabo CD, George OC. Anthropometric variables in breast lesions of women of reproductive age in university of Port Harcourt teaching hospital. Int J Med Med Res (IJMMR). 2022;8(2):18–23. DOI: 10.11603/ijmmr.2413-6077.2022.2.13154

Модифікована методика Pitanquy-Ribeiro у мастопексії: перспективи покращення естетичних і функціональних результатів

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Анотація. Однією з невирішених проблем в естетичній мамопластиці залишається профілактика гравітаційного птозу в віддаленому післяопераційному періоді. Метою дослідження було покращити результати лікування мастоптозу шляхом удосконалення класичних операційних методів, які сприяють профілактиці повторного птозу у віддалений післяопераційний період. До основної групи увійшло 50 пацієнток з діагнозом мастоптоз. Пацієнткам була проведена операція за методикою Pitanguy-Ribeiro з модифікацією: фіксації укорочення краніального переміщення та фіксація до фасції великого грудного м'яза. Результати дослідження показують необхідність підбору методик мастопексії для кожної пацієнтки індивідуально, щоб отримати естетичні і функціональні результати на тривалий період. В роботі представлено розподіл пацієнток за методикою проведення мастопексії та порівняння показників раннього і пізнього післяопераційного птозу для кожної групи. Встановлено, що мастопексія проведена за методикою Pitanquy-Ribeiro є ефективною на короткостроковий період і має значний ризик розвитку пізнього післяопераційного птозу. Редукційна мастопексія має високий ризик розвитку птозу в пізній післяопераційний період, а мастопексія за методикою Pitanquy-Ribeiro з модифікацією є ефективною технікою, що дає стабільні результати в порівнянні з іншими розглянутими методиками щодо профілактики післяопераційного птозу, а саме – повну відсутність раннього птозу та мінімальну частоту пізнього птозу, що свідчить про її перевагу. Проведене дослідження дозволяє виділити методику Pitanquy-Ribeiro з модифікацією як найбільш ефективну для корекції мастоптозу, забезпечуючи довгострокову стабільність, профілактику рецидиву птозу та високий рівень задоволеності пацієнтів. Запропонована модифікація може бути рекомендована як оптимальна техніка для проведення мастопексії. Результати дослідження можуть бути використані для вдосконалення хірургічних технік, розробки рекомендацій щодо індивідуального підбору методик мастопексії та планування профілактичних заходів у віддаленому післяопераційному періоді

Ключові слова: птоз молочних залоз; хірургічна корекція; фіксація фасції; естетична мамопластика

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Evaluating the hypoglycaemic, anti-inflammatory, and antioxidant effects of *Hibiscus sabdariffa* in alloxan-induced diabetic rats

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Abstract. *Hibiscus sabdariffa* is beneficial in treating diabetes mellitus. This study investigated the hypoglycaemic, anti-inflammatory, and antioxidant effects of *Hibiscus sabdariffa* in alloxan-induced diabetic rats. Thirty Wistar rats were divided into six groups of five and acclimatised for two weeks before the experiment commenced. Group I: non-diabetic control; Group II: diabetic control; Group III: non-diabetic with 200 mg/kg of *Hibiscus sabdariffa*; Group IV: non-diabetes with 300 mg/kg of *Hibiscus sabdariffa*; Group V: diabetic with 200 mg/kg of *Hibiscus sabdariffa*; Group VI: diabetic with 300 mg/kg of *Hibiscus sabdariffa*. The rats received a single intraperitoneal injection of alloxan (150 mg/kg of body weight), and diabetic rats were treated with *Hibiscus sabdariffa* for 21 days. Fasting blood glucose levels, insulin levels, superoxide dismutase, catalase, malondialdehyde, interleukin-6, and tumour necrosis factor-alpha were measured, and organ and blood samples were collected. The results were analysed using analysis of variance with $p < 0.05$ considered significant, and data were visualised using GraphPad. This study demonstrated that *Hibiscus sabdariffa* exerts significant effects on diabetic parameters, pro-inflammatory cytokines, and antioxidant enzymes. Daily oral treatment for 21 days lowered fasting blood glucose, interleukin-6, tumour necrosis factor-alpha, and malondialdehyde levels. It also enhanced insulin production, superoxide di smutase, and catalase activity in the skeletal muscle, liver, pancreas, and kidney. It can be concluded that

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Hibiscus sabdariffa has the potential to manage hyperglycaemia and inflammation while improving antioxidant enzyme activity. Furthermore, it may serve as a natural source or agent for the treatment or prevention of diabetes

Keywords: oxidative stress biomarkers; pro-inflammatory cytokines; bioactive compounds; pancreatic β-cells

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease characterised by elevated blood glucose levels and is associated with complications affecting the heart, blood vessels, eyes, kidneys, and nerves. DM is primarily classified into two types: type 1 diabetes mellitus (T1DM), which typically results from the destruction of insulin-producing β-cells, and type 2 diabetes mellitus (T2DM), which is characterised by insulin resistance and accounts for over 90% of all diabetes cases [1]. According to the International Diabetes Federation (IDF), diabetes affects approximately 537 million people aged 20-79 years, with this number projected to rise to 643 million by 2030 and 783 million by 2045 [2]. As of 2025, conventional antidiabetic medications do not always provide optimal treatment outcomes. For instance, D.T. Liss *et al*. [3] reported that nearly 40% of patients with T2DM discontinued their second-line medication within a year, highlighting challenges related to the effectiveness and tolerability of current pharmacotherapies.

Oxidative stress disrupts cellular signalling and contributes to endothelial dysfunction by damaging DNA, proteins, cell membranes, and plasma lipids. This damage leads to the activation of inflammatory mediators, including nuclear factor-kappa B (NF-κB) [4]. Once activated, NF-κB stimulates the production of pro-inflammatory cytokines, including interleukin-6 (IL-6) and tumour necrosis factoralpha (TNF-α). Elevated levels of these cytokines are commonly observed in pre-diabetic and diabetic individuals [5]. Given the detrimental effects of oxidative stress and inflammation in diabetes, developing antioxidant and anti-inflammatory therapeutic strategies has become a critical area of research.

Hibiscus sabdariffa (HS), commonly known as Roselle, is a plant rich in bioactive compounds. It has been reported to exhibit multiple therapeutic properties, including antihypertensive, antimicrobial, hepatoprotective, antioxidant, antihyperlipidaemic, anticancer, anti-inflammatory, and antidiabetic activities. The antioxidant and anti-inflammatory properties of bioactive compounds in medicinal plants have been extensively studied in the context of diabetes management [6-8]. Research by A. Hamadjida *et al*. [9] identified triterpenes, flavonoids, phenolic compounds, polysaccharides, organic acids, vitamins, and tannins as the major bioactive compounds present in the calyces of HS, contributing to its antidiabetic properties in alloxan-induced diabetic rats. Similarly, N. Herdiani & E.A. Wikurendra [10] demonstrated that Roselle petal extract effectively reduces malondialdehyde (MDA) levels – a biomarker of lipid peroxidation – in diabetic rats, suggesting its potential to mitigate oxidative stress.

Despite the documented benefits of HS, its specific effects on insulin signalling pathways and the interrelationship between oxidative stress and inflammation remain underexplored. Therefore, this study aimed to investigate the impact of *Hibiscus sabdariffa* on insulin signalling, oxidative stress, and inflammation in alloxan-induced diabetic rats.

MATERIALS AND METHODS

The study was conducted in May 2024 at the University of Ilorin, Nigeria. Thirty albino Wistar rats, weighing between 150 and 212 g, were used in this experiment. They were obtained from Temmy Concept, Gaa Akanbi, Ilorin, Kwara State. The rats were housed in a controlled environment (ambient room temperature and a 12-hour light/dark cycle) and divided into six groups. They were fed a standard rat diet and provided with ad libitum access to water. Cages were cleaned daily, and food and water were replenished, while the rats were acclimatised for two weeks before the experiment commenced.

Ethical approval for this experiment was obtained from the University of Ilorin Ethical Review Committee through the Faculty of Basic Medical Sciences (protocol identification code UERC/BMS/238 and approval number UERC/ASN/2024/2935). The study complied with the Institutional Animal Care and Use Committee (IACUC) guidelines, ensuring the humane treatment and welfare of all animals used in the research. These guidelines align with internationally recognised ethical standards for the use of animals in scientific research, including the principles of Replacement, Reduction, and Refinement (3Rs) [11].

The calyces of HS were purchased from Ipata Market, Ilorin, Kwara State, Nigeria, and identified at the Herbarium of the Department of Plant Biology, University of Ilorin. The calyces were air-dried in a dust-free environment at the Pharmacy Department of the University of Ilorin. The dried calyces were ground into a fine powder using a mechanical grinder. The powdered material was soaked in ethanol for 48 hours with occasional agitation. The mixture was then filtered using filter paper, and the resulting extract was concentrated to a final concentration of 100 mg/mL. The extract was stored at 4°C in tightly sealed, amber-coloured glass bottles until use. Extract administration was performed orally. Male Wistar rats were randomly assigned to six groups of five animals each (n = 5) (Table 1).

Table 1. Distribution of rats (n = 30)

Groups	Description	No. of animal	Treatment
Group I	Non-diabetic control	5	Normal saline
Group II	Diabetic control	5	Normal saline
Group III	Non-diabetic	5	HS (200 mg/kg)
Group IV	Non-diabetic	5	HS (300 mg/kg)

Table 1. Continued

Groups	Description	No. of animal	Treatment
Group V	Diabetic	5	HS (200 mg/kg)
Group VI	Diabetic	5	HS (300 mg/kg)

Source: compiled by the authors

Diabetes mellitus was induced with an intraperitoneal injection of alloxan at a dose of 150 mg/kg of body weight, prepared at a concentration of 20 mg/mL in normal saline. The rats underwent a 36-hour fasting period prior to injection, and the injection was administered in the morning at pH 4.5, according to the method described by O.L. Sheriff *et al*. [12]. The diabetic state was assessed by measuring blood glucose levels 72 hours post-induction using an On-Call Plus glucometer. Rats with blood glucose levels above 200 mg/dL were classified as diabetic.

Fasting blood glucose (FBG) was measured before and after the experiment using an On-Call Plus glucometer. At the end of the experiment, ketamine was used as the anaesthetic agent, and blood samples were collected. The serum was separated by centrifuging the blood at 3,000 rpm for 20 minutes at 4°C. Serum insulin levels were measured using the Insulin Mouse ELISA Kit (Thermo Fisher Scientific, MA, USA), following the manufacturer's instructions. Lipid peroxidation refers to the oxidative degradation of lipids caused by reactive oxygen species. MDA is a key by-product of this process and serves as a reliable marker of lipid oxidative damage.

To assess lipid peroxidation, MDA levels were quantified using the Thiobarbituric Acid Reactive Substances (TBARS) method. Specifically, MDA levels were determined in skeletal muscle, pancreas, kidney, and liver homogenates and normalised to protein content [13]. Superoxide dismutase (SOD) and catalase are key endogenous antioxidant enzymes that neutralise free radicals. SOD activity was assessed according to the method described by H.P. Misra & I. Fridovich [14], based on the inhibition of epinephrine auto-oxidation at pH 10.2. Catalase activity was determined using the method of A.K. Sinha [15], based on dichromate reduction.

Plasma levels of the pro-inflammatory cytokines IL-6 and TNF-α were measured using a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) kit (EDM Millipore, MA, USA). The assay involved incubating samples with a biotin-conjugated solution, followed by streptavidin-HRP, and measuring absorbance spectrophotometrically at 450 nm. Plasma samples were collected using the retro-orbital technique under anaesthesia, whereby a needle was inserted into the retro-orbital sinus to obtain 2-3 mL of blood. Plasma was carefully separated by centrifugation of the blood at 2,000 rpm for 15 minutes. Statistical analysis was conducted using SPSS version 17 (SPSS Inc., Chicago, USA) and GraphPad Prism version 5 (GraphPad Software, Inc., La Jolla, USA). Data are presented as mean ± SEM. One-way ANOVA with Tukey's multiple comparison tests was employed for statistical comparisons, and a p-value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The FBG levels of diabetic and non-diabetic rats were measured on days 0 and 21 to assess the effect of HS administered at doses of 200 and 300 mg/kg. On day 0, the non-diabetic control group had an average FBG of 90 ± 3.17 mg/dL, which decreased significantly to 78 ± 2.26 mg/dL by day 21 (p = 0.005). Similarly, the non-diabetic group treated with 300 mg/kg HS showed a significant reduction in FBG from 86 ± 1.12 to 69.5 ± 1.20 mg/dL (p = 0.002), indicating the glucose-lowering potential of HS. In contrast, the non-diabetic group treated with 200 mg/kg HS showed a slight increase in FBG levels from 85.5 ± 0.18 to 90.5 ± 0.07 mg/dL, which was not statistically significant (p = 0.12).

For the diabetic groups, the control rats exhibited persistently elevated FBG levels, with no significant change between day 0 (239.5 ± 3.10 mg/dL) and day 21 (235 ± 0.20 mg/dL, p = 0.45). Diabetic rats treated with 200 mg/kg HS showed increased FBG levels from 368 ± 0.9 to 426 ± 2.09 mg/dL, but this change was not statistically significant (p = 0.15). However, 300 mg/kg HS treatment led to a statistically significant reduction in FBG levels, from 367.6 ± 2.14 mg/dL on day 0 to 88.5 ± 3.018 mg/dL on day 21 (p = 0.001). The results are shown in Figure 1.

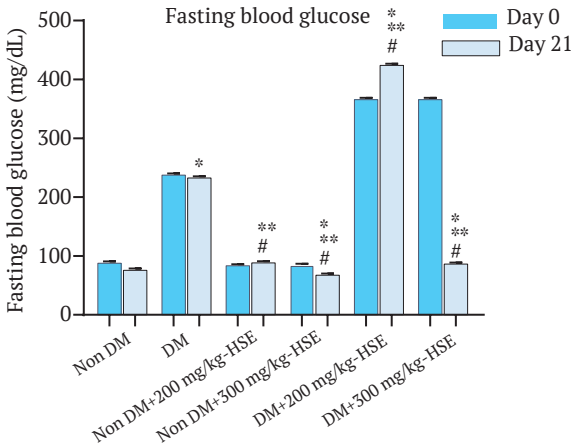

Figure 1. Hypoglycaemic effect of HS on FBG in alloxan-induced diabetic Wistar rats

Notes: * – p < 0.05 vs control group; ** – p < 0.05 vs diabetic control; # – p < 0.05 vs blood glucose on day 0. Data are presented as mean ± SEM (n = 5)

Source: compiled by the authors

The serum insulin level in the control group was 2.59 ± 0.05 µIU/mL, which was significantly higher (p < 0.05) than in the diabetic control group (0.79 ± 0.06 µIU/mL). Treatment with HS resulted in increased serum insulin levels in diabetic rats in a dose-dependent manner. Diabetic rats treated with 200 mg/kg HS had 1.96 ± 0.06 µIU/mL (p < 0.01), while those treated with 300 mg/kg HS had 2.68 ± 0.06 µIU/mL (p < 0.001). This is shown in Figure 2. In non-diabetic rats, serum insulin levels were also significantly elevated (p < 0.05) following HS treatment. The

control group had 2.59 ± 0.05 µIU/mL, while the 200 mg/kg HS group had 3.41 ± 0.06 µIU/mL ($p < 0.001$), and the 300 mg/kg HS group had 3.91 ± 0.05 µIU/mL ($p < 0.001$).

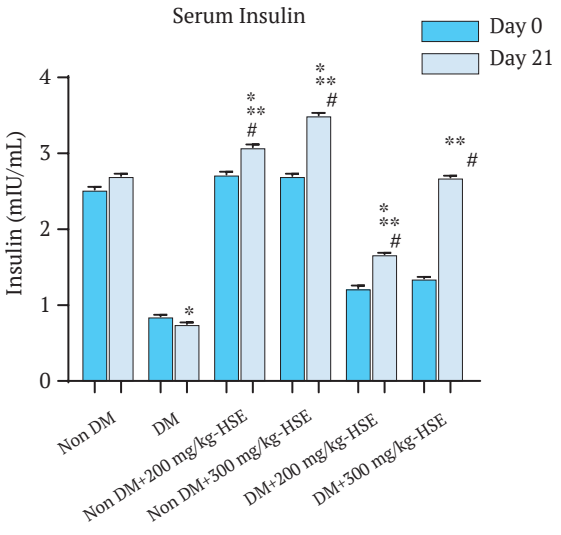

Figure 2. Hypoglycaemic effect of HS on insulin levels in alloxan-induced diabetic Wistar rats
Notes: * – $p < 0.05$ vs control group; ** – $p < 0.05$ vs diabetic control; # – $p < 0.05$ vs blood glucose on day 0. Data are presented as mean ± SEM (n = 5)
Source: compiled by the authors

The antioxidant parameters, including catalase activity, SOD activity, SOD inhibition, and MDA concentrations, were assessed in skeletal muscle, pancreas, liver, and kidney tissues of alloxan-induced diabetic Wistar rats treated with *Hibiscus sabdariffa* at doses of 200 and 300 mg/kg. Comparisons were made between the non-diabetic control, non-diabetic HS-treated groups, and diabetic groups with and without HS treatment. Catalase activity, a key antioxidant marker, was significantly higher in the control group (71.9 ± 0.33 U/mL) compared to the diabetic control (1.6 ± 0.02 U/mL, $p < 0.001$). Treatment with HS at 200 and 300 mg/kg restored catalase activity in diabetic rats (34.9 ± 0.29 and 10.4 ± 0.30 U/mL, respectively, $p < 0.01$ for both). Similarly, MDA levels, an indicator of oxidative stress, were elevated in the diabetic control group (0.358 ± 0.03 mmol/mL) and significantly reduced following HS treatment at 300 mg/kg (0.213 ± 0.31 mmol/mL, $p < 0.05$) (Fig. 3).

In the pancreas (Fig. 4), the diabetic control group exhibited significantly reduced catalase activity (1.0 ± 0.12 U/mL) compared to the control group (0.7 ± 0.08 U/mL, $p < 0.001$). Treatment with 200 mg/kg HS significantly increased catalase activity in diabetic rats (21.9 ± 0.81 U/mL, $p < 0.01$). Additionally, MDA levels were highest in the non-diabetic 300 mg/kg group (0.468 ± 0.22 mmol/mL), with a marked reduction in the diabetic group treated with 300 mg/kg HS (0.096 ± 0.04 mmol/mL, $p < 0.001$), demonstrating the dose-dependent antioxidative effect of HS.

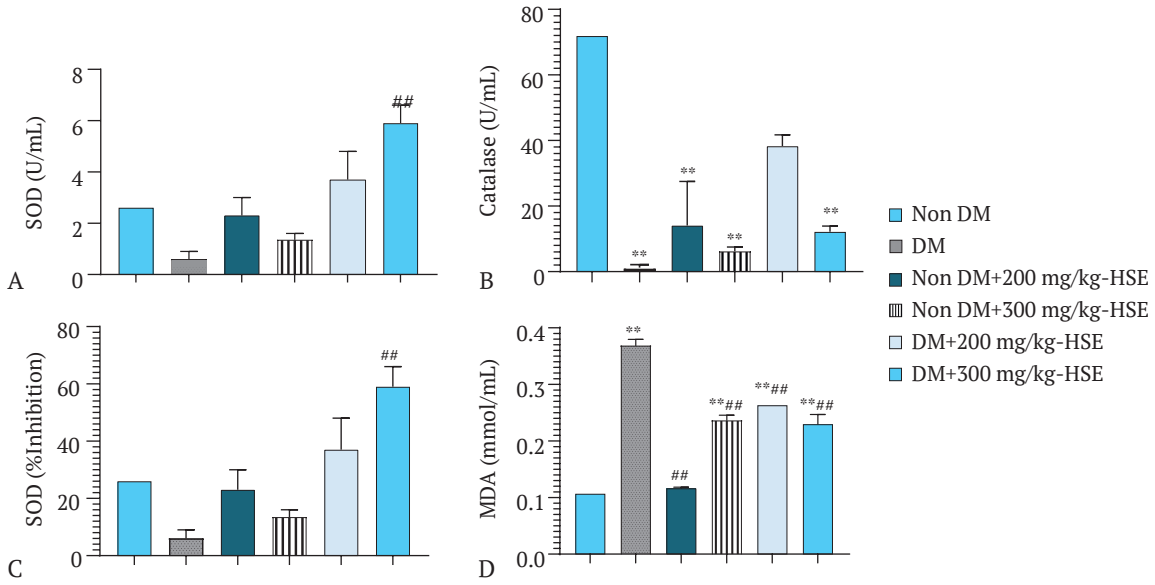

Figure 3. Antioxidant effect of HS on antioxidant enzyme parameters in skeletal muscle
Notes: A – Catalase; B – SOD; C – SOD inhibition; D – MDA; * – $p < 0.05$ vs control group; ** – $p < 0.05$ vs diabetic control; # – $p < 0.05$ vs blood glucose on day 0. Data are presented as mean ± SEM (n = 5)
Source: compiled by the authors

The diabetic control group showed the lowest catalase activity in the liver (1.0 ± 0.45 U/mL), which was significantly lower than that of the control group (55.9 ± 0.23 U/mL, $p < 0.001$). Administration of HS to diabetic rats improved catalase activity to 32.4 ± 0.67 U/mL (200 mg/kg, $p < 0.05$) and 18.5 ± 0.42 U/mL (300 mg/kg, $p < 0.01$). A similar trend was observed for SOD activity, which improved with HS treatment but remained significantly lower than in the control group. This is shown in Figure 5.

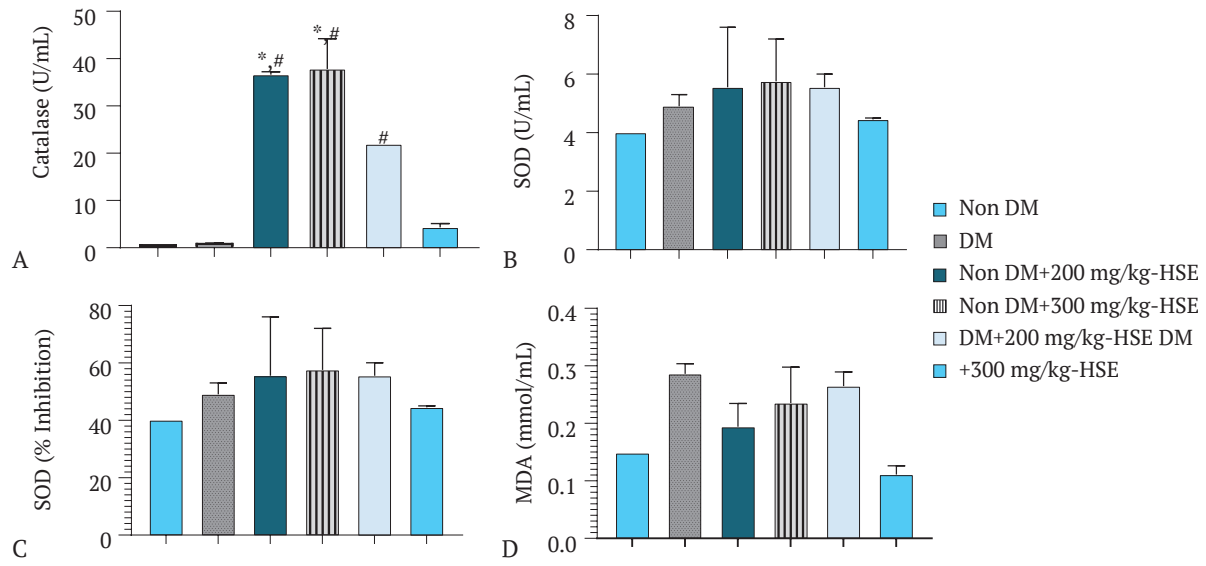

Figure 4. Antioxidant effect of HS on antioxidant enzyme parameters in the pancreas
Notes: A – Catalase; B – SOD; C – SOD inhibition; D – MDA; * – $p < 0.05$ vs control group; ** – $p < 0.05$ vs diabetic control; # – $p < 0.05$ vs blood glucose on day 0. Data are presented as mean ± SEM (n = 5)
Source: compiled by the authors

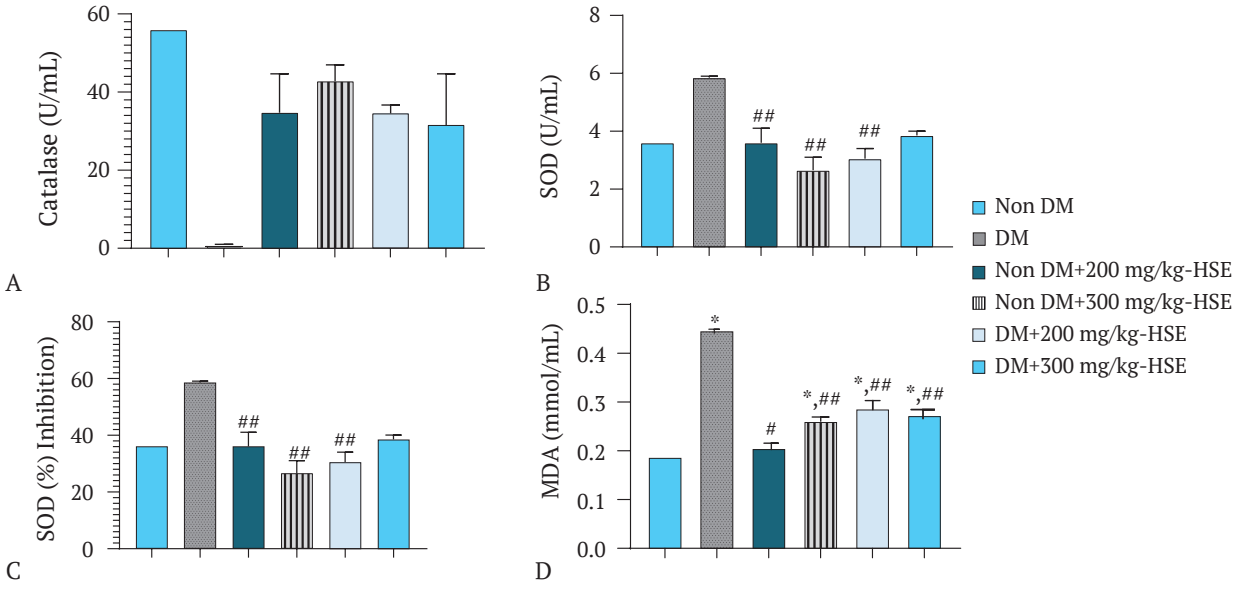

Figure 5. Antioxidant effect of HS on antioxidant enzyme parameters in the liver
Notes: A – Catalase; B – SOD; C – SOD inhibition; D – MDA; * – $p < 0.05$ vs control group; ** – $p < 0.05$ vs diabetic control; # – $p < 0.05$ vs blood glucose on day 0. Data are presented as mean ± SEM (n = 5)
Source: compiled by the authors

In the kidney (Fig. 6), HS treatment resulted in significant improvements in catalase activity in diabetic rats (17.6 ± 0.30 U/mL for 200 mg/kg and 3.6 ± 0.32 U/mL for 300 mg/kg) compared to the control group (1.4 ± 0.02 U/mL, $p < 0.001$). MDA levels, which were elevated in the diabetic control group (0.400 ± 0.41 mmol/mL), were significantly reduced following 200 mg/kg HS treatment (0.234 ± 0.26 mmol/mL, $p < 0.01$).

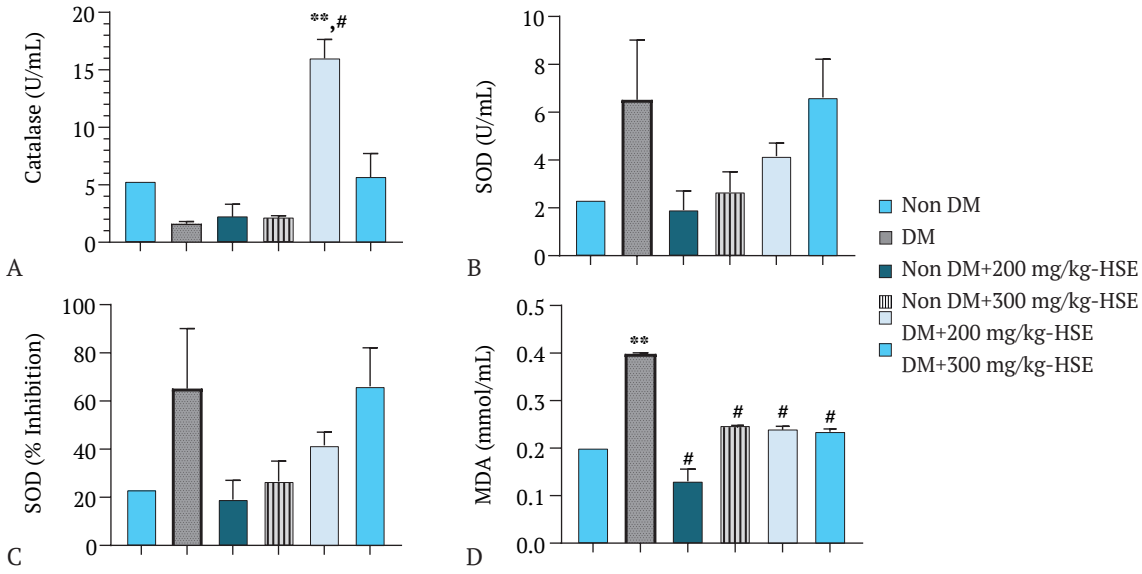

Figure 6. Antioxidant effect of HS on antioxidant enzyme parameters in the kidney
Notes: A – Catalase; B – SOD; C – SOD inhibition; D – MDA; * – $p < 0.05$ vs control group; ** – $p < 0.05$ vs diabetic control; # – $p < 0.05$ vs blood glucose on day 0. Data are presented as mean ± SEM (n = 5)
Source: compiled by the authors

The effect of HS treatment on TNF-α and IL-6 levels was evaluated in control, diabetic, and HStreated groups. The treatment demonstrated a capacity to modulate inflammatory cytokine levels in both diabetic and non-diabetic rats. Figure 7 illustrates that the TNF-α level in the control group was 42.22 ± 3.53 pg/mL, while it increased significantly in the diabetic control group to 110.19 ± 9.46 pg/mL ($p = 0.007$). In the non-diabetic groups treated with HS, TNF-α levels decreased to 20.14 ± 2.61 pg/mL in the 200 mg/kg group ($p = 0.010$) and 25.67 ± 3.44 pg/mL in the 300 mg/kg group ($p = 0.035$). In diabetic rats, HS treatment also reduced TNF-α levels. The group treated with 200 mg/kg HS had a TNFα level of 81.03 ± 3.85 pg/mL, while the 300 mg/kg HS-treated group showed a level of 75.86 ± 3.71 pg/mL. Although these reductions, compared to the diabetic control group, did not reach statistical significance ($p = 0.081$ and $p = 0.074$, respectively), the downward trend suggests an antiinflammatory effect.

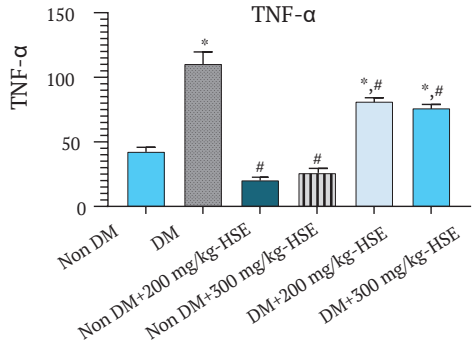

Figure 7. Anti-inflammatory effect of HS on inflammatory parameters (TNF-α)
Notes: * – $p < 0.05$ vs control group; ** – $p < 0.05$ vs diabetic control; # – $p < 0.05$ vs blood glucose on day 0. Data are presented as mean ± SEM (n = 5)
Source: compiled by the authors

Figure 8 shows that the IL-6 level in the control group was 0.113 ± 0.003 pg/mL. In the diabetic control group, IL-6 levels increased significantly to 0.150 ± 0.001 pg/mL ($p = 0.001$). Non-diabetic rats treated with HS exhibited reductions in IL-6 levels, with the 200 mg/kg group recording 0.103 ± 0.0001 pg/mL ($p = 0.047$) and the 300 mg/kg group recording 0.105 ± 0.001 pg/mL ($p = 0.107$). In diabetic rats, HS treatment at 200 mg/kg reduced IL-6 levels to 0.118 ± 0.001 pg/mL ($p < 0.001$), while the 300 mg/kg group recorded a further reduction to 0.106 ± 0.001 pg/mL ($p < 0.001$).

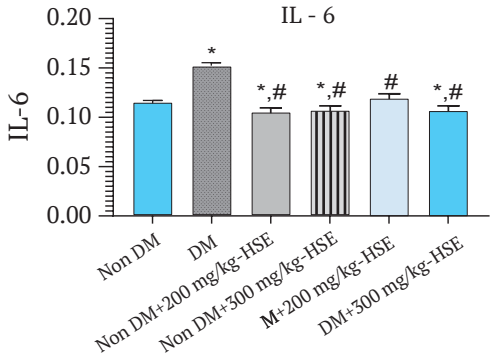

Figure 8. Anti-inflammatory effect of HS on inflammatory parameters (IL-6)
Notes: * – $p < 0.05$ vs control group; ** – $p < 0.05$ vs diabetic control; # – $p < 0.05$ vs blood glucose on day 0. Data are presented as mean ± SEM (n = 5)
Source: compiled by the authors

Elevated fasting blood glucose serves as a hallmark of impaired glucose metabolism and is a critical diagnostic indicator of diabetes mellitus. In this study, alloxan-induced diabetes resulted in a significant elevation of FBG levels in the diabetic control group compared to the normal control

group, consistent with its mechanism of β-cell destruction. However, administering *Hibiscus sabdariffa* extract at a dose of 300 mg/kg significantly reduced FBG levels, bringing them closer to the values observed in the nondiabetic control group. This effect aligns with findings reported by G.I. Kasimu *et al*. [16], who demonstrated the hypoglycaemic efficacy of HS in untreated diabetic rats. The observed reduction in FBG may be attributed to improved insulin secretion, enhanced glucose transport, or the regeneration of pancreatic β-cells, supported by the bioactive phytochemicals (polyphenols – especially anthocyanins – polysaccharides, and organic acids) in HS extract [17]. These findings emphasise the therapeutic potential of HS in managing hyperglycaemia and improving insulin sensitivity in diabetic conditions. A study by T. Suárez-Diéguez *et al*. [18] evaluated HS extracts in diabetic rats over an 80-day period. The authors found that doses of 200, 400, and 600 mg/kg significantly reduced fasting blood glucose levels by 35.2, 41.63, and 50.1%, respectively. Additionally, the highest dose improved lipid metabolism by lowering total cholesterol, triglycerides, VLDL, and LDL while increasing HDL levels. This suggests that HS extract can modulate glucose and lipid metabolism while offering potential as a functional ingredient or nutraceutical for managing diabetes. In another study, B.O. Ajiboye *et al*. [19] investigated the effects of flavonoid extracts from HS on nephropathy in streptozotocin-induced rats. The extracts mitigated kidney damage, indicating potential benefits for diabetic complications.

The present studies demonstrate that HS extracts can effectively reduce blood glucose levels and improve lipid profiles in diabetic models. However, the results of this study specifically highlight potential mechanisms, such as β-cell regeneration and improved insulin sensitivity, which are not explored in detail in other studies. This distinction adds a unique dimension to understanding how HS exerts its antidiabetic effects. Additionally, while the other studies focus on specific aspects such as nephropathy or lipid metabolism, the present research provides a more comprehensive view by addressing the hypoglycaemic, anti-inflammatory, and antioxidant effects of the extract. This holistic approach offers a more thorough insight into the potential therapeutic benefits of HS in diabetes management. Insulin is a key hormone involved in glucose storage in the liver, muscle, and adipose tissue. Following diabetes induction with alloxan, insulin levels decreased in the DM groups compared to the non-DM groups. The subsequent increase in insulin levels, particularly at the higher HS dose, aligns with reports from D. Jamrozik *et al*. [20] and M. Bule *et al*. [21], who proposed that gallic acid, a component of HS (Roselle), is responsible for stimulating insulin secretion through β-cell regeneration in the islets of Langerhans., This process enhances insulin sensitivity and reduces insulin resistance. D.B. Koval *et al*. [22] conducted a study similar to the present one, using the same species of rats but obtaining slightly different statistical results. The authors also emphasised the benefits of the chemical compound microcrystalline cellulose rather than those of plant-based treatments.

Diabetes-induced oxidative stress was evident in this study, as reflected by increased levels of MDA, a marker of lipid peroxidation, and reduced activity of antioxidant enzymes such as SOD and CAT in the diabetic control group. These alterations were observed across key organs involved in glucose regulation, including skeletal muscle, the pancreas, liver, and kidney. Elevated MDA levels in diabetic rats suggest heightened peroxidative injury, contributing to the development of DM. Oxidative stress activates IL-6 and TNF-α, worsening insulin sensitivity and exacerbating hyperglycaemia. D. Jamrozik *et al*. [20] reported that patients with high FBG had increased MDA levels and decreased SOD and CAT activity, alongside elevated IL-6 and TNF-α, forming a cycle that perpetuates the diabetic state. In this study, oxidative stress – characterised by elevated MDA levels and reduced SOD and CAT activities – was significantly mitigated in the HS-treated groups. The improvement in antioxidant activity across multiple organs indicates the protective role of HS against oxidative damage. Comparative studies have further elucidated the antioxidant properties of HS in diabetic models. Ajiboye *et al.* [19] demonstrated that an aqueous HS extract ameliorated diabetic nephropathy in streptozotocin-induced type 1 diabetic rats by reducing lipid peroxidation and increasing catalase and glutathione activity in the kidney, suggesting that HS exerts protective effects via modulation of oxidative stress pathways. Additionally, a systematic review by D. Jamrozik *et al*. [20] and M. Bule *et al*. [21] highlighted that HS exhibits hypoglycaemic, antioxidant, hypotensive, and anti-lipidaemic activities, indicating its potential as a complementary therapy in diabetes management. These findings align with the results of the present experiment, reinforcing the notion that HS's antioxidant properties contribute significantly to its therapeutic effects in diabetes.

Inflammation, evidenced by elevated IL-6 and TNF-α levels in diabetic rats, was significantly reduced following HS treatment, further demonstrating its anti-inflammatory properties. This is consistent with previous studies that have reported the anti-inflammatory effects of HS [23, 24]. The observed anti-inflammatory response is likely due to the presence of phytochemical compounds in HS extract, which modulate inflammatory pathways [25]. This study underscores the multifaceted role of HS in mitigating hyperglycaemia, enhancing insulin secretion, reducing oxidative stress, and suppressing inflammation in diabetic rats. The interplay between fasting blood glucose, insulin levels, oxidative stress, and inflammation is well-documented in the pathogenesis of DM. This study also identifies a correlation between antioxidant enzymes and pro-inflammatory cytokines such as IL-6 and TNF-α in diabetes. The administration of *Hibiscus sabdariffa* over a 21-day period significantly affected FBG, insulin levels, antioxidant enzymes, and pro-inflammatory cytokines in alloxan-induced diabetic rats.

CONCLUSIONS

This study demonstrates the potential of *Hibiscus sabdariffa* as a therapeutic agent for managing diabetes and its complications. Alloxan administration induced hyperglycaemia, oxidative stress, and inflammation in diabetic rats, replicating key pathological features of diabetes mellitus. However, treatment with HS extract at 300 mg/kg significantly reduced fasting blood glucose levels, likely through enhanced insulin secretion and the regeneration of pancreatic β-cells. This suggests that HS may contribute to improved glucose homeostasis, making it a promising

candidate for diabetes management. Beyond its hypoglycaemic effect, HS exhibited strong antioxidant and anti-inflammatory properties. The extract reduced malondialdehyde levels, a marker of lipid peroxidation while increasing the activities of key antioxidant enzymes such as superoxide dismutase and catalase. These antioxidative effects suggest that HS helps counteract diabetes-induced oxidative damage, protecting vital metabolic organs such as the liver, pancreas, kidneys, and skeletal muscles. Additionally, the extract significantly reduced the proinflammatory cytokines IL-6 and TNF-α, indicating its potential to alleviate inflammation, a major contributor to insulin resistance and diabetes progression.

Comparative studies further reinforce these findings, with prior research demonstrating similar hypoglycaemic, antioxidative, and anti-inflammatory effects of HS in various diabetic models. The presence of bioactive compounds, such as anthocyanins and polyphenols, in HS, likely underpins these effects by modulating multiple pathological pathways, including insulin sensitivity, glucose metabolism, and oxidative stress responses. These findings have significant implications, particularly in regions with limited access to conventional antidiabetic medications. As a natural, cost-effective alternative, HS holds promise for improving diabetes management while mitigating its complications. However, further research is necessary to optimise its dosage, evaluate its long-term effects, and validate its efficacy in human clinical trials. Additionally, investigating its interactions with standard antidiabetic drugs may provide insights into its potential role as a complementary treatment. Overall, *Hibiscus sabdariffa* offers a promising natural intervention for diabetes care, warranting further investigation in translational and clinical settings.

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CONFLICT OF INTEREST

None.

REFERENCES

[1] Laakso M. Biomarkers for type 2 diabetes. Mol Metab. 2019;27:139–46. DOI: 10.1016/j.molmet.2019.06.016
[2] International Diabetes Federation (IDF). Diabetes and kidney disease (Atlas report) [Internet]. 2023 [cited 2024 August 13]. Available from: https://diabetesatlas.org/atlas/diabetes-and-kidney-disease/
[3] Liss DT, Cherupally M, O'Brien MJ, Kang RH, Aikman C, Wallia A, et al. Treatment modification after initiating second-line medication for type 2 diabetes. Am J Manag Care. 2023;29(12):661–8. DOI: 10.37765/ajmc.2023.89466
[4] Yulianti E, Sunarti, Wahyuningsih MSH. The effect of *Kappaphycus alvarezii* active fraction on oxidative stress and inflammation in streptozotocin and nicotinamide-induced diabetic rats. BMC Complement Med Ther. 2022;22:15. DOI: 10.1186/s12906-021-03496-8
[5] Araujo LS, da Silva MV, da Silva CA, Borges MDF, da Cunha Palhares HM, Rocha LP, et al. Analysis of serum inflammatory mediators in type 2 diabetic patients and their influence on renal function. PloS One. 2020;15(3):e0229765. DOI: 10.1371/journal.pone.0229765
[6] Izquierdo-Vega JA, Arteaga-Badillo DA, Sánchez-Gutiérrez M, Morales-González JA, Vargas-Mendoza N, Gómez-Aldapa CA, et al. Organic acids from Roselle (*Hibiscus sabdariffa* L.) – a brief review of its pharmacological effects. Biomedicines. 2020;8(5):100. DOI: 10.3390/biomedicines8050100
[7] Islam MM. Food and medicinal values of Roselle (*Hibiscus sabdariffa* L. Linne Malvaceae) plant parts: A review. Open J Nutr Food Sci. 2019;1(1):1003.
[8] Montalvo-González E, Villagrán Z, González-Torres S, Iñiguez-Muñoz LE, Isiordia-Espinoza MA, Ruvalcaba-Gómez JM, et al. Physiological effects and human health benefits of *Hibiscus sabdariffa*: A review of clinical trials. Pharmaceuticals. 2022;15(4):464. DOI: 10.3390/ph15040464
[9] Hamadjida A, Metechie LC, Tchiengang FDT, Otto GLN, Eteme ON, Njintang NY, et al. Antidiabetic potential of *Hibiscus sadariffa* extract in alloxan-induced diabetic rats. GSC Biol Pharm Sci. 2023;23(1):193–203. DOI: 10.30574/gscbps.2023.23.1.0158
[10] Herdiani N, Wikurendra EA. Effect of Roselle petal extract on decreased levels of MDA in rats with type 2 diabetes. J Health Sci. 2021;14(1):48–52. DOI: 10.33086/jhs.v14i1.1688
[11] Institutional Animal Care and Use Committee Guidebook [Internet]. 2002 [cited 2024 August 13]. Available from: https://grants.nih.gov/grants/olaw/guidebook.pdf
[12] Sheriff OL, Olayemi O, Taofeeq AO, Riskat KE, Ojochebo DE, Ibukunoluwa AO. A new model alloxan-induced diabetes mellitus in rats. J Bangladesh Soc Physiol. 2020;14(2):56–62. DOI: 10.3329/jbsp.v14i2.44785
[13] Kil HN, Eom SY, Park JD, Kawamoto T, Kim YD, Kim H. A rapid method for estimating the levels of urinary thiobarbituric acid reactive substances for environmental epidemiologic survey. Toxicol Res. 2014;30(1):7–11. DOI: 10.5487/TR.2014.30.1.007
[14] Misra HP, Fridovich I. The role of superoxide anion in the auto-oxidation of epinephrine and simple assay for superoxide dismutase. J Biol Chem. 1972;247(10):3170–5.
[15] Sinha AK. Colorimetric assay of catalase. Anal Biochem. 1972;47(2):389–94. DOI: 10.1016/0003-2697(72)90132-7
[16] Kasimu GI, Ababubakar MB, Audu BB, Mainasara AS. Effect of co-administration of glibenclamide and aqueous calyx extract of hibiscus sabdariffa on oxidative stress markers in streptozotocin-induced diabetic rats. Niger J Physio Sci. 2021;36(1):77–84.

[17] Adeyemi DO, Adewole OS. *Hibiscus sabdariffa* renews pancreatic β-cells in experimental type 1 diabetic model rats. Morphologie. 2019;103(341(2)):80–93. DOI: 10.1016/j.morpho.2019.04.003
[18] Suárez-Diéguez T, Palma-Morales M, Camacho Bernal GI, Valdez López EN, Rodríguez-Pérez C, del Socorro Cruz-Cansino N, et al. Modulation of the hyperglycemia condition in diabetic lab rats with extracts of the creole Jamaica Flower (*Hibiscus sabdariffa* L.) from the Morelia region (Mexico). Antioxidants. 2024;13(8):1010. DOI: 10.3390/antiox13081010
[19] Ajiboye BO, Famusiwa CD, Nifemi DM, Ayodele BM, Akinlolu OS, Fatoki TH, et al. Nephroprotective effect of *Hibiscus sabdariffa* leaf flavonoid extracts via KIM-1 and TGF-1β signaling pathways in streptozotocin-induced rats. ACS Omega. 2024;9(17):19334–44. DOI: 10.1021/acsomega.4c00254
[20] Jamrozik D, Borymska W, Kaczmarczk-Żebrowska I. *Hibiscus sabdariffa* in diabetes prevention and treatment – does it work? An evidence-based review. Foods. 2022;11(14):2134. DOI: 10.3390/foods11142134
[21] Bule M, Albelbeisi AH, Nikfar S, Amini M, Abdollahi M. The Antidiabetic and antilipidemic effects of *Hibiscus sabdariffa*: A systematic review and meta-analysis of randomized clinical trials. Food Res Int. 2020;130:108980. DOI: 10.1016/j.foodres.2020.108980
[22] Koval DB, Malyarchuk HR, Levenets OO. The effect of microcrystalline cellulose on the microflora of the colon. Int J Med Med Res (IJMMR). 2021;7(2):91–6. DOI: 10.11603/ijmmr.2413-6077.2021.2.12516
[23] Janson B, Pramsomthong J, Malakul W, Boonsong T, Tunsophon S. *Hibiscus sabdariffa* L. calyx extract prevents the adipogenesis of 3T3-L1 adipocytes, and obesity-related insulin resistance in high-fat diet-induced obese rats. Biomed Pharmacother. 2021;138:111438. DOI: 10.1016/j.biopha.2021.111438
[24] Prasomthong J, Limpeanchob N, Daodee S, Chonpathompikunlert P, Tunsophon S. *Hibiscus sabdariffa* extract improves hepatic steatosis, partially through IRS-1/Akt and Nrf2 signaling pathways in rats fed a high fat diet. Sci Rep. 2022;12:7022. DOI: 10.1038/s41598-022-11027-9
[25] Hamadjida A, Ayissi Mbomo RE, Minko SE, Ntchapa F, Mingoas JPK, Nnanga N. Antioxidant and anti-inflammatory effects of *Boswellia dalzielii* and *Hibiscus sabdariffa* extracts in alloxan-induced diabetes rats. Metab Open. 2024;21:100278. DOI: 10.1016/j.metop.2024.100278

Оцінка гіпоглікемічної, протизапальної та антиоксидантної дії *Hibiscus sabdariffa* у щурів з алоксановим діабетом

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Анотація. *Hibiscus sabdariffa* корисний при лікуванні цукрового діабету. Дослідження було присвячено вивченю гіпоглікемічної, протизапальної та антиоксидантної дій *Hibiscus sabdariffa* у щурів з алоксановим діабетом. 30 щурів лінії Вістар були розділені на шість груп по п'ять щурів і акліматизовані протягом двох тижнів перед початком експерименту. Група I: контроль без діабету; група II: контроль з діабетом; група III: без діабету з 200 мг/кг *Hibiscus sabdariffa*; група IV: без діабету з 300 мг/кг *Hibiscus sabdariffa*; група V: з діабетом з 200 мг/кг *Hibiscus sabdariffa*; група VI: з діабетом з 300 мг/кг *Hibiscus sabdariffa*. Щури отримували одноразову внутрішньоочеревинну ін'єкцію алоксану (150 мг/кг маси тіла), а щури з діабетом отримували *Hibiscus sabdariffa* протягом 21 дня. У дослідженні вимірювались рівень глюкози в крові натще, рівень інсуліну, супероксиддисмутази, каталази, малонового діальдегіду, інтерлейкіну-6 та фактора некрозу пухлин-альфа, а також відбиралися зразки органів та крові. Результати були проаналізовані за допомогою дисперсійного аналізу з рівнем значущості <0,05, а дані були візуалізовані за допомогою GraphPad. Це дослідження продемонструвало, що *Hibiscus sabdariffa* має значний вплив на діабетичні параметри, прозапальні цитокіни та антиоксидантні ферменти. Щоденний пероральний прийом протягом 21-го дня знижував рівень глюкози в крові натще, інтерлейкіну-6, фактора некрозу пухлин-альфа та малонового діальдегіду. Він також посилював вироблення інсуліну, активність супероксиддисмутази та каталази в скелетних м'язах, печінці, підшлунковій залозі та нирках. Можна зробити висновок, що *Hibiscus sabdariffa* має потенціал для боротьби з гіперглікемією та запаленням, одночасно покращуючи активність антиоксидантних ферментів. Крім того, він може служити природним джерелом або засобом для лікування або профілактики діабету

Ключові слова: біомаркери оксидативного стресу; прозапальні цитокіни; біологічно активні сполуки; β-клітини підшлункової залози

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Experimental modelling of full-thickness skin wounds in pigs

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Abstract. In the modern context, there is a growing need to develop a relevant experimental model of a skin wound that closely replicates the regeneration processes occurring in human wounds. The aim of this study was to develop a method to prevent premature contraction of wound edges during the experimental modelling of a full-thickness skin wound in pigs, thereby creating optimal conditions for evaluating the effectiveness of local treatment approaches. An experimental study was conducted on a white pig weighing 15 kg. A full-thickness skin wound measuring 5×5 cm was created on the animal's back under thiopental sodium anaesthesia at a dosage of 80 mg/kg. Tissue samples were collected from the wound site via punch biopsy under general anaesthesia, fixed in 10% neutral formalin, and embedded in paraffin using standard histological techniques. Deparaffinised sections were stained with haematoxylin and eosin. A computer program was developed in Python to calculate the wound area using the Monte Carlo method. To visualise the results and observe trends, graphical representations in the form of diagrams were used. The study demonstrated the feasibility of modulating contraction in full-thickness skin defects by applying incisions. The most effective method involved tangential incisions at each corner of the wound, each measuring up to 1 cm in length. This technique reduced the degree of wound edge contraction. On day 28 of observation, the wound area in the experimental group was 69.3% of the original size, compared to 39.3% in the control group. To accurately assess the effectiveness of treatments for full-thickness skin wounds in porcine models, it is essential to maintain a wound of appropriate size for at least 28 days to allow for observation of scar tissue formation. The proposed wound model enables controlled modulation of contraction and preserves an adequate wound surface area for the duration necessary to study scar formation processes

Keywords: excisional wound model; porcine model; wound edge contraction; wound area; wound healing; dermal matrix

INTRODUCTION

The repair of full-thickness skin wounds is governed by complex mechanisms of tissue regeneration and repair. Novel therapeutic agents are continually being developed, particularly those targeting the restoration of the damaged dermal matrix. To evaluate the effectiveness of such treatments, various animal models are used that aim to replicate the characteristics of human skin wounds. However, the skin of small laboratory animals – such as rodents and rabbits – differs significantly from human skin, with wound healing in these species primarily occurring through contraction rather than re-epithelialisation. Additionally, their limited skin surface area often necessitates the use of a large number of animals to obtain sufficient experimental material, which can increase variability and reduce the reliability of results.

In contrast, pig skin closely resembles human skin in both structure and function. It features a thick epidermis, dense collagen and elastic fibres in the dermis, and the presence of hair and sweat glands. D.W. Hamilton *et al*. [1] emphasise that pigs and humans share similar physiological and anatomical characteristics, resulting in comparable mechanisms of wound healing. However, the authors also highlight the need for further research to establish pigs as a reliable model for studying human wound healing processes. Moreover, T. Ryk [2] suggests that pigs hold promise as potential organ donors for humans, further underscoring their biomedical relevance.

J. Shiff *et al*. [3] also support the use of the porcine wound model, arguing that it more accurately reflects the processes of wound repair in humans compared to rodent

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models. M. Elloso *et al*. [4] highlight the advantages of the porcine model for advancing the understanding of wound biology and developing new therapeutic strategies. This model plays a crucial role in preclinical research by effectively bridging the gap between *in vitro* studies and clinical trials. Additionally, M. Tucci *et al*. [5] point out that the body size of pigs offers sufficient skin surface to allow for the creation of multiple wounds on a single animal. This minimises the influence of extraneous factors and reduces inter-subject variability.

According to R.B. Diller & A.J. Tabor [6], one of the key factors in wound regeneration is the condition of the extracellular matrix, which is actively involved in all phases of the healing process. Restoration of dermal integrity through the regeneration of the dermal matrix can significantly improve treatment outcomes by promoting the formation of a structured and elastic scar with tensile strength and elasticity comparable to those of intact dermis. In cases where the dermis is damaged, E.M. Tottoli *et al*. [7] argue that advanced technologies are required, as routine clinical methods of local wound treatment are often insufficient to address the complexity of full-thickness skin defects effectively.

P. Bargavi *et al*. [8] note that when the microarchitecture of a bioengineered product closely mimics the natural extracellular matrix (ECM), it can effectively promote cell growth, ECM deposition, and the formation of new tissue. Similarly, J. Xu *et al*. [9] provide evidence that ECM substitutes create a microenvironment resembling native epidermal or dermal tissue, thereby supporting cell migration, angiogenesis, proliferation, differentiation, and ECM production during the wound healing process. C. Dai *et al*. [10] describe various commercially available skin substitutes designed to replace the ECM, derived from autologous, allogeneic, or xenogeneic sources.

To evaluate the efficacy of dermal substitutes, full-thickness wound models are commonly employed. The fundamental principles for creating such models are outlined in the work of T.Y. Kuo *et al*. [11], who provide recommendations regarding the optimal wound size, location on the pig's body, and the advisable number of wounds. Adhering to these recommendations is essential to avoid negatively impacting the wound healing process. For instance, placing wounds too close to one another may impair local blood circulation, while increasing the number and total area of wounds can induce systemic effects in the animal. These systemic changes may lead to infectious complications and can compromise the validity of experimental data by influencing the effectiveness of local treatment under conditions of general physiological stress in the animal.

Since one of the primary criteria for evaluating local treatment is the rate of wound area reduction, it is essential to understand the underlying mechanisms driving this process in experimental models. However, contemporary literature pays insufficient attention to the role of wound edge contraction in area reduction, which may lead to misinterpretation of results when assessing the effectiveness of local treatment strategies in wound repair. Given this issue, and the lack of solutions proposed in the current literature to address it, the author aimed to investigate methods for minimising the influence of wound edge contraction on granulation tissue formation. The objective was to establish a more relevant experimental model of a full-thickness skin wound that would allow for accurate assessment of the effectiveness of a dermal matrix substitute derived from porcine dermis, as a promising method for restoring the native extracellular matrix in damaged dermal tissue.

MATERIALS AND METHODS

A white pig weighing 15 kg was used for the experimental study. The animal was housed in the vivarium of I.Ya. Horbachevsky Ternopil National Medical University, Ministry of Health of Ukraine, and maintained on a standard diet in accordance with sanitary-hygienic regulations and Good Laboratory Practice (GLP) standards [12]. The surgical intervention was conducted during morning hours between March and May 2024, in full compliance with the general principles and provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes [13], the Bioethics Guidelines [14], and the Law of Ukraine No. 3447-IV [15]. The Bioethics Commission of I.Ya. Horbachevsky Ternopil National Medical University confirmed that no violations of ethical standards occurred during the study (Protocol No. 80, dated 10 January 2025).

A full-thickness skin wound measuring 5×5 cm was created on the pig's back by excising a skin flap along with subcutaneous fat down to the superficial fascia, under thiopental sodium anaesthesia at a dosage of 80 mg/kg. The dynamics of wound healing were monitored over time without any intervention at the wound edges. Wound area measurements were taken on days 1, 7, 14, and 28 of the experiment. The wounds were divided into two groups: Group I received only aseptic dressings, while Group II was treated with an acellular dermal matrix derived from porcine skin. To investigate the effect of mechanical manipulation of wound edges on wound contraction, the wound variations illustrated in Figure 1 were created on the pig's back.

Figure 1. Variants of forming full-thickness wounds
Notes: wound No. 1: 5×5 cm, 1 cm incisions were made perpendicular to the wound edge; wound No. 2: 5×5 cm with linear incisions at the corners of the wound 1 cm long; wound No. 3: 5×5 cm, without affecting the wound edges (control group)
Source: created by the author

To assess the degree of structural changes within the wound, a morphological study was conducted. On the 28th day of the experiment, under general anaesthesia induced by sodium thiopental, wound tissue samples were collected using a 6 mm diameter punch biopsy. The specimens were fixed in 10% neutral formalin solution and embedded in paraffin blocks. Cross-sections 5-6 μm thick were prepared using a microtome. The resulting histological sections were stained with haematoxylin and eosin for microscopic examination (eyepiece 10×, objective 20×). To calculate the wound area, the Monte Carlo method was employed [16]. For this purpose, a custom computer program was developed using the Python programming language. The program is based on an algorithm comprising the following stages:

- Selection of a bounding figure: A simple geometric shape (e.g., a square or rectangle) that completely encloses the wound area is selected. The area of this shape can be calculated using standard geometric formulas.
- Generation of random points: A large number of random points are generated within the bounding figure. The coordinates of these points are determined using random number generators.
- Determination of point inclusion: Each randomly generated point is evaluated to determine whether it lies within the wound boundary. This is achieved by analysing the coordinates of the point in relation to the mathematical description of the wound's perimeter.
- Calculation of point fraction: The proportion of points falling within the wound area is calculated relative to the total number of generated points.
- Estimation of wound area: The wound area is then estimated by multiplying the area of the bounding figure by the fraction of points that lie within the wound boundary (1).

$$S_{figure} = S_{bounding\ figure} \cdot \frac{N_{successful}}{N_{total}}, \quad (1)$$

where S_{figure} – the area of the wound under study; $S_{bounding\ figure}$ – the area of the bounding figure; $N_{successful}$ – the number of points falling within the wound area; N_{total} – the total number of generated points.

The use of this algorithm enables accurate estimation of wound area, even for irregularly shaped surfaces, thereby providing an objective assessment of wound size at various stages of treatment. The image resolution used was 37 pixels/cm. The bounding figure was defined as a square with a side length of 6 cm. The number of pixels (points) within the bounding figure was calculated as the product of the area of the bounding figure and the number of pixels per cm² (2):

$$N = 6^2 \cdot 37^2 = 49{,}284. \quad (2)$$

To analyse trends in the change of wound surface area across the observation groups, a graphical method was employed using diagrams to visually represent the data.

RESULTS AND DISCUSSION

Based on formulas (1) and (2), the program generated a total of 49,284 random points. Each point was evaluated to determine whether it fell within the wound image or outside of it, in accordance with the aforementioned formulas. An example of the program's execution and output is presented in Figure 2.

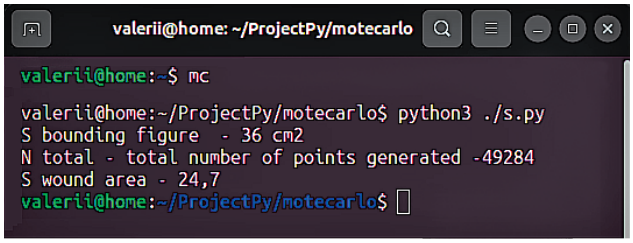

Figure 2. The result of the program for calculating the wound area using the Monte Carlo method
Source: created by the author

During the study, the dynamics of wound area reduction were monitored in two observation groups: Group I consisted of wounds closed without the use of a dermal matrix substitute, while Group II involved closure with an acellular dermal matrix derived from porcine skin (Fig. 3).

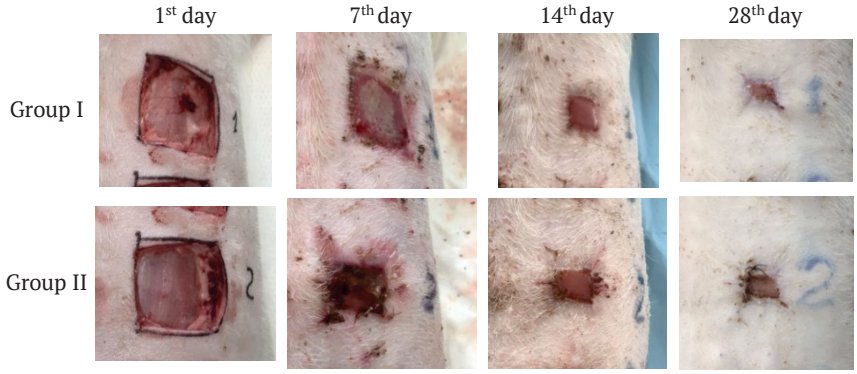

Figure 3. Dynamics of change in the area of the full-thickness wound of the first and second groups
Source: created by the author

During the observation period from day 1 to day 28, it was noted that the wound areas in both groups decreased at a similar rate. Visual assessment confirmed that the wound areas were so closely aligned that precise digital measurement was deemed unnecessary, as measurement accuracy in this context was not considered critical. By day 28, there was virtually no difference in wound area between the two observation groups. Of particular note, a marked reduction in wound area in both groups was already apparent by day 7, which is consistent with the findings of R. Elia *et al.* [17].

Under such conditions, it becomes impractical to evaluate the therapeutic effectiveness of the acellular dermal matrix in treating full-thickness skin defects. Figure 4 presents the histological findings from a biopsy taken from a Group I wound, while Figure 5 shows the histological result from a Group II wound on day 28 of the experiment.

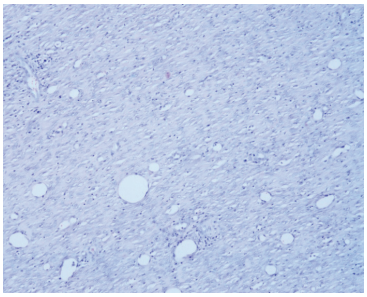

Figure 4. Biopsy from a wound of Group I
Source: created by the author

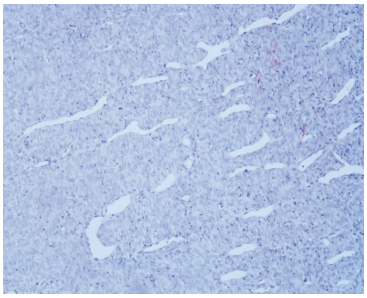

Figure 5. Biopsy from a wound of Group II
Source: created by the author

Both histological preparations revealed young connective tissue characterised by a high density of fibroblasts and focal lymphomacrophage infiltration. The connective tissue was well vascularised, with newly formed blood vessels visible. These findings suggest that the reduction in wound area was not primarily due to re-epithelialisation over newly formed granulation tissue, but rather due to contraction of the wound edges. The absence of a significant difference in granulation tissue formation between the two observation groups supports the hypothesis that an experimental model of a full-thickness wound in pig skin must meet specific temporal criteria. As noted by V. Coger *et al*. [18], the regeneration of full-thickness skin defects follows defined time intervals. Therefore, for such a model to remain relevant, it is essential to preserve a sufficient wound area for a certain duration – ensuring that closure does not occur predominantly through wound edge contraction. S.O. Udegbunam *et al*. [19] also emphasise the influence of wound edge contraction on the restoration of skin integrity. The strong tendency of full-thickness skin defects to contract is further highlighted by H.A. Wallace *et al*. [20], who reported that wound contraction contributed to 88% of closure, while scar formation accounted for only 12% of the wound surface.

A detailed analysis of the wound contraction mechanism is presented by S.E. Cross *et al*. [21], who demonstrated that excision of granulation tissue within the wound bed had no effect on contraction. Similarly, cutting the wound edges down to the deep fascia did not prevent contraction; on the contrary, it led to rapid adhesion of the wound edges to the wound bed, followed by resumed inward migration. The study showed that granulation tissue formed in the wound centre does not significantly contribute to contraction. Instead, a narrow rim of newly proliferated fibroblasts, located beneath the wound margin and measuring 1-2 mm in width, is responsible for wound closure. This mass of fibroblasts forms a subcutaneous “picture frame”, anchoring the dermal edges to the underlying deep fascia and pulling the intact dermis inward through directed collective migration.

It is important to note that in several classical excisional wound models – particularly in mice, as demonstrated by D.S. Masson-Meyers *et al*. [22] – healing occurs predominantly through contraction, which constitutes a significant component of the wound closure process. Traditionally, it was believed that complete wound closure by contraction was characteristic only of animals with highly mobile skin, such as guinea pigs, rabbits, and rats. However, the present findings clearly indicate that even animals with relatively immobile skin, such as pigs, also experience substantial contraction, which can result in a marked reduction of the wound area. In the course of the conducted observations on full-thickness defect closure in pig skin, it was found that the contraction of wound edges in pigs was sufficiently pronounced to draw the surrounding skin inward, thereby significantly contributing to the closure of the wound defect.

S.A. Park *et al*. [23] and X. Wang *et al*. [24] addressed a similar issue in rodents by developing a splinted wound model to suppress dermal contraction and improve the translational relevance of the mouse model for studying human wound healing. This technique involves the creation of full-thickness wounds on the dorsal surface of the mouse, followed by the placement of a silicone splint at the wound site, which is then secured to the skin with sutures to prevent contraction. Consequently, wound healing in this model proceeds via granulation tissue formation and re-epithelialisation, closely mimicking the human healing process. However, no studies have been reported in the literature describing the application of similar mechanical interventions to prevent wound contraction in porcine models. This highlights a significant gap and the need for further research to develop and validate methods that can suppress contraction in pigs, thereby increasing the relevance of this model for studying full-thickness wound healing in humans.

According to the hypothesis proposed by the author of this study, mechanical damage to the wound edges would result in disruption of the collagen fibres to which myofibroblasts are attached. It was anticipated that such disruption would weaken the contractile forces responsible for wound narrowing and thereby slow the reduction of wound area. This hypothesis was informed by the findings of S.M. Karppinen *et al*. [25] and F. Chang *et al*. [26], who reported that myofibroblasts play a central role in wound contraction. These cells exert contractile forces by attaching to the extracellular matrix via integrins and generating tension through stress fibres rich in alpha-smooth muscle actin.

Figure 6 illustrates the dynamics of changes in wound surface area during the observation period following mechanical disruption of the wound edges in wound No. 1 and wound No. 2. Wound No. 3 served as the control. In determining the number and orientation of incisions, the author followed several guiding principles: to ensure that the additional trauma to the wound edges did not increase the risk of infection; to preserve the overall wound contour, thereby enabling accurate measurement of the wound area throughout the observation period; and to retain the possibility of fixing a bioengineered product to the wound edges for improved attachment if necessary.

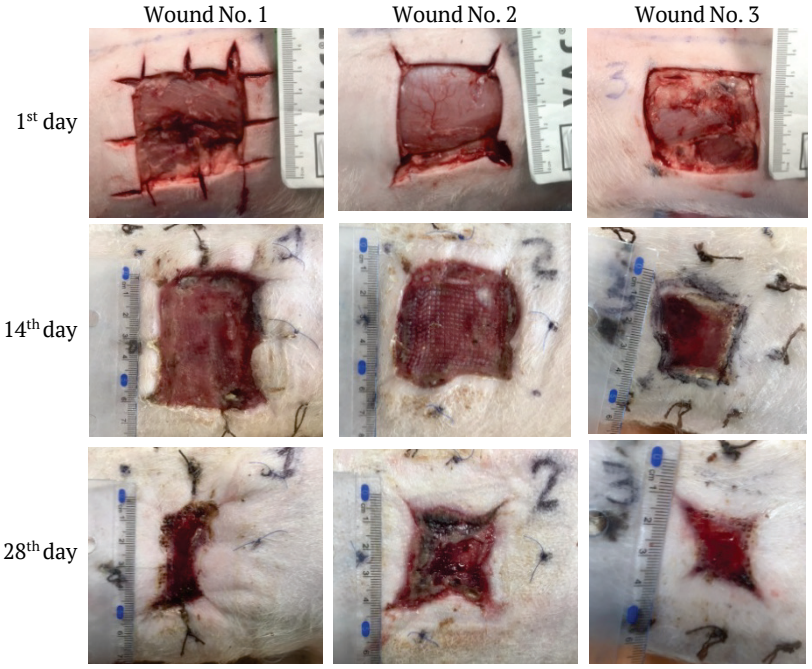

Figure 6. Dynamics of change in wound area

Source: created by the author

The application of different methods of mechanical intervention on the wound edges resulted in varying effects, as evidenced by differing degrees of wound area reduction over the observation period. The use of the Monte Carlo method enabled accurate and efficient calculation of wound surface area, even for irregularly shaped wounds. The results of wound area measurements obtained using this method are presented in Table 1.

Table 1. Wound area in sq. cm

	Wound No. 1		Wound No. 2		Wound No. 3	
	$N_{successful}$	S_{figure}	$N_{successful}$	S_{figure}	$N_{successful}$	S_{figure}
1st day	33,814	24.7	34,088	24.9	34,499	25.2
14th day	25,053	18.3	27,928	20.4	22,999	16.8
28th day	11,363	8.3	23,615	17.25	13,553	9.9

Source: created by the author

By day 14, a reduction in wound area was observed across all wounds. The measured areas were as follows: wound No. 1 – 18.3 cm², wound No. 2 – 20.4 cm², and wound No. 3 (control) – 16.6 cm². These results indicate that the incisions made at the wound edges did not halt the contraction process but rather reduced its rate. Specifically, wound No. 1 showed a 25.9% reduction in area, wound No. 2 a reduction of 18.1%, and the control wound No. 3 a reduction of 33.3%. Between days 14 and 28, the contraction of wound edges continued, albeit at differing rates. Although more incisions were made on the edges of wound No. 1 than wound No. 2, its area continued to decrease, reaching 33.6% of its original size by day 28. This value was only slightly higher than that of the control wound (wound No. 3), which had reduced to 39.3% of its initial area. Notably, wound No. 2 preserved the largest residual area, maintaining 69.3% of its initial surface area by day 28. To assess the results, the relative change in wound area was evaluated as a percentage of the original wound size rather than using absolute values. These findings are presented in Table 2 and visualised in Figure 7.

Table 2. Wound area indicators in % relative to the initial value

	Wound No. 1	Wound No. 2	Wound No. 3
1st day	100	100	100
14th day	74.1	81.9	66.7
28th day	33.6	69.3	39.3

Source: created by the author

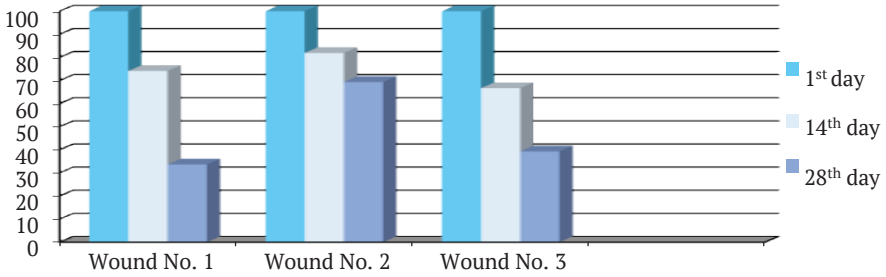

Figure 7. Wound area indicators in % relative to the initial value
Source: created by the author

The diagram presented in Figure 7 clearly illustrates the trend in wound surface area changes across the different wounds. A common pattern is evident: up to day 14, the rate of wound contraction is lower than during the period from day 14 to day 28. The findings demonstrate that disrupting the integrity of the wound edges can reduce the contraction process. However, analysis of the results for wound No. 1 indicates that a greater number of incisions, compared to wound No. 2, did not yield the expected outcome. The author suggests that it is not the number of incisions but rather their anatomical location that plays a more significant role in preventing or reducing the rate of wound edge contraction. Additionally, it is important to consider that the rate of contraction increases after day 14. Therefore, to effectively control this process, supplementary interventions at the wound edges may be required during this later stage of healing.

CONCLUSIONS

Wound contraction is a fundamental healing mechanism aimed at reducing the size of the tissue defect and, consequently, the volume of tissue requiring regeneration. In an experimental model involving the creation of a 5×5 cm excisional wound on the back of a pig, contraction occurs through the retraction of adjacent skin into the wound bed. This characteristic of full-thickness wound repair in porcine skin represents a major limitation of this model for studying wound regeneration processes relevant to humans. Despite the dense attachment of the dermis and subcutaneous fat to the superficial fascia in pigs, the skin still exhibits a significant capacity to migrate centrally during wound closure, even when all skin layers are involved. Reducing the contractile potential of the wound edges is therefore a key objective in developing a more representative experimental model of a full-thickness skin defect. One potential strategy is to disrupt the integrity of collagen fibres at the wound edge, which play a critical role in driving centripetal contraction of the wound. In this study, the application of tangential incisions at each corner of the wound – each up to 1 cm in length – proved effective in slowing the contraction process. By day 28, 69.3% of the original wound area was preserved in the intervention group, compared to only 39.3% in the control group. This proposed wound model allows for greater control over the contraction process and preserves a sufficient wound surface area for an adequate duration, thereby enabling meaningful evaluation of granulation tissue formation, re-epithelialisation, and scar development in response to the test treatment. During the healing process, the excisional full-thickness wound tends to assume an irregular, star-shaped configuration. This indicates that the surrounding skin does not contract uniformly; rather, skin edges equidistant from the wound centre move inward at varying speeds. This irregularity complicates planimetric analysis during experimental observation. It is also noteworthy that the inhibitory effect of edge disruption begins to diminish once the wound edges become closely opposed to the wound bed – a process that typically begins around days 10 to 12. From this point, contraction accelerates again, despite prior mechanical disruption of the wound margins. This observation highlights the need for further investigation into additional or sustained interventions that may be required beyond this critical period to effectively control wound narrowing and ensure the maintenance of a consistent wound area for experimental purposes.

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CONFLICT OF INTEREST

None.

REFERENCES

[1] Hamilton DW, Walker JT, Tinney D, Grynyshyn M, El-Warrak A, Truscott E, et al. The pig as a model system for investigating the recruitment and contribution of myofibroblasts in skin healing. Wound Rep Reg. 2022;30(1):45–63. DOI: 10.1111/wrr.12981
[2] Ryk T. Molecular genetic status of pigs of Ukrainian breeds suitable for use in xenotranplantation. Sci Rep Natl Univ Life Environ Sci Ukr. 2024;20(2). DOI: 10.31548/dopovidi.2(108).2024.003
[3] Shiff J, Schwartz K, Hausman B, Seshadri DR, Bogie KM. Development and use of a porcine model with clinically relevant chronic infected wounds. J Tissue Viability. 2023;32(4):527–35. DOI: 10.1016/j.jtv.2023.08.004
[4] Elloso M, Hutter MF, Jeschke N, Rix G, Chen Y, Douglas A, et al. Challenges of porcine wound models: A review. Int J Transl Med. 2025;5(1):4. DOI: 10.3390/ijtm5010004
[5] Tucci M, Hildebrandt D, Lichtenhan J, Benghuzzi H. Evaluation of full thickness wounds following application of a visco-liquid hemostat in a swine model. Pathophysiology. 2024;31(3):458–70. DOI: 10.3390/pathophysiology31030034
[6] Diller RB, Tabor AJ. The role of the extracellular matrix (ECM) in wound healing: A review. Biomimetics. 2022;7(3):87. DOI: 10.3390/biomimetics7030087
[7] Tottoli EM, Dorati R, Genta I, Chiesa E, Pisani S, Conti B. Skin wound healing process and new emerging technologies for skin wound care and regeneration. Pharmaceutics. 2020;12(8):735. DOI: 10.3390/pharmaceutics12080735
[8] Bargavi P, Ramya R, Chitra S, Vijayakumari S, Chandran RR, Durgalakshmi D, et al. Bioactive, degradable and multi-functional three-dimensional membranous scaffolds of bioglass and alginate composites for tissue regenerative applications. Biomater Sci. 2020;8:4003–25. DOI: 10.1039/D0BM00714E
[9] Xu J, Fang H, Zheng S, Li L, Jiao Z, Wang H, et al. A biological functional hybrid scaffold based on decellularized extracellular matrix/gelatin/chitosan with high biocompatibility and antibacterial activity for skin tissue engineering. Int J Biol Macromol. 2021;187:840–9. DOI: 10.1016/j.ijbiomac.2021.07.162
[10] Dai C, Shih S, Khachemoune A. Skin substitutes for acute and chronic wound healing: An updated review. J Dermatolog Treat. 2020;31(6):639–48. DOI: 10.1080/09546634.2018.1530443
[11] Kuo TY, Huang CC, Shieh SJ, Wang YB, Lin MJ, Wu MC, et al. Skin wound healing assessment via an optimized wound array model in miniature pigs. Sci Rep. 2022;12(1):445. DOI: 10.1038/s41598-021-03855-y
[12] Order of the Ministry of Health of Ukraine No. 944. On Approval of the Procedure for Conducting Preclinical Studies of Medicinal Products and Expertise of Materials of Preclinical Studies of Medicinal Products [Internet]. 2009 December 14 [cited 2024 October 7]. Available from: https://ips.ligazakon.net/document/view/re17348?an=2&ed=2009_12_14
[13] European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes [Internet]. 1986 March 18 [cited 2024 October 7]. Available from: https://zakon.rada.gov.ua/laws/show/994_137#Text
[14] Patrieva L, Pidpala T, Kalynychenko H. Bioethics guidelines. Mykolaiv: Mykolaiv National Agrarian University; 2021. P. 125.
[15] Law of Ukraine No. 3447-IV. On the Protection of Animals from Cruelty [Internet]. 2006 February 21 [cited 2024 October 7]. Available from: https://zakon.rada.gov.ua/laws/show/3447-15#Text
[16] Gould H, Tobochnik J, Harrison DE. An introduction to computer simulation methods: Applications to physical systems part 1 and part 2. Comput Phys. 1988;2(1):90–1. DOI: 10.1063/1.4822668
[17] Elia R, Maruccia M, Di Summa PG, Trisciuzzi R, Lovero G, Cazzato G, et al. Conventional versus regenerative methods for wound healing: A comparative experimental study on a sheep model. Medicina. 2024;60(11):1836. DOI: 10.3390/medicina60111836
[18] Coger V, Million N, Rehbock C, Sures B, Nachev M, Barcikowski S, et al. Tissue concentrations of zinc, iron, copper, and magnesium during the phases of full thickness wound healing in a rodent model. Biol Trace Elem Res. 2019;191(1):167–76. DOI: 10.1007/s12011-018-1600-y
[19] Udegbunam SO, Ogbobe S, Okereke NH, Enejere AS, Udegbunam IR, Ezeobialu TH. Assessment of wound contraction, re-epithelialization and histological changes in full thickness excision wounds of rats treated with different concentrations of hydrogen peroxide. Trop J Pharm Res. 2021;20(8):1623–9. DOI: 10.4314/tjpr.v20i8.11
[20] Wallace HA, Basehore BM, Zito PM. Wound healing phases. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2022. P. 35–43.
[21] Cross SE, Naylor IL, Coleman RA, Teo TC. An experimental model to investigate the dynamics of wound contraction. Br J Plast Surg. 1995;48(4):189–97. DOI: 10.1016/0007-1226(95)90001-2
[22] Masson-Meyers DS, Andrade TAM, Caetano GF, Guimaraes FR, Leite MN, Leite SN, et al. Experimental models and methods for cutaneous wound healing assessment. Int J Exp Pathol. 2020;101(1–2):21–37. DOI: 10.1111/iep.12346
[23] Park SA, Raghunathan VK, Shah NM, Teixeira L, Motta MJ, Covert J, et al. PDGF-BB does not accelerate healing in diabetic mice with splinted skin wounds. PLoS One. 2014;9(8):e104447. DOI: 10.1371/journal.pone.0104447
[24] Wang X, Ge J, Tredget EE, Wu Y. The mouse excisional wound splinting model, including applications for stem cell transplantation. Nat Protoc. 2013;8(2):302–9. DOI: 10.1038/nprot.2013.002
[25] Karppinen SM, Heljasvaara R, Gullberg D, Tasanen K, Pihlajaniemi T. Toward understanding scarless skin wound healing and pathological scarring. F1000Res. 2019;8:787. DOI: 10.12688/f1000research.18293.1
[26] Chang F, Yan L, Zha Y, Hong X, Zhu K, Fei Y, et al. Development of a wound epithelialization healing model: Reducing the impact of contraction healing on the wound surface. J Burn Care Res. 2024;45(4):1016–25. DOI: 10.1093/jbcr/irae065

Особливості експериментального моделювання повношарової рани шкіри свині

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Анотація. В сучасному світі існує необхідність створення ревалентної експериментальної моделі рани шкіри, яка б максимально відтворювала процеси регенерації, що відбуваються в рані людини. Мета статті полягала у спробі створення попередження завчасної контракції країв рани при експериментальному моделюванні повношарової рани шкіри свині, що дозволить створити оптимальні умови для вивчення ефективності способу місцевого лікування. Проведено експериментальне дослідження на свині білої породи, вагою 15 кг. Повношарову рану розміром 5×5 см змодельовано на спині тварини під тіопентал-натрієвим наркозом з розрахунку 80 мг/кг. За допомогою панч-біопсії під загальним обезболенням вилучено тканини з рани, що фіксувалися в 10 % розчині нейтрального формаліну і ущільнювалися парафіном за стандартною методикою. Депарафінізовані зрізи було пофарбовано гематоксиліном і еозином. Для обчислення площі рани методом Монте-Карло було розроблено комп'ютерну програму мовою програмування Python. Використано графічний метод у вигляді діаграм для візуалізації результатів дослідження і спостереження тенденцій. Отримано можливість коригуючого впливу на процес контракції країв повношарового дефекту шкіри за допомогою надрізів. Найбільш оптимальним є варіант рани, що передбачає тангенціальні надрізи в кожному куті рани довжиною до 1 см. Застосована методика впливу на краї рани довзолила зменшити ступінь скорочення країв рани. На 28 добу спостереження площа рани становила 69,3 % від початкової, відповідно площа контрольної рани становила 39,3 % від її початкового значення. Для визначення ефективності лікування повношарової рани шкіри в експерименті на свині необхідно зберігати рану відповідної площі протягом не менше 28 діб для вивчення процесу формування рубцевої тканини. Запропонована модель рани дає можливість контролювати перебіг контракції, зберігати на необхідний час достатню площу ранової поверхні для вивчення процесу формування рубцевої тканини

Ключові слова: ексцизійна модель рани; модель на свині; контракція країв рани; площа рани; заживлення рани; дермальний матрикс

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

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

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

INTRODUCTION

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

The study of antioxidants was carried out by I. Gulcin *et al*. [1]. The researchers concluded that antioxidants are molecules that can prevent or slow down the oxidation of macromolecules. Now it is known that antioxidants are of natural (lycopene, vitamins E and C, carotene) and synthetic origin. Synthetic antioxidants are used to slow down the oxidation processes in foods, that is, they do not have any benefit for the human body, unlike natural ones. Natural antioxidants are found in large quantities in foods such as broccoli, blueberries, dark chocolate, pomegranates, strawberries, nuts, and citrus fruits. The most common of them are vitamin C or ascorbic acid, vitamin A or carotenoids, vitamin E – tocopherol, polyphenols-flavonoids, anthocyanins, lycopene, coenzyme, ubiquitin [2].

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

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

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

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

MATERIALS AND METHODS

20 sexually mature Wistar rats were selected for this study. The animals were fertilised after the introduction of antioxidants, and the direct effect of these substances on the foetus and female was also investigated. The study was conducted for 7 months at the Centre for Bone Marrow Transplantation of Radiation Medicine – LTT Laboratory. Excel was used to process the obtained data and research results. Key indicators were measured, such as the concentration of hormones, antioxidant defence enzymes (for example, SOD, glutathione peroxidase), oxidative markers, and changes in animal blood tests. Changes in sperm and spermatozoa were considered, and the sperm collection procedure itself included: animal anaesthesia (inhaled anaesthesia), electrical stimulation that caused ejaculation, sperm collection in a sterile test tube, and sperm treatment. Mobility, morphology, and biochemical properties were evaluated.

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

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

RESULTS AND DISCUSSION

It was shown that experimental rats had levels of SOD and glutathione activity during all trimesters of pregnancy due to induced stress compared to the intact group of rats (Table 1). In the study group, the initial SOD level was 2 ± 0.04.

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

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

Source: compiled by the author

SOD has a protective effect on pregnant rats, in particular, on the condition of the mother and foetus, reducing oxidative stress. Namely, cell protection – SOD neutralises the action of free radicals. Preventing damage to female and placental cells. Improves rat foetal development – the antioxidant properties of SOD contribute to the physiological development of the foetus, reducing the development of abnormalities. Regulates immune responses – SOD can reduce the risk of inflammatory processes that can negatively affect the course of pregnancy. In the study group, the baseline level of glutathione peroxidase was 7 ± 0.37 (Table 2). Glutathione peroxidase exerted a protective effect on the thyroid gland by regulating oxidative stress. In Wistar rats, increased activity of this substance was found to reduce thyroid cell damage and stabilise the levels of the hormones triiodothyronine and thyroxine, which helped to maintain stable levels of thyroid-stimulating hormones.

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

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

Source: compiled by the author

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

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

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

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

Source: compiled by the author

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

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

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

Flavonoids such as quercetin and resveratrol have a powerful antioxidant effect, markedly reducing oxidative stress in rats. They increased the activity of antioxidant systems by 35%, reduced the level of lipid peroxidation by 30%, and improved heart and liver function. They also increased the number and quality of spermatozoa by 20%, increased their motility by 25%. They helped to stabilise the level of reproductive hormones, namely testosterone and oestrogen, which is important for fertility. Selenium is involved in the synthesis of glutathione peroxidase, which protects cells from damage. Wistar rats treated with additional portions of selenium showed a 40% increase in glutathione peroxidase activity and a 30% decrease in lipid peroxidation. Alpha-lipoic acid reduced oxidative damage and improved cognitive function in rats, protecting nerve cells from stress damage. L-carnitine – its high concentrations occur in the testicular appendage (2,000 times more than in sperm). It also has a positive effect on sperm quality and therefore fertility.

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

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

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

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

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

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

Table 4. Positive and negative external factors influenced the antioxidants

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

Source: compiled by the author

Certain diseases were modelled in which antioxidants did not show a protective effect or even negatively affected the body. Especially in the late stages of cancer, antioxidants reduced the effectiveness of chemotherapy or radiotherapy by 40%. This is because antioxidants can protect cancer cells from oxidative stress, which is used to kill them. The introduction of antioxidants increased the occurrence of cardiac pathology by 20%. Antioxidants reduced cell sensitivity to insulin by 20%. The effect of antioxidants on the body in diabetes was studied by N. Rusli *et al*. [11]. The researchers emphasised the importance of the therapeutic effects of antioxidants in the treatment of diabetic peripheral neuropathy, which may be useful for further clinical research and the development of new treatment approaches. Chronic inflammatory diseases – asthma or rheumatoid arthritis. In some inherited diseases, such as cystic fibrosis or phenylketonuria, antioxidants cannot eliminate the underlying cause of the disease because these conditions are associated with a deficiency or dysfunction of certain enzymes. In cases of acute infections (such as viral or bacterial infections), antioxidants may not have a significant effect, because the immune system and inflammatory mechanisms are central to fighting infection. Excessive use of antioxidants can even suppress the immune response.

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

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

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

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

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

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

Table 5. Doses that positively affected fertility

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

Source: compiled by the author

Thus, the study showed that correcting the hormonal profile with antioxidants was an important tool for maintaining reproductive function and overall thyroid health in animals of all ages. A disease such as thyroid inflammation was also modelled on Wistar rats. Antioxidants play a crucial role in the development and progression of thyroid inflammation. Studies have shown that cells in an inflamed thyroid gland have a reduced activity of antioxidant enzymes, which leads to an ineffective defence system that cannot neutralise reactive oxygen species. Studies have consistently shown that antioxidant levels in patients with thyroid inflammation are lower than in healthy patients. In addition, analysis of serum oxidative status showed that oxidant levels were significantly elevated in rats with thyroid inflammation, while antioxidant levels were reduced. Reduced antioxidant activity in the cells of the inflamed thyroid gland led to accelerated lipid peroxidation, which made these tissues more susceptible to damage. In addition, a lack of antioxidant protection components and increased lipid peroxidation contributed to the development of thyroid inflammation. Antioxidants such as selenium, vitamins C and E have been used to reduce oxidative stress, which contributes to the development of inflammation, with specific concentrations listed in Table 6. These vitamins reduced oxidative stress levels by 45%, acting synergistically to protect cell membranes and neutralise free radicals.

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

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

Source: compiled by the author

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

CONCLUSIONS

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

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

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REFERENCES

[1] Gulcin I, Izol E, Yapıcı I. Natural antioxidants and in vitro antioxidant assays. Istanbul: Nobel Tip Kitabevleri; 2024. 258 P. DOI: 10.69860/nobel.9786053359333

[2] Tkach G, Shvets O, Bal-Prylypko L, Slobodianiuk N, German S. Preclinical study of the effects of vitamin-mineral complex use during normal pregnancy in white rats. Anim Sci Food Technol. 2022;13(4):59–68. DOI: 10.31548/animal.13(4).2022.59-68

[3] Mukherjee S, Chopra H, Goyal R, Jin S, Dong Z, Das T, et al. Therapeutic effect of targeted antioxidant natural products. Discov Nano. 2024;19:144. DOI: 10.1186/s11671-024-04100-x
[4] Jeruto RD, Onyango LA, Nakhumicha A, Omwoma S. Comparing synthetic and natural antioxidants in vegetable oils: Effects on oxidation and oil quality. Asian J Appl Chem Res. 2024;15(4):119–37. DOI: 10.9734/ajacr/2024/v15i4301
[5] Weissig V. Free radicals and antioxidants: A new paradigm has emerged. Med Res Arch. 2024;12(7):5456. DOI: 10.18103/mra.v12i7.5456
[6] Noel KI. A reciprocal relationship between oxidative stress, antioxidants, and cancer: A review. Siriraj Med J. 2024;76(8):550–6. DOI: 10.33192/smj.v76i8.268647
[7] Dewi EF, Mahriani M. The effect of giving the antioxidant vitamin C in tris-egg yolk extender during the preservation period on the quality of the epididymal spermatozoa of mice (*Mus musculus* L.). Berkala Sainstek. 2023;11(4):207–15. DOI: 10.19184/bst.v11i4.39109
[8] Kukurt A, Karapehlivan M, Gelen V. The use of astaxanthin as a natural antioxidant on ovarian damage. In: Kukurt A, Karapehlivan M, Gelen V, editors. Animal models and experimental research in medicine. London: IntechOpen; 2023. DOI: 10.5772/intechopen.108854
[9] Goodarzi S, Rafiei S, Javadi M, Haghighian HK, Noroozi S. A review on antioxidants and their health effects. J Nutr Food Secur. 2018;3(2):106–12.
[10] European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes [Internet]. 1986 March 18 [cited 2024 August 18]. Available from: https://rm.coe.int/168007a67b
[11] Rusli N, Ng CF, Makpol S, Wong YP, Isa IL, Remli R. Antioxidant effect in diabetic peripheral neuropathy in rat model: A systematic review. Antioxidants. 2024;13(9):1041. DOI: 10.3390/antiox13091041
[12] Kuznetsov TY, Shinkarenko V. Electrochemical study of antiradical properties of endogenous antioxidants. In: 15th Mendeleev Readings. Poltava: Poltava V.G. Korolenko National Pedagogical University; 2022; P. 29–31.
[13] Klepko AV, Kondratova YuA, Gudkov IM. The role of rabbit seminal plasma natural antioxidants in the realization of spermatozoid activity after ionizing irradiation. Factors Exp Evol Org. 2020;26:132–8. DOI: 10.7124/FEEO.v26.1255
[14] Stetska VO, Holota YuV, Gonchar SYu, Korbush MYu, Dovbynchuk TV, Serhiychuk TM, et al. Comparison of long-term effect of two dysbiosis models in Wistar rats. Microbiol Biotechnol. 2019;46(2):6–15. DOI: 10.18524/2307-4663.2019.2(46).163699
[15] Grinevich YuP. Lipid peroxidation in rat blood following a single administration of different active. Nucl Phys Energy. 2014;15(4):353–8.
[16] Diorditsa Ya. Antioxidant system activity in liver of rats under conditions of acute hepatitis during correction with antioxidant complexes. Visnyk Lviv Univ Biol Ser. 2019;81:12–20. DOI: 10.30970/vlubs.2019.81.02
[17] Kryukova EO, Zemlyanskaya OV. Study of the effect of antioxidants on the human body after oxidative stress. In: Proceedings of the 25th all-Ukrainian scientific and methodological conference. Kyiv: National Technical University of Ukraine "Igor Sikorsky Kyiv Polytechnic Institute"; 2022. P. 119–21.
[18] Fedosieieva OV. Morphogenesis of the thyroid gland of rats of the early period after the prenatal action of dexamethasone. Rep Vinnytsia Nat Med Univ. 2021;25(1):22–6. DOI: 10.31393/reports-vnmedical-2021-25(1)-04
[19] Gerasymchuk MR. Manifestations of experimental hypothyroidism during the influence of moderate physical activity. Pathologia. 2015;33(1):80–3.
[20] Romanova KB, Hancheva OV, Kolesnyk YuM. Pathogenetic characteristics of the hormonal profile in rats subjected to restraint stress of different duration. Mod Med Technol. 2023;2:12–5. DOI: 10.34287/MMT.2(57).2023.2
[21] Prodanchuk GM, Usenko TV. The creation of historical control of hematological parameters of peripheral blood Wistar han rats. Orig Res Mech Intoxication. 2015;72(4):35–40.

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

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

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

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Determination of risk factors for osteopenic changes in women pregnant using assisted reproductive technologies

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Abstract. The study aimed to investigate the factors that influence the risk of osteopenic changes in women who became pregnant with the help of assisted reproductive technologies. The study involved 150 women aged 25-40 who became pregnant after in vitro fertilisation, intrauterine insemination and oocyte donation. Bone mineral density was assessed by ultrasound densitometry in the distal forearm at the early (8-12 weeks) and late (32-36 weeks) stages of pregnancy. The T-score values showed a significant decrease in the third trimester in all groups, the largest – in patients who became pregnant due to oocyte donation (-1.3 ± 0.3 in the distal forearm). An analysis of socio-demographic characteristics revealed that age over 35, low physical activity, vitamin D and calcium deficiency, and bad habits such as smoking and excessive caffeine consumption significantly increased the risk of developing osteopenic changes. Women with regular consumption of dairy products, calcium supplements and a normal level of physical activity had significantly better bone mineral density. Biochemical markers confirmed higher levels of calcium, magnesium and vitamin D in the oocyte donation group, while the intrauterine insemination group demonstrated a deficiency of these elements and increased markers of bone resorption. The hormonal profile also had a significant impact on bone health: high oestrogen levels in the oocyte donation group were associated with less severe osteopenic changes, while elevated parathyroid hormone levels in the intrauterine insemination group contributed to increased bone loss. Patients with the highest levels of prolactin had better bone mineral density preservation due to the positive effect of this hormone on calcium metabolism. The findings emphasise the need for comprehensive monitoring of bone health in women who have become pregnant using assisted reproductive technologies

Keywords: bone density; biochemical markers; hormonal profile; intrauterine insemination; oocyte donation

INTRODUCTION

Assisted reproductive technologies are a significant achievement of modern medicine, overcoming infertility and providing the possibility of having a child in patients with reproductive disorders. However, pregnancy resulting from the use of such methods is accompanied by significant changes in a woman's physiological and metabolic state. Bone tissue is a particularly vulnerable system during this period, as it changes due to the body's increased need for calcium and other minerals. Women who have become pregnant through in vitro fertilisation, intrauterine insemination or oocyte donation may face an increased risk of osteopenic changes due to the specifics of their hormonal and metabolic background. The study of factors affecting the state of bone tissue in such patients is an urgent task.

The hormonal profile is crucial for maintaining bone health. O. Deinichenko *et al*. [1] studied the effect of oes-

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trogen on bone mineral density in women who became pregnant through oocyte donation. They found that high levels of oestrogen slowed bone loss. The impact of mineral metabolism on bone health is also an important factor. A. Tsymbal & Y. Kotlova [2] analysed the mineral composition of blood in pregnant women and found that calcium and vitamin D deficiency significantly increases the risk of osteopenic changes. The authors emphasised that regular consumption of calcium-containing foods and food supplements has a positive effect on mineral density.

Physical activity is also substantial in maintaining bone health. L. Zhytnik *et al*. [3] demonstrated that pregnant women who exercise regularly have higher bone mineral density than those who are sedentary. Biochemical markers of bone resorption are an important tool for assessing bone health. J.E. Stern *et al*. [4] demonstrated that high levels of C-telopeptides correlate with intense bone loss in pregnant women.

Psychosocial and demographic factors also significantly affect the risk of osteopenic changes. Y. Wang *et al*. [5] demonstrated that women over 35 years of age who have bad habits, such as smoking or excessive caffeine consumption, are prone to a significant decrease in bone mineral density. The authors noted that social factors, such as access to good nutrition, are also substantial in the prevention of osteopenia, but they did not address the hormonal and metabolic characteristics of women who became pregnant using assisting technology. The impact of physiological stress on bone metabolism was investigated by B.M. Nørgård *et al*. [6], noting that elevated cortisol levels during pregnancy can contribute to a decrease in mineral density. Their study confirmed the importance of controlling stress factors. M.E. Graham *et al*. [7] highlighted the correlation between prolactin levels and the preservation of bone mineral density. The study demonstrated that high levels of this hormone positively affect calcium metabolism. The hormonal balance and its effect on calcium metabolism were studied by B. Sun *et al*. [8], noting that elevated levels of parathyroid hormone increase bone resorption. The authors emphasised that women with endocrine disorders during pregnancy need careful monitoring.

Hormonal, metabolic, and social factors are significant in maintaining bone health in pregnant women, especially those who become pregnant through assisted reproductive technologies. However, existing studies do not provide a comprehensive analysis of these aspects in this category of patients. In particular, the effect of oestrogen on bone mineral density was considered without considering concomitant changes in prolactin, parathyroid hormone and cortisol levels, which also affect calcium metabolism. In addition, although a correlation between calcium and vitamin D deficiency and the risk of osteopenic changes was established, the specifics of mineral metabolism in women after in vitro fertilisation remain understudied.

Thus, there is a need for a more detailed study of the complex impact of hormonal, metabolic and socio-demographic factors on bone health in women who became pregnant using reproductive technologies. The study aimed to determine the main risk factors for the development of osteopenic changes in women who became pregnant with the help of assisted reproductive technologies, incorporating the influence of hormonal, metabolic and socio-demographic context. The objectives of the study included assessing bone mineral density at different stages of pregnancy, analysing the impact of hormonal profiles, and developing recommendations for the prevention of osteopenic changes in this group of patients.

MATERIALS AND METHODS

The study was conducted in 2024 at the Kyiv Perinatal Centre [9], a municipal non-profit enterprise (MNE), involving 150 women aged 25 to 40 who became pregnant with the help of assisted reproductive technologies (ART). The types of ART used in the patients included in vitro fertilisation (IVF) (57 women), intrauterine insemination (IUI) (45 women) and oocyte donation (48 women). The inclusion criteria were confirmed pregnancy obtained through ART, absence of diagnosed bone diseases or endocrine disorders, and voluntary consent to participate. Patients with existing chronic metabolic disorders or systemic diseases that could affect bone metabolism were excluded from the study. To determine bone mineral density (BMD), ultrasound densitometry of the forearm was performed using the OsteoSys SONOST 3000 apparatus (OsteoSys, South Korea). The study was conducted in two stages: in the first trimester of pregnancy (8-12 weeks) and in the third trimester (32-36 weeks). The measurements were performed in the distal forearm, which was used to detect early osteopenic changes and assess their dynamics during pregnancy.

Additionally, a questionnaire was administered to patients to collect data on risk factors for osteopenic changes. The questionnaire consisted of several sections. The socio-demographic section collected data on age, level of education, place of residence (urban or rural) and occupation. Reproductive history included information about the duration of fertility treatment, the number of previous ART attempts, the number of embryos transferred, the success of previous pregnancies, and complications. Dietary habits were assessed based on the consumption of dairy products, calcium supplements, vitamin D, and diet during pregnancy. Physical activity level was determined by the frequency and duration of activities such as walking, yoga or swimming. A separate section of the questionnaire was devoted to bad habits, including smoking, alcohol consumption, and excessive caffeine consumption. Stress levels were assessed subjectively on a scale from 0 to 10, and the availability of psychological support was also considered. All participants in the study participated voluntarily after providing informed consent, and the confidentiality of the data was guaranteed. All manipulations were conducted following the Declaration of Helsinki for Ethical Principles of Medical Research [10].

Biochemical studies were performed to assess the levels of calcium (total and ionised), phosphorus, magnesium, bone resorption markers (C-telopeptides) and 25-hydroxyvitamin D. Blood was drawn on an empty stomach using sterile BD Vacutainers (Becton Dickinson, USA). Analyses were performed on an automated biochemical analyser Roche Cobas 6000 (Roche Diagnostics, Germany). The hormonal profile, including levels of oestrogen, progesterone, parathyroid hormone and prolactin, which are important regulators of bone metabolism, was assessed separately. IBM SPSS Statistics software (version 27, USA) was used for statistical analysis [11]. Multivariate regression analysis

was used to assess the relationship between risk factors and osteopenic changes. The mean values were compared using Student's t-test, and the Mann-Whitney U-test was used to analyse the distribution of data between groups. Statistical significance was determined at the level of $p < 0.05$.

RESULTS

The analysis of the dynamics of BMD in the first and third trimesters of pregnancy achieved with ART revealed significant changes in T-score in different measurement areas (Table 1).

Table 1. Dynamics of T-score in the distal forearm in the first and third trimesters of pregnancy

ART Group	Measurement area	First trimester (T-score)	Third trimester (T-score)
IVF	Distal forearm	-0.3 ± 0.2	-1.0 ± 0.2
IUI	Distal forearm	-0.4 ± 0.1	-1.1 ± 0.3
Oocyte donation	Distal forearm	-0.5 ± 0.2	-1.3 ± 0.3

Source: compiled by the authors

In the first trimester, the mean T-score values in the distal forearm, measured by ultrasound densitometry, remained within normal limits, ranging from -0.3 ± 0.2 to -0.5 ± 0.2, depending on the type of ART method used. The lowest values were recorded in patients who became pregnant through oocyte donation, which is explained by specific hormonal changes caused by superovulation and hormone replacement therapy. This group demonstrates a more pronounced release of calcium from bone tissue due to hormonal fluctuations associated with preparation for pregnancy. Baseline BMD may be lower due to prolonged treatment of concomitant endocrine or gynaecological pathologies, which often precede the oocyte donation procedure.

Patients who became pregnant as a result of IVF had slightly higher T-score values in the first trimester than in the oocyte donation group. This is explained by the lower intensity of hormonal changes after embryo implantation compared to the processes that occur when using donor oocytes. However, the level of BMD in this group is also reduced, which may be due to the long period of preparation for pregnancy and the use of hormonal ovulation stimulants. The IUI group demonstrated a mean T-score of -0.4 ± 0.1 in the distal forearm in the first trimester of pregnancy. Compared to IVF and oocyte donation, this reproductive care method has a less pronounced effect on hormonal balance, which is reflected in relatively smaller changes in BMD in the first trimester.

In the third trimester of pregnancy, a significant decrease in the T-score was recorded in all groups, indicating an increased release of calcium from bone tissue for the needs of the foetus. The most pronounced changes were noted in the oocyte donation group, where the mean T-score in the distal forearm decreased to -1.3 ± 0.3 in the third trimester of pregnancy. This could be determined by more profound hormonal changes caused using hormone replacement therapy, as well as possibly lower initial BMD in these patients. In the IVF group, the average T-score in the distal forearm decreased to -1.0 ± 0.2, which also indicates significant changes in the structure of bone tissue associated with the physiological needs of the foetus and changes in hormonal levels.

The IUI group also demonstrated a decrease in BMD in the third trimester, although the values were slightly higher than in the IVF and oocyte donation groups. In particular, the T-score in the distal forearm in this group was -1.1 ± 0.3. This indicates less bone loss in patients whose pregnancy occurred as a result of intrauterine insemination, which may be due to less intensive hormonal preparation for pregnancy compared to other ART methods. Similar changes were observed when measuring the T-score in the distal forearm, although their intensity varied depending on the group. In the first trimester, the mean values ranged from -0.3 ± 0.2 in the IVF group to -0.5 ± 0.2 in the oocyte donation group, while in the third trimester, these values decreased to -1.0 ± 0.2 and -1.3 ± 0.3, respectively. This indicates a different level of bone vulnerability depending on the location: in the lumbar spine, the decrease in BMD is faster, which is explained by the increased load on this area during pregnancy. In the femoral neck, although less pronounced, significant BMD loss was also noted, due to general systemic changes in bone metabolism.

The data obtained demonstrate that the most intense loss of BMD was observed in the distal forearm, where the average decrease in T-score between the first and third trimesters was 0.7-0.8 units, depending on the group. This indicates a high metabolic activity of bone tissue in this area and its sensitivity to physiological and hormonal changes associated with pregnancy. In the third trimester, osteopenic changes were recorded in 30-40% of patients in the IVF and IUI groups. At the same time, in the group of women who became pregnant through oocyte donation, the frequency of such changes reached 50%. Women who became pregnant through oocyte donation were more vulnerable to BMD loss due to the specifics of hormonal support used in this group. These changes require enhanced monitoring and implementation of preventive measures to minimise the risk of osteoporosis in the postpartum period.

Thus, the distribution of osteopenic changes by measurement areas and groups shows that pregnancy achieved through ART is accompanied by significant changes in bone health. This requires the introduction of regular monitoring of bone health and preventive measures to reduce the risk of bone loss. The analysis of socio-demographic, nutritional, behavioural factors and physical activity levels of patients demonstrated a significant impact of these parameters on bone health in women who became pregnant with assisted reproductive technologies (Table 2).

Table 2. Risk factors and their impact on the T-score

Factor	Group	Average T-score
Age	25-30 years	-0.4 ± 0.1
	35-40 years	-0.9 ± 0.2
Calcium intake	Regular	-0.3 ± 0.1
	Irregular	-0.7 ± 0.2
Vitamin D levels	Normal (20-50 ng/mL)	-0.5 ± 0.1
	Deficiency (<20 ng/mL)	-1.0 ± 0.3
Physical activity	Moderate	-0.4 ± 0.2
	Low	-0.8 ± 0.2
Smoking	None	-0.5 ± 0.1
	Present	-1.0 ± 0.2
Caffeine consumption	<3 cups of coffee/day	-0.5 ± 0.1
	>3 cups of coffee/day	-0.9 ± 0.2

Source: compiled by the authors

Socio-demographic variables such as age, education level and place of residence had a moderate effect on bone mineral density. Older women (35-40 years old) had lower mean T-score values (-0.9 ± 0.2) compared to younger women (25-30 years old), where the T-score was -0.4 ± 0.1. This demonstrates the significant impact of age-related changes on bone health, which is associated with a decrease in the level of oestrogen and other hormones that are central to maintaining bone mineral density. In older age, the activity of osteoblasts, which form new bone tissue, decreases and the activity of osteoclasts, which contribute to bone resorption, increases. In addition, the deterioration of calcium and vitamin D absorption due to age-related changes in metabolism also exacerbates this effect.

Dietary habits were substantial in maintaining bone health. Patients who regularly consumed calcium-enriched dairy products and additionally used calcium supplements showed higher T-scores (mean value -0.3 ± 0.1). In patients whose diets were poor in calcium, this figure decreased to -0.7 ± 0.2. Vitamin D intake also proved to be a critical factor. Women with vitamin D deficiency (serum levels below 20 ng/mL) demonstrated significantly lower T-scores (-1.0 ± 0.3), while patients with normal vitamin D levels (20-40 ng/mL) had a mean T-score of -0.5 ± 0.1. This confirms the importance of a balanced diet with sufficient calcium and vitamin D for maintaining bone mineral density.

The level of physical activity had a significant impact on bone health in patients. Women who regularly performed moderate physical exercises such as yoga, swimming or walking at least three times a week had higher T-scores (-0.4 ± 0.2) compared to those who were sedentary, with a T-score of -0.8 ± 0.2. Of particular importance was regular physical activity in the third trimester of pregnancy, when the physiological decrease in bone mineral density was most pronounced. Maintaining an active lifestyle during this period contributed to the improvement of bone tissue condition, helping to compensate for the natural loss of minerals.

Bad habits, such as smoking and excessive caffeine consumption, had a significant negative impact on bone health, which was manifested in a decrease in mineral density and an increased susceptibility to osteopenic changes. Excessive caffeine intake (more than three cups of coffee per day) was associated with a decrease in T-score to -0.9 ± 0.2, as caffeine increases calcium excretion from the body through the urine, which reduces the availability of this trace element to maintain bone structure. In women who restricted their caffeine intake, the T-score remained at -0.5 ± 0.1, which indicates the importance of a moderate approach to the consumption of high-caffeine drinks.

Smoking also had a significant impact on bone tissue. Smokers had an average T-score of -1.0 ± 0.2, which is significantly lower than women who did not have this habit. Nicotine and toxic substances contained in tobacco smoke inhibit the activity of osteoblasts (cells responsible for bone formation) and disrupt bone remodelling processes. In addition, smoking reduces oestrogen levels, which is an important factor in protecting bone tissue, especially during pregnancy. The impact of these factors makes it difficult for the body to adapt naturally to the increased calcium requirements during pregnancy, leading to a more intense decrease in mineral density.

An analysis of the hormonal profile of women who became pregnant with the help of ART revealed a significant impact of key hormone levels on bone health during pregnancy. Hormones such as oestrogen, progesterone, parathyroid hormone and prolactin have a direct impact on the regulation of calcium metabolism and BMD, especially in conditions of increased stress on the woman's body. Table 3 shows that the levels of oestrogen and prolactin were highest in the oocyte donation group, while the lowest values of oestrogen and progesterone were observed in the IUI group, which may explain the higher incidence of osteopenic changes in this group.

Table 3. Average values of hormonal parameters depending on the type of ART

Hormone	IVF (M ± SD)	IUI (M ± SD)	Oocyte donation (M ± SD)
Oestrogen (pg/mL)	345 ± 26	298 ± 30	372 ± 28
Progesterone (ng/mL)	22 ± 5	18 ± 4	28 ± 4
Parathormone (pg/mL)	36 ± 6	42 ± 5	32 ± 6
Prolactin (ng/mL)	78 ± 7	72 ± 8	90 ± 9

Source: compiled by the authors

Oestrogen has proven to be a key factor that significantly affects the preservation of BMD in women during pregnancy achieved through ART. An analysis of the hormonal profile of patients showed that high oestrogen levels in the third trimester of pregnancy were associated with a reduced risk of osteopenic changes. In the oocyte donation group, where oestrogen levels were the highest (372 ± 28 pg/mL), the incidence of osteopenic manifestations was only 25%, while in the distal forearm, the T-score remained at -0.5 ± 0.2, which is significantly better than in other groups. This is attributed to the ability of oestrogen to inhibit the activity of osteoclasts, the cells responsible for bone resorption, and to maintain a balance between bone resorption and bone formation.

In the IUI group, where the average oestrogen level was lower (298 ± 30 pg/mL), the incidence of osteopenic changes reached 40%. In this group, the T-score in the distal forearm decreased to -1.1 ± 0.3, indicating insufficient hormonal support and a higher risk of bone loss. The effect of oestrogen on bone tissue is confirmed by its role in inhibiting the activation of osteoclasts and stimulating osteoblasts, the cells responsible for the formation of new bone tissue. This mechanism ensures the preservation of bone structure and strength, especially during pregnancy, when the mother's body is under increased stress due to foetal growth.

In addition, women with higher oestrogen levels had a less pronounced decrease in BMD in the distal forearm. In the oocyte donation group, the mean T-score in this area was -0.7 ± 0.2, while in the IUI group, it was -1.0 ± 0.3. This indicates the importance of maintaining sufficient oestrogen levels to prevent osteopenic changes in bones with high metabolic activity. High oestrogen levels were also associated with a lower incidence of complications such as fractures or significant bone loss after pregnancy.

Progesterone, as one of the key hormones in pregnancy maintenance, had a moderate positive effect on BMD. In the oocyte donation group, where progesterone levels reached 28 ± 4 ng/mL, better T-score values in the distal forearm (-0.5 ± 0.2) were observed compared to the IVF and IUI groups. This is due to the ability of progesterone to stimulate the synthesis of bone matrix proteins, in particular collagen, which provides bone strength and elasticity. Progesterone also has antioxidant properties that protect bone tissue from oxidative stress damage.

Parathyroid hormone levels, on the contrary, were associated with an increased risk of osteopenic changes, especially in the IUI group, where its values were the highest (42 ± 5 pg/mL). Excessive parathyroid hormone promotes the mobilisation of calcium from bone tissue to meet the needs of the body, especially the foetus, which leads to a decrease in BMD. In this group, the incidence of osteopenic changes reached 40%, and the mean T-score was -1.1 ± 0.3. This emphasises the need to control parathyroid hormone levels to prevent significant bone loss.

Prolactin, another important hormone that regulates calcium metabolism, also showed a significant correlation with BMD. In the oocyte donation group, where prolactin levels reached 90 ± 9 ng/mL, the best bone preservation was observed. High levels of prolactin support adaptive mechanisms of calcium metabolism, ensuring adequate calcium intake for the fetus without significant damage to the mother's bone tissue. Patients with high prolactin levels demonstrated a lower incidence of osteopenic changes, with a mean T-score of -0.5 ± 0.1.

An analysis of biochemical markers of bone metabolism in pregnant women who became pregnant using different types of ART revealed significant differences in key parameters (Table 4). These differences reflect the impact of different technologies on bone metabolism through changes in hormonal profile and biochemical status. The levels of calcium, phosphorus, magnesium, bone resorption markers and vitamin D reflected the state of bone tissue and its remodelling trends in pregnancy.

Table 4. Indicators of biochemical markers of bone metabolism depending on the type of ART

Metric	IVF	IUI	Oocyte donation
Total calcium (mmol/L)	2.20 ± 0.15	2.10 ± 0.20	2.30 ± 0.10
Ionised calcium (mmol/L)	1.10 ± 0.08	1.02 ± 0.05	1.15 ± 0.07
Magnesium (mmol/L)	0.80 ± 0.05	0.70 ± 0.04	0.85 ± 0.06
Vitamin D (ng/mL)	28.0 ± 5.0	20.0 ± 4.5	32.0 ± 6.0
C-telopeptidy (ng/mL)	0.3 ± 0.07	0.50 ± 0.09	0.30 ± 0.05
Phosphorus (mmol/L)	1.20 ± 0.10	1.15 ± 0.12	1.25 ± 0.08

Source: compiled by the authors

An analysis of biochemical markers of bone metabolism in pregnant women who became pregnant using different types of ART revealed significant differences in key parameters. These differences reflect the impact of different technologies on bone metabolism through changes in hormonal profile and biochemical status. The levels of calcium, phosphorus, magnesium, bone resorption markers and vitamin D reflected the state of bone tissue and its remodelling trends in pregnancy. The results showed that patients who became pregnant through oocyte donation had the highest mean calcium levels, both in total and ionised form. This could be determined by increased nutritional control in this group of women and possibly by the more frequent use of calcium supplements. At the same time, calcium levels were lowest in the IUI group, indicating potentially inadequate calcium intake or absorption. This trend was accompanied by higher rates of bone resorption, as evidenced by the high levels of C-telopeptides in this group.

Magnesium levels also differed between groups. The highest magnesium values were observed in the oocyte donation group, which could contribute to bone stability. Magnesium plays an important role in bone remodelling and maintaining calcium balance, and its deficiency can lead to bone deterioration. In the IUI group, magnesium was at the lowest level, which, together with low vitamin D levels, could contribute to an increased risk of osteopenic changes. The

role of vitamin D in bone metabolism should be noted separately [12]. In the oocyte donation group, its level was the highest, which ensured better calcium absorption and bone stability. In the IUI group, on the contrary, vitamin D levels were significantly reduced, which could contribute not only to bone loss but also to an increased risk of osteopenia.

The dependence of biochemical markers on the type of ART is also confirmed by differences in bone resorption markers. In the IUI group, the level of C-telopeptides was the highest, indicating active processes of bone destruction. This could be due to micronutrient deficiencies, which, in turn, exacerbates the negative impact on bone structure. In IVF patients, these indicators were at an average level, which may reflect a moderate adaptation to changes in metabolism. The results emphasise the need for careful monitoring of biochemical markers of bone metabolism in pregnant women, especially those who became pregnant with IUI. Decreased levels of vitamin D, calcium and magnesium require timely correction with special dietary recommendations and nutraceutical support. Patients with oocyte donation, although they have the best biochemical profile, also need to be monitored to maintain a positive balance.

The final analysis of the results shows a complex effect of the type of assisted reproductive technology on biochemical markers of bone metabolism in pregnant women. The group that became pregnant through oocyte donation demonstrated the most favourable mineral metabolism, including higher levels of calcium, magnesium and vitamin D, suggesting a potential adaptive advantage of this method in the context of bone health. Participants who used intrauterine insemination had increased bone resorption activity and the lowest calcium levels, which may indicate an increased risk of osteopenic changes in this group. Overall, the results emphasise the need for an individualised approach to the management of pregnancies achieved through ART, incorporating the type of technology used and its impact on biochemical status. An important aspect is to ensure adequate levels of vitamin D, calcium and magnesium through diet or supplementation, especially for high-risk groups. This will reduce the probability of osteopenic changes and maintain optimal bone health during pregnancy.

DISCUSSION

Pregnancy achieved by ART has a significant effect on BMD, namely in the distal forearm. In the third trimester, the greatest decrease in T-score was observed in the oocyte donation group (-1.3 ± 0.3), which corresponds to the findings of S. Golombok [13], emphasising the significant effect of hormone therapy on bone metabolism. K. Berg & K. Whitehead [14] noted that such hormonal changes cause significant bone resorption. The results confirm these observations, demonstrating that hormonal support during oocyte donation is an additional risk for bone loss.

The decrease in BMD in the distal forearm was significant in all groups. In the oocyte donation group, the T-score in this area decreased to -1.0 ± 0.2. Although the study evaluated only one anatomical area, the results are consistent with the findings of S. Butscheidt *et al*. [15], emphasising the importance of the metabolic activity of bone tissue when assessing bone loss. P. Anagnostis *et al*. [16] also noted that even within the same zone, the indicators can vary depending on the local load. This confirms the feasibility of an integrated approach to monitoring bone health in women during pregnancy.

Socio-demographic factors significantly affect BMD. Women aged 35-40 years had a mean T-score of -0.9 ± 0.2, while younger patients (25-30 years) demonstrated a value of -0.4 ± 0.1. M. Carsote *et al*. [17] highlighted an age-related decrease in oestrogen levels, which contributes to bone loss. S. Lee *et al*. [18] emphasised that an additional factor is vitamin D deficiency in older age. The data of the current study confirm these findings, demonstrating the relationship between age, hormonal changes and bone health.

Dietary habits demonstrated a significant impact on BMD preservation. Patients who regularly consumed calcium demonstrated a mean T-score of -0.3 ± 0.1, while in patients with low calcium intake, this figure decreased to -0.7 ± 0.2. M. Fukushima *et al*. [19] noted that calcium is a key element in maintaining bone mass. N. Mishra *et al*. [20] noted that calcium deficiency is especially dangerous during pregnancy when the need for minerals increases. The results confirmed these observations, emphasising the need for dietary adjustment to prevent osteopenia.

Physical activity was an important factor in the preservation of BMD. In women with moderate physical activity, the T-score was -0.4 ± 0.2, while in sedentary participants this figure decreased to -0.8 ± 0.2. A.L.C. Morato *et al*. [21] emphasised the positive effect of physical activity on osteogenesis. K. Maehara *et al*. [22] emphasised that regular exercise reduces the risk of bone loss during pregnancy. The current data confirmed these findings, demonstrating a correlation between physical activity and BMD preservation.

Bad habits, such as smoking and excessive caffeine consumption, had a significant impact on BMD. In smokers, the average T-score was -1.0 ± 0.2, while in non-smokers, this figure was -0.5 ± 0.1. T.A. Ottun *et al*. [23] noted that nicotine inhibits the activity of osteoblasts. Y. Qian *et al*. [24] stated that caffeine promotes calcium excretion from the body. Current results are consistent with these findings, demonstrating the need to limit bad habits to maintain bone health.

The hormonal profile was instrumental in the preservation of BMD. In the oocyte donation group, the oestrogen level reached 372 ± 28 pg/mL, which correlated with a lower incidence of osteopenic changes. K. Lampropoulou-Adamidou *et al*. [25] emphasised that oestrogen inhibits osteoclast activity. H. Basma *et al*. [26] noted that progesterone maintains the bone matrix. The results of the study confirm these findings, demonstrating the importance of hormonal balance in the prevention of bone loss.

The level of parathyroid hormone in the IUI group reached 42 ± 5 pg/mL, which was associated with a significant decrease in BMD. C.-H. Cheng *et al*. [27] noted that excess parathyroid hormone activates bone resorption by stimulating osteoclasts, which leads to rapid bone loss. P. Wu *et al*. [28] highlighted that prolactin, although partially reduces this effect, does not always provide adequate protection in the case of high concentrations of parathyroid hormone. The current study demonstrated that parathyroid hormone levels in women from the IUI group correlate with higher resorption markers, which supports the hypothesis of increased bone loss. This finding contradicts some studies that point to the compensatory role of prolactin, emphasising the importance of further studying the mechanisms of hormonal influence on

bone metabolism. Thus, the results of the current study are more detailed, as they incorporate both hormonal and biochemical parameters, which improves the description of pathogenetic mechanisms.

The analysis of biochemical markers demonstrated that the oocyte donation group had higher levels of calcium and magnesium. B. Miles *et al*. [29] noted that adequate levels of calcium and magnesium reduce the activity of bone resorption, preventing excessive bone loss. Y. Kaneuchi *et al*. [30] stated that vitamin D is critical for calcium absorption, ensuring its efficient transport to bone tissue and regulation of parathyroid hormone levels. Current results demonstrated that women with higher levels of these micronutrients in the oocyte donation group had significantly lower resorption markers than participants in other groups. This demonstrates the advantage of a comprehensive approach to nutraceutical support, compared to less focused recommendations often used in other studies.

In the VMI group, a high level of resorption markers was observed, indicating significant disorders in bone metabolism. D.A. Ferreira *et al*. [31] noted that this is a consequence of vitamin D deficiency, which plays a key role in calcium absorption and bone mass maintenance. A.A. Abdulrazaq *et al*. [32] added that active resorption is a direct consequence of the hyperproduction of parathyroid hormone, which stimulates osteoclastic activity. The current study determined that the level of resorption markers in women from the IUI group exceeded the average values reported in other studies, which may indicate more severe disorders. These findings demonstrate that vitamin D deficiency has a more significant impact than previously thought, highlighting the importance of a comprehensive approach to correcting mineral metabolism.

In general, the results obtained indicate that pregnancy achieved through ART is accompanied by significant changes in bone health. A. Cohen *et al*. [33] emphasised the importance of an individual approach to the prevention of osteopenia, focusing on the adaptation of nutritional, physical and medication strategies. M. Rahimi *et al*. [34] reported that regular monitoring, including the use of biochemical markers of bone metabolism, allows for the timely identification of risks and implementation of preventive measures that help maintain tissue mineral density even in high-risk groups. These studies are consistent with these findings, while also detailing the relationship between specific hormonal changes and biochemical analysis, which allows for a deeper understanding of the impact of different types of ART on bone health. This approach demonstrates significantly greater accuracy and practical value for managing bone health.

The findings highlight the complex impact of pregnancy achieved through assisted reproductive technologies on bone health. The analysis showed that key factors, such as hormonal changes, dietary habits, physical activity and micronutrient levels, have a significant impact on bone mineral density. The data of the current study correlate with the findings of previous studies, but at the same time detail and expand on them, emphasising the importance of a comprehensive approach to the prevention of osteopenic changes. The use of modern biochemical markers, a detailed analysis of hormonal changes, and the development of individualised recommendations for each group of patients was noted to significantly improve the effectiveness of pregnancy management.

CONCLUSIONS

The study determined a significant decrease in BMD in women who became pregnant with ART, especially in the third trimester of pregnancy. The most significant changes were recorded in the distal forearm. In the oocyte donation group, the mean T-score decreased from -0.5 ± 0.2 to -1.3 ± 0.3. In the IVF and IUI groups, the T-score decreased from -0.3 ± 0.2 and -0.4 ± 0.1 to -1.0 ± 0.2 and -1.1 ± 0.3, respectively. Age, dietary and behavioural factors had a significant impact on bone health. In women over 35 years of age, the mean T-score was -0.9 ± 0.2, while in younger age groups this figure was -0.4 ± 0.1. Regular calcium intake was associated with less BMD loss (decrease in T-score by 0.3 ± 0.1 units), while its deficiency was associated with greater losses (decrease by 0.7 ± 0.2 units). Vitamin D deficiency had a significant effect on the scores, as women with vitamin D deficiency had a mean T-score of -1.0 ± 0.3, which was 0.5 units lower than in patients with normal vitamin D levels. Regular physical activity contributed to BMD maintenance: the mean T-score was -0.4 ± 0.2 in physically active women compared to -0.8 ± 0.2 in sedentary women. Limitations of the study are related to the selectivity of the participants and the fact that it included only women who became pregnant through ART, which limits the generalisability of the findings to the wider population of pregnant women. In addition, the lack of analysis of genetic factors and long-term monitoring of BMD after childbirth leaves some questions open. Prospects for future research include the development of long-term strategies for monitoring BMD in the postpartum period, evaluating the effectiveness of dietary and physical interventions, and incorporation of genetic, ethnic and hormonal characteristics to create personalised approaches to the prevention of osteopenia and osteoporosis in pregnant women.

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CONFLICT OF INTEREST

None.

REFERENCES

[1] Deinichenko O, Siusiuka V, Krut Y, Pavlyuchenko M, Kyryliuk O, Boguslavska N. Prediction of the development of fetal growth retardation in pregnant women with chronic arterial hypertension. Reprod Health Woman. 2022;7:14–20. DOI: 10.30841/2708-8731.7.2022.272466

[2] Tsymbal A, Kotlova Y. Assessment of risk factors for osteopenia development in premature babies. Mod Med Technol. 2023;4:27–36. DOI: 10.34287/mmt.4(59).2023.4

[3] Zhytnik L, Maasalu K, Duy BH, Pashenko A, Khmyzov S, Reimann E, et al. De novo and inherited pathogenic variants in collagen-related osteogenesis imperfecta. Mol Gen Genomic Med. 2019;7(3):e559. DOI: 10.1002/mgg3.559
[4] Stern JE, Luke B, Tobias M, Gopal D, Hornstein MD, Diop H. Adverse pregnancy and birth outcomes associated with underlying diagnosis with and without assisted reproductive technology treatment. Fertil Steril. 2015;103(6):1438–45. DOI: 10.1016/j.fertnstert.2015.02.027
[5] Wang Y, Chen L, Tao Y, Luo M. Risk factors of ectopic pregnancy after in vitro fertilization-embryo transfer in Chinese population: A meta-analysis. PLoS ONE. 2024;19(1):e0296497. DOI: 10.1371/journal.pone.0296497
[6] Nørgård BM, Catalini L, Jølving LR, Larsen MD, Friedman S, Fedder J. The efficacy of assisted reproduction in women with a wide spectrum of chronic diseases – a review. Clin Epidemiol. 2021;13:477–500. DOI: 10.2147/clep.s310795
[7] Graham ME, Jelin A, Hoon AH, Floet AMW, Levey E, Graham EM. Assisted reproductive technology: Short- and long-term outcomes. Dev Med Child Neurol. 2022;65(1):38–49. DOI: 10.1111/dmcn.15332
[8] Sun B, Li L, Zhang Y, Wang F, Sun Y. Pregnancy outcomes in women with primary ovarian insufficiency in assisted reproductive technology therapy: A retrospective study. Front Endocrinol. 2024;15:1343803. DOI: 10.3389/fendo.2024.1343803
[9] Kyiv Perinatal Centre [Internet]. [cited 2024 August 7]. Available from: https://www.perinatalcenter.com.ua/en/
[10] The World Medical Association. Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects [Internet]. [cited 2024 August 7]. Available from: https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/
[11] IBM. SPSS Statistics [Internet]. [cited 2024 August 7]. Available from: https://www.ibm.com/products/spss-statistics
[12] Vohra G, Rajendran N, Kumari A. Association of mineral metabolism biomarkers in patients with chronic kidney disease and renal transplant recipients: A single-centre prospective study. Int J Med Med Res (IJMMR). 2024;10(2):57–64. DOI: 10.61751/ijmmr/2.2024.57
[13] Golombok, S. Assisted reproductive technologies. In: The SAGE encyclopedia of lifespan human development. Thousand Oaks, CA: SAGE Publications, Inc.; 2018. P. 162–3. DOI: 10.4135/9781506307633.n65
[14] Berg K, Whitehead K. Pregnancy associated osteoporosis. Practising Midwife. 2024;27(5):35–8. DOI: 10.55975/chwn3013
[15] Butscheidt S, Tsourdi E, Rolvien T, Delsmann A, Stürznickel J, Barvencik F, et al. Relevant genetic variants are common in women with pregnancy and lactation-associated osteoporosis (PLO) and predispose to more severe clinical manifestations. Bone. 2021;147:115911. DOI: 10.1016/j.bone.2021.115911
[16] Anagnostis P, Lampropoulou-Adamidou K, Bosdou JK, Trovas G, Galanis P, Chronopoulos E, et al. Comparative effectiveness of therapeutic interventions in pregnancy and lactation-associated osteoporosis: A systematic review and meta-analysis. J Clin Endocrinol Metab. 2023;109(3):879–901. DOI: 10.1210/clinem/dgad548
[17] Carsote M, Turturea MR, Valea A, Buescu C, Nistor C, Turturea IF. Bridging the gap: Pregnancy- and lactation-associated osteoporosis. Diagnostics. 2023;13(9):1615. DOI: 10.3390/diagnostics13091615
[18] Lee S, Hong N, Kim KJ, Park CH, Lee J, Rhee Y. Bone density after teriparatide discontinuation with or without antiresorptive therapy in pregnancy- and lactation-associated osteoporosis. Calcif Tissue Int. 2021;109:544–53. DOI: 10.1007/s00223-021-00869-6
[19] Fukushima M, Kawajiri M, Yoshida M, Takeishi Y, Nakamura Y, Yoshizawa T. Prevalence of pregnancy- and lactation-associated osteoporosis in the postpartum period: A systematic review and meta-analysis. Drug Discoveries Ther. 2024;18(4):220–8. DOI: 10.5582/ddt.2024.01037
[20] Mishra N, Masroor H, Gupta M. Osteoporosis in pregnant and lactating females: An update. Turk J Osteoporos. 2024;30(1):1–15. DOI: 10.4274/tod.galenos.2023.24392
[21] Morato ALC, Verruma CG, Furtado CLM, Reis RMD. In vitro maturation of oocytes: What is already known? Biol Reprod. 2024;112(2):18–30. DOI: 10.1093/biolre/ioae147
[22] Maehara K, Iwata H, Kimura K, Mori E. Experiences of transition to motherhood among pregnant women following assisted reproductive technology: A qualitative systematic review. JBI Evidence Synth. 2021;20(3):725–60. DOI: 10.11124/jbies-20-00545
[23] Ottun TA, Adewunmi AA, Olumodeji AM, Jinadu FO. Factors associated with clinical pregnancy following assisted reproductive technology: A comparative cross-sectional study. J West Afr Coll Surg. 2024;15(1):37–43. DOI: 10.4103/jwas.jwas_148_23
[24] Qian Y, Wang L, Yu L, Huang W. Pregnancy- and lactation-associated osteoporosis with vertebral fractures: A systematic review. BMC Musculoskeletal Disord. 2021;22:926. DOI: 10.1186/s12891-021-04776-7
[25] Lampropoulou-Adamidou K, Trovas G, Triantafyllopoulos IK, Yavropoulou MP, Anastasilakis AD, Anagnostis P, et al. Teriparatide treatment in patients with pregnancy- and lactation-associated osteoporosis. Calcif Tissue Int. 2021;109:554–62. DOI: 10.1007/s00223-021-00871-y
[26] Basma H, Dbouk S, Issa Z. Pregnancy and lactation associated osteoporosis: A case report. Front Med Case Rep. 2023;4(5):1–8. DOI: 10.47746/fmcr.2023.4503
[27] Cheng CH, Chen LR, Chen KH. Osteoporosis due to hormone imbalance: An overview of the effects of estrogen deficiency and glucocorticoid overuse on bone turnover. Int J Mol Sci. 2022;23(3):1376. DOI: 10.3390/ijms23031376

[28] Wu P, Sharma GV, Mehta LS, Chew-Graham CA, Lundberg GP, Nerenberg KA, et al. In-hospital complications in pregnancies conceived by assisted reproductive technology. J Am Heart Assoc. 2022;11(5). DOI: 10.1161/jaha.121.022658
[29] Miles B, Panchbhavi M, Mackey JD. Analysis of fracture incidence in 135 patients with pregnancy and lactation osteoporosis (PLO). Cureus. 2021;13(10):e19011. DOI: 10.7759/cureus.19011
[30] Kaneuchi Y, Iwabuchi M, Hakozaki M, Yamada H, Konno SI. Pregnancy and lactation-associated osteoporosis successfully treated with romosozumab: A case report. Medicina. 2022;59(1):19. DOI: 10.3390/medicina59010019
[31] Ferreira DA, Taborda F, Mendonça T, Farinha F. Pregnancy and lactation-associated osteoporosis in a Systemic Lupus Erythematosus patient. Lupus. 2022;31(14):1829–33. DOI: 10.1177/09612033221136102
[32] Abdulrazaq AA, Ainsworth AJ, Britton JW, Shenoy CC, Babayev SN, Cascino GD, et al. Seizure control in women with epilepsy undergoing assisted reproductive technology. Epilepsia. 2023;64(10):e207–13. DOI: 10.1111/epi.17748
[33] Cohen A, Kamanda-Kosseh M, Dempster DW, Zhou H, Müller R, Goff E, et al. Women with pregnancy and lactation-associated osteoporosis (PLO) have low bone remodeling rates at the tissue level. J Bone Miner Res. 2019;34(9):1552–61. DOI: 10.1002/jbmr.3750
[34] Rahimi M, Daneshvar S, Khabbazi A. Pregnancy-associated osteoporosis following in vitro fertilization: A case report. Clin Case Rep. 2024;12(3):e8702. DOI: 10.1002/ccr3.8702

Визначення факторів ризику виникнення остеопенічних змін у жінок, які завагітніли за допомогою допоміжних репродуктивних технологій

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Анотація. Метою дослідження було вивчення факторів, які впливають на ризик виникнення остеопенічних змін у жінок, які завагітніли за допомогою допоміжних репродуктивних технологій. У дослідженні взяли участь 150 жінок віком 25-40 років, які завагітніли після використання екстракорпорального запліднення, внутрішньоматкової інсемінації та донорства ооцитів. Мінеральну щільність кісткової тканини було оцінено методом ультразвукової денситометрії у ділянці дистального передпліччя на ранніх (8-12 тижнів) і пізніх (32-36 тижнів) етапах вагітності. Показники T-score демонстрували значне зниження у третьому триместрі у всіх групах, найбільше – у пацієнток, які завагітніли завдяки донорству ооцитів (-1,3±0,3 у дистальному передпліччі). Аналіз соціально-демографічних характеристик виявив, що вік старше 35 років, низький рівень фізичної активності, дефіцит вітаміну D і кальцію, а також шкідливі звички, такі як куріння та надмірне споживання кофеїну, суттєво підвищували ризик розвитку остеопенічних змін. Жінки з регулярним споживанням молочних продуктів, кальцієвмісних добавок і нормальним рівнем фізичної активності мали значно кращі показники мінеральної щільності кісткової тканини. Біохімічні маркери підтвердили вищі рівні кальцію, магнію та вітаміну D у групі донорства ооцитів, тоді як у групі внутрішньоматкової інсемінації виявлено дефіцит цих елементів і підвищення маркерів кісткової резорбції. Гормональний профіль також суттєво впливав на стан кісткової тканини: високий рівень естрогену у пацієнток групи донорства ооцитів був асоційований із менш вираженими остеопенічними змінами, тоді як підвищений рівень паратгормону у групі внутрішньоматкової інсемінації сприяв посиленню втрати кісткової маси. У пацієнток із найвищими рівнями пролактину спостерігалося краще збереження мінеральної щільності кісткової тканини завдяки позитивному впливу цього гормону на кальцієвий обмін. Отримані результати підкреслюють необхідність комплексного моніторингу стану кісткової тканини у жінок, які завагітніли за допомогою допоміжних репродуктивних технологій

Ключові слова: щільність кісткової тканини; біохімічні маркери; гормональний профіль; внутрішньоматкова інсемінація; донорство ооцитів

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Epidemiological trends of salmonellosis in the cross-border regions of Ukraine and Poland (2014-2023)

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Abstract. The epidemiology of salmonellosis, despite being extensively studied, remains a relevant public health concern. The aim of this research was to compare the incidence of salmonellosis in Poland and Ukraine at both the national level and within selected cross-border regions of the two countries during the period 2014-2023. The average annual incidence of salmonellosis over the 10-year period was 15.02 per 100,000 population in Ukraine, with 64,108 confirmed cases; in Poland, the incidence was 22.74 per 100,000, with 86,956 confirmed cases ($p = 0.0025$). In the cross-border regions, the number of confirmed cases and incidence rates (per 100,000) were as follows: Volyn – 1,879 cases / 18.02; Lviv – 3,305 cases/13.18; Lublin Voivodeship – 6,067 cases/27.62; and Podkarpackie – 7,869 cases/37.13 ($p < 0.001$). The study analysed trends in prevalence and the serological profiles of *Salmonella* detected in humans, food products, and other environmental sources. The dominant serotype during outbreaks and isolated cases in the Volyn and Lviv regions was *S. enterica* subsp. *enterica* Serovar Enteritidis, accounting for 87.51 and 77.90% of cases, respectively. The Serovar *S.* Typhimurium was identified in 10.04% of cases in Volyn and in 18.08% in Lviv. The most common transmission vectors of *Salmonella* in the Volyn region were: eggs and egg products (29.51% of all *Salmonella*-positive items), meat and meat products (27.40%), prepared dishes (12.3%), and confectionery (11.0%). In the Lviv region, the most frequently contaminated items were: prepared dishes (27.12%), meat products (17.53%), confectionery (8.49%), and eggs and egg products (7.12%). The findings of this study may serve as a foundation for evidence-based epidemiological practices and support the development of coordinated actions to enhance epidemiological surveillance and control of salmonellosis both in cross-border regions and nationally in each country

Keywords: *Salmonella* serovars; incidence; epidemiological analysis; food products

INTRODUCTION

Human and animal health, food safety, and nutrition are inextricably linked and form a key component of the One Health concept. This approach unites the efforts of specialists from various health-related fields and institutions –

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operating at local, national, and global levels – to improve the health of people, domestic animals, wildlife, and plants as integral parts of the environment [1, 2]. The causative agent of salmonellosis remains a pathogen of ongoing significance in medicine, both in terms of its biological properties and its role in the epidemiological process of disease [3]. *Salmonella* species – bacterial pathogens with a broad host range and diverse pathogenicity factors – are characterised by a wide variety of sources, reservoirs, transmission routes, and a high degree of ubiquity, which forms the biological basis for their persistence and resilience [4, 5].

Researchers M. Milczarek *et al*. [6] examined the incidence of salmonellosis in Poland during 2018-2019 and reported that the highest incidence was recorded in the Podkarpackie Voivodeship at 42.2 per 100,000 population, while the lowest was in the Lublin Voivodeship at 9.4 per 100,000. The same group of scientists [7, 8] observing the dynamics of the manifestation of the salmonellosis epidemic process in 2020 and 2021 made the conclusion that COVID-19 pandemic and the related restrictions introduced in Poland, as well as increased hygiene approaches, could had contributed to a decrease in the number of salmonellosis cases registered in Poland in 2020 compared to 2019. In Ukraine, O. Zublenko & T. Petrusevych [9] analysed the structure of acute intestinal infections from 2015 to 2019 and found that the average incidence of salmonellosis was 19.3 per 100,000, ranking second among causative pathogens within this group, with yearly fluctuations in intensity during the observation period. N. Polishchuk *et al*. [10] conducted an epidemiological analysis of non-typhoidal salmonellosis incidence in the Zaporizhia region between 2018 and 2022. They found that regional incidence rates consistently exceeded national averages, ranging from 19.88 per 100,000 in 2020 to 8.24 per 100,000 in 2022. In a study by E. Baharev [11] the long-term (1981-2018) data on *Salmonella* serovars isolated by diagnostic institutions of the North-Western Black Sea region, in particular Odesa, Mykolaiv and Kherson regions was studied. He concluded that the total number of *Salmonella* serovars isolated from humans is on average 10 times higher than the total number of *Salmonella* isolated from animals in this region.

Despite these efforts, the number of scientific studies analysing the epidemiology of salmonellosis in Ukraine remains limited. In particular, within the territory of Western Ukraine, there has been no comprehensive analysis of salmonellosis incidence or of the serovar profile of *Salmonella* strains isolated from patients, carriers, and food products linked to outbreaks. Furthermore, the geographical proximity of two Ukrainian regions (Volyn and Lviv) to each other and to two Polish voivodeships (Lublin and Podkarpackie) presents an opportunity not only for socio-economic collaboration but also for the comparative study of epidemiological patterns of salmonellosis across borders. In this context, the aim of the article was to analyse key aspects of the epidemic process of salmonellosis in four cross-border regions of Ukraine and Poland over the period 2014-2023. To achieve this goal, the following research objectives were set: to compare incidence rates in the four adjacent cross-border regions – Volyn and Lviv (Ukraine) and Lublin and Podkarpackie Voivodeships (Poland) – over a 10-year period; to compare national incidence rates of salmonellosis in Poland, Ukraine, and the European Union; to determine the relative contribution of various categories of food products and environmental objects as transmission factors in the Volyn and Lviv regions between 2014 and 2023.

MATERIALS AND METHODS

The epidemiological analysis and comparisons were conducted using official statistical data from the State Sanitary Inspectorates of the Lublin and Podkarpackie Voivodeships, as published on the official websites of the State Sanitary Inspectorate of the Ministry of Health of Poland (DSIMOZP) [12, 13]. Annual reports from the regional Public Health Centres (PHCs) of the Ministry of Health of Ukraine (MoH) in the Volyn and Lviv regions from 2014 to 2023 were also collected. These data were obtained directly by the authors and are not publicly accessible. To gather data on morbidity within the European Union, the Surveillance Atlas of Infectious Diseases [14] provided by the European Centre for Disease Prevention and Control (ECDC) was used. The selection of the four analysed regions was based on their geographical proximity: each region shares borders with at least one of the others.

The inclusion criterion for data selection was that the information originated from official state reporting forms of the Central Institutes of Health under the respective Ministries of Health in Ukraine and Poland. In Ukraine, the results of bacteriological investigations are annually summarised in official forms in accordance with current legislation, notably Order of the Ministry of Health of Ukraine No. 132 [15]. In Poland, data on salmonellosis are compiled in standard reporting forms A02.0 and A02.1 and are published annually by the Department of Epidemiology and Surveillance of Infectious Diseases in the report "Infectious diseases and poisonings in Poland" [16].

There was no need to adapt data between countries, as the incidence rate – calculated as the ratio of newly detected cases of disease over a specified period to the average annual population, expressed per 100,000 – is determined using identical methodology in both Ukraine and Poland. Due to the lack of publicly available primary data on the number and serovars of *Salmonella* isolated from patients and food products associated with outbreaks in Poland, only the relevant official data from the Volyn and Lviv regions were included in the analysis. The selection criteria were based on officially registered salmonellosis outbreaks and the *Salmonella* strains isolated, identified, and confirmed by the laboratories of the Central Centre for Disease Control of the Ministry of Health of Ukraine. These procedures were conducted in accordance with the approved outbreak investigation protocol as outlined in Order of the Ministry of Health of Ukraine No. 190 [17] and Law of Ukraine No. 1645-III [18]. Microbiological investigations were performed following the national standard protocol, as established by Order of the Ministry of Health of Ukraine No. 425 [19]. For statistical data processing, Microsoft Excel was used. The primary statistical methods applied included calculation of the arithmetic mean, standard error of the mean, representativeness error, t-value, and confidence probabilities (p). Statistical significance was considered at $p < 0.05$. Relative values were not used in the analysis.

RESULTS

Between 2014 and 2023, a total of 64,108 cases of salmonellosis were registered in Ukraine, compared to 86,956 cases in Poland. Among the studied cross-border regions, the highest number of confirmed cases was recorded in the Podkarpackie (7,869) and Lublin (6,067) Voivodeships. In the corresponding neighbouring regions of Ukraine – Lviv and Volyn – the number of confirmed cases was 3,305 and 1,879, respectively, which is 2.4 and 3.2 times lower (Table 1).

Table 1. Number of confirmed cases of salmonellosis (absolute number) and incidence (per 100 thousand population) in Lviv, Volyn regions and Ukraine; Lublin and Podkarpackie Voivodeships and Poland (2014-2023)

Years	Lviv* region (Ukraine)		Volyn* region (Ukraine)		Ukraine**		Lublin* Voivodeship (Poland)		Podkarpackie* Voivodeship (Poland)		Poland**	
	Number of cases	Incidence	Number of cases	Incidence	Number of cases	Incidence	Number of cases	Incidence	Number of cases	Incidence	Number of cases	Incidence
2014	299	11.85	141	13.2	8,412	18.54	607	28.2	588	27.6	8,392	21.83
2015	369	15.05	231	21.66	8,350	19.46	535	24.6	615	28.37	8,652	22.6
2016	494	19.61	274	26.13	8,941	20.91	794	36.5	731	33.05	10,027	26.07
2017	369	14.61	284	27.31	7,391	17.35	811	28.7	775	35.86	10,000	26.03
2018	333	13.1	269	25.91	7,713	18.18	609	28.56	898	41.15	9,957	25.95
2019	675	26.88	349	33.7	8,586	20.34	830	38.48	1,172	53.83	9,243	24.1
2020	116	4.62	79	7.65	3,758	8.95	344	15.79	708	32.72	5,468	14.3
2021	203	8.1	79	7.71	3,350	8.03	417	20.0	743	39.8	8,294	21.7
2022	225	8.98	81	7.91	3,195	7.71	410	20.2	550	26.4	6,575	17.4
2023	222	8.98	92	9.01	4,412	10.76	710	35.2	1,089	52.5	10,348	27.4
Total	3,305		1,879		64,108		6,067		7,869		86,956	

Notes: * – for the incidence rates for Ukraine and Poland, the t-value was 3.542 ($p = 0.0025$); ** – for the incidence rates for Volyn, Lviv, Lublin, and Podkarpackie regions, the t-value was 15.366 ($p < 0.001$)
Source: compiled by the authors based on personally collected data, as well as taking into account the Annual report "Infectious diseases and poisonings in Poland" [16]

The data obtained show that, across the four adjacent regions of Ukraine and Poland, the average annual incidence of salmonellosis in the population during 2014-2023 was as follows (per 100,000 population): Volyn region – 18.02 ± 3.15; Lviv region – 13.18 ± 2.03; Lublin Voivodeship – 27.62 ± 2.40; and Podkarpackie Voivodeship – 37.13 ± 3.09. During the pre-pandemic period (2014-2019), the incidence rates in these regions were higher: Volyn – 24.65 ± 2.79; Lviv – 16.85 ± 2.08; Lublin – 30.84 ± 2.21; and Podkarpackie – 36.64 ± 4.00. In contrast, during the COVID and post-COVID period (2020-2023), these values declined significantly: Volyn – 8.07 ± 0.32; Lviv – 7.67 ± 1.04; Lublin – 22.08 ± 4.26; and Podkarpackie – 37.86 ± 5.60. These differences in average values between regions were statistically significant ($p < 0.001$). Nationally, the incidence of salmonellosis in Poland remained consistently higher over the 10-year period: the average annual incidence was 22.74 ± 1.32 per 100,000 population, compared to 15.02 ± 1.23 in Ukraine. A notable decline in incidence was observed in both countries during the COVID and post-COVID years (2020-2023). Specifically, during 2014-2019, the average incidence in Poland and Ukraine was 24.43 ± 0.77 and 19.13 ± 0.55, respectively, while in 2020-2023, the incidence declined to 20.2 ± 2.84 in Poland and 8.86 ± 0.68 in Ukraine ($p = 0.0025$).

Across the European Union as a whole, the average annual incidence of salmonellosis ranged from 20.34 ± 0.20 per 100,000 during 2014-2019 to 16.25 ± 0.83 during 2020-2023, with a 10-year average of 18.70 ± 0.74 per 100,000 population ($p < 0.001$). During 2014-2023, a total of 26 outbreaks of salmonellosis were recorded in the Volyn region and 78 in the Lviv region. In all cases, the causative agent was *Salmonella enterica* subsp. *enterica* Serovar Enteritidis. Data on all *Salmonella* serovars isolated from clinically ill patients and carriers in the Volyn region during 2014-2023 are presented in Table 2.

Table 2. Data on the number of outbreaks and Salmonella serovars that caused the outbreaks, and Salmonella serovars from clinically ill patients and carriers not associated with outbreaks in the Volyn region (2014-2023)

Years	Number of foutbreaks	Serovars involved in outbreaks	Serovars isolated from clinically ill patients and carriers not associated with outbreaks, n (%)										Total
		Enteritidis	Enteritidis	Typhimurium	Virchow	Stanley	Muenchen	Derby	Newport	Postdam	Agona	Java	
2014	2	45 (21.74)	124 (59.90)	32 (15.46)	1 (0.48)	0 (0.0)	5 (2.42)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	207 (100.00)
2015	3	80 (26.76)	170 (56.86)	42 (14.05)	1 (0.33)	0 (0.0)	5 (1.67)	0 (0.0)	1 (0.33)	0 (0.0)	0 (0.0)	0 (0.0)	299 (100.00)
2016	4	64 (18.88)	229 (67.55)	40 (11.82)	0 (0.0)	0 (0.0)	3 (0.88)	1 (0.29)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.59)	339 (100.00)
2017	4	58 (20.42)	185 (65.14)	20 (7.04)	3 (1.06)	1 (0.35)	5 (1.76)	0 (0.0)	1 (0.35)	0 (0,0)	11 (3.87)	0 (0.0)	284 (100.00)
2018	4	32 (12.45)	213 (82.88)	8 (3.11)	3 (1.17)	0 (0.0)	1 (0.39)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	257 (100.00)
2019	7	112 (32.18)	112 (64.08)	12 (3.45)	0 (0.0)	0 (0.0)	1 (0.29)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	348 (100.00)
2020	1	8 (9.88)	62 (76.54)	10 (12.35)	0 (0.0)	0 (0.0)	1 (1.23)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	81 (100.00)
2021	1	5 (6.10)	62 (75.61)	14 (17.07)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.22)	82 (100.00)
2022	0	0 (0.0)	72 (84.71)	12 (14.12)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.18)	0 (0.0)	0 (0.0)	0 (0.0)	85 (100.00)
2023	0	0 (0.0)	77 (77.78)	19 (19.19)	2 (2.02)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.01)	0 (0.0)	0 (0.0)	99 (100.00)
Total	26	404 (19.41)	1,417 (66.98)	209 (10.04)	10 (0.48)	1 (0.05)	21 (1.01)	1 (0.05)	3 (0.14)	1 (0.05)	11 (0.53)	3 (0.14)	2,081 (100.00)

Source: data provided personally by the authors of the work

The predominant *Salmonella* serovars isolated in the Volyn region during 2014-2023 were as follows: *S.* Enteritidis accounted for the largest proportion, with a total of 1,821 isolates (87.51%); *S.* Typhimurium – 209 isolates (10.04%); and *S.* Muenchen – 21 isolates (1.01%). Other identified serovars – *S.* Virchow, *S.* Stanley, *S.* Derby, *S.* Newport, *S.* Postdam, *S.* Agona, and *S.* Java – each constituted less than 1% of the total isolates. Notably, *S.* Typhi and *S.* Paratyphi were not isolated in the Volyn region during the entire observation period. Data on all *Salmonella* serovars isolated from clinically ill patients and carriers in the Lviv region during 2014-2023 are presented in Table 3.

Table 3. Data on the number of outbreaks and Salmonella serovars that caused the outbreaks, and Salmonella serovars from clinically ill patients and carriers not associated with outbreaks in Lviv region (2014-2023)

Years	Number of outbreaks	Serovars involved in outbreaks	Serovars isolated from clinically ill patients and carriers not associated with outbreaks, n (%)														Total
		Enteritidis	Enteritidis	Typhimurium	Infantis	Haifa	Hadar	Tshiongwe	Kottbus	Montevideo	Coeln	Moscow	Java	Anatum	Typhi		
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		17
2014	14	35 (9.54)	273 (74.39)	43 (11.72)	8 (2.18)	0 (0.0)	1 (0.27)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.27)	1 (0.27)	3 (0.82)	0 (0.0)	2 (0.54)		367 (100.00)
2015	9	64 (18.39)	203 (58.33)	72 (20.69)	4 (1.15)	1 (0.29)	0 (0.0)	0 (0.0)	1 (0.29)	0 (0.0)	0 (0.0)	3 (0.86)	0 (0.0)	0 (0.0)	0 (0.0)		348 (100.00)
2016	11	78 (19.65)	210 (52.90)	80 (20.15)	16 (4.03)	1 (0.25)	0 (0.0)	1 (0.25)	0 (0.0)	1 (0.25)	8 (2.02)	0 (0.0)	1 (0.25)	1 (0.25)	0 (0.0)		397 (100.00)
2017	17	98 (28.49)	131 (38.08)	104 (30.23)	7 (2.03)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.29)	1 (0.29)	0 (0.0)	1 (0.29)	0 (0.0)	0 (0.0)	1 (0.29)		344 (100.00)

Table 3. Continued

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
2018	7	18 (78.26)	5 (21.71)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	23 (100.00)
2019	14	94 (83.19)	14 (12.50)	3 (2.68)	1 (0.89)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	112 (100.00)
2020	0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (100.00)
2021	1	16 (76.19)	5 (23.81)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	21 (100.00)
2022	2	20 (83.33)	4 (16.67)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	24 (100.00)
2023	3	18 (52.94)	15 (44.12)	0 (0.0)	1 (2.97)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	34 (100.00)
Total	78	441 (26.41)	860 (51.50)	302 (18.08)	37 (2.22)	2 (0.12)	1 (1.01)	1 (0.06)	2 (0.12)	2 (0.12)	9 (0.54)	5 (0.30)	4 (0.24)	1 (0.06)	3 (0.18)	2,081 (100.00)

Source: data provided personally by the authors of the work

In the Lviv region, the most prevalent *Salmonella* serovars identified during 2014-2023 were as follows: *S.* Enteritidis – 1,301 isolates (77.90%); *S.* Typhimurium – 302 isolates (18.08%); and *S.* Infantis – 37 isolates (2.22%). Other detected serovars – *S.* Haifa, *S.* Hadar, *S.* Tshiongwe, *S.* Kottbus, *S.* Montevideo, *S.* Coeln, *S.* Moscow, *S.* Java, and *S.* Anatum – each accounted for less than 1% of the total. Notably, three isolates (0.18%) of *S.* Typhi were identified in the Lviv region over the study period. During outbreaks of salmonellosis in the Volyn and Lviv regions between 2014 and 2023, several potential transmission factors were identified. These included food products, drinking water, surface washdowns, wastewater, and soil (Table 4).

Table 4. Distribution of Salmonella in food products and other research objects during outbreak investigations in Volyn region in 2014-2023 (n, %)

Years	Research object, number of positive cases detected (%)												
	Meat and meat products	Milk and milk products	Eggs and products thereof	Fish and fish products	Vegetables, fruits	Confectionery	Culinary products	Drinking water	Water from open bodies of water	Wastewater	Soil	Swab samples	Total
2014	3 (33.33)	0	1 (11.11)	0	0	3 (33.33)	0	0	0	0	0	2 (22.22)	9 (100.0)
2015	4 (33.33)	1.00 (8.33)	2 (16.67)	4 (33.33)	0	0	0	1 (8.33)	0	0	0	0	12 (100.0)
2016	2 (22.22)	0	0	1 (11.11)	0	2 (22.22)	4 (44.44)	0	0	0	0	0	9 (100.0)
2017	1 (10.00)	1 (10.00)	6 (60.00)	0	0	1,00 (10.00)	1 (10.00)	0	0	0	0	0	10 (100.0)
2010	1 (16.67)	0	2 (33.33)	1 (16.67)	0	1 (16.67)	1 (16.67)	0	0	0	0	0	6 (100.0)
2019	3 (25.00)	0	6 (50.00)	1 (8.33)	0	0	2 (16.67)	0	0	0	0	0	12 (100.0)
2020	1 (50.00	0	1 (50.00)	0	0	0	0	0	0	0	0	0	2 (100.0)
2020	0	1.00 (100.0)	0	0	0	0	0	0	0	0	0	0	1 (100.0)
2022	0	0	0	0	0	0	0	0	0	0	0	0	0.00
2023	0	0	0	0	0	0	0	0	0	0	0	0	0.00
Total	15 (24.59)	3 (4.92)	18 (29.51)	7 (11.48)	0	7 (11.48)	8 (13.11)	1 (1.64)	0	0	0	2 (3.28)	61 (100.0)

Source: data provided personally by the authors of the work

As shown in Table 4, in the Volyn region the largest proportion of *Salmonella*-positive samples was attributed to eggs and egg products, with 18 samples (29.51%) identified. This was followed by meat products with 15 positive samples (24.59%) and culinary products with 8 samples (13.11%). Additionally, fish and fish products, as well as confectionery, each accounted for 7 positive samples (11.48%). In total, 61 *Salmonella*-positive samples were detected in Volyn between 2014 and 2023 during the investigation of food products, water, and other objects associated with salmonellosis outbreaks. No positive samples were identified in the "vegetables and fruits" category.

A somewhat different pattern was observed in the Lviv region (Table 5). Over the same period, a total of 365 *Salmonella*-positive samples linked to outbreaks were identified – 5.98 times more than in Volyn. Notably, in Lviv, positive cases were recorded across all tested categories. The most frequently identified transmission vectors were culinary products (99 samples, 27.12%), meat products (64 samples, 17.53%), and confectionery products (31 samples, 8.49%). Other categories included milk and dairy products (22 samples, 6.03%), eggs (26 samples, 7.12%), fish (17 samples, 4.66%), vegetables and fruits (7 samples, 1.92%), and drinking water (8 samples, 2.19%).

Table 5. Distribution of *Salmonella* in food products and other research objects during outbreak investigations in Lviv region in 2014-2023 (n, %)

Years	Research object, number of positive cases detected (%)												
	Meat and meat products	Milk and milk products	Eggs and products thereof	Fish and fish products	Vegetables, fruits	Confectionery	Culinary products	Drinking water	Water from open water sources	Wastewater	Soil	Swab samples	Total
2014	14 (20.90)	4 (5.97)	6 (8.96)	3 (4.48)	2 (2.99)	7 (10.45)	15 (22.39)	1 (1.49)	1 (1.49)	5 (7.46)	0	9 (13.43)	67 (100.0)
2015	12 (18.18)	6 (9.09)	7 (10.61)	4 (6.06)	1 (1.52)	6 (9.09)	13 (19.70)	0	3 (4.55)	4 (6.06)	1 (1.52)	9 (13.64)	66 (100.0)
2016	10 (15.38)	5 (7.69)	5 (7.69)	1 (1.54)	1 (1.54).	9 (13.85)	16 (24.62)	2 (3.08)	2 (3.08)	6 (9.23)	0	8 (12.31)	65 (100.0)
2017	13 (15.85)	7 (8.54)	8 (9.76)	5 (6.10)	3 (3.66)	8 (9.76)	17 (20.73)	2 (2.44)	2 (2.44)	8 (9.76)	2 (2.44)	7 (8.54)	82 (100.0)
2010	7 (20.59)	0	0	3 (8.82)	0	0	10 (29.41)	0	0	0	0	14 (41.18)	34 (100.0)
2019	7 (17.95)	0	0	1 (2.56)	0	1 (2.56)	22 (56.41)	1 (2.56)	0	0	0	7 (17.95)	39 (100.0)
2020	0	0	0	0	0	0	0	0	0	0	0	0	0
2020	0	0	0	0	0	0	0	0	0	0	0	0	0
2022	0	0	**0**	0	0	0	0	0	3 (100.0)	0	0	0	3 (100.0)
2023	1 (11.11)	0	0	0	0	0	6 (66.67)	2 (22.22)	0	0	0	0	9 (100.0)
Total	64 (17.53)	22 (6.03)	26 (7.12)	17 (4.66)	7 (1.92)	31 (8.49)	99 (27.12)	8 (2.19)	11 (3.01)	23 (6.30)	3 (0.82)	54 (14.79)	365 (100.0)

Source: data provided personally by the authors of the work

Thus, the significantly higher incidence rates observed in the neighbouring Podkarpackie and Lublin Voivodeships, compared to the Volyn and Lviv regions, are more likely indicative of a higher level of diagnostic capacity and the consistent application of a unified methodological approach to data collection and reporting across all administrative levels in Poland. These findings highlight the need for strengthened cooperation between state diagnostic institutions within the cross-border region. Such collaboration would enable more effective management of salmonellosis incidence and its underlying causes, thereby enhancing the overall quality of epidemiological surveillance and public health response.

DISCUSSION

In the countries of the European Union, epidemiological surveillance of salmonellosis plays a vital role, as this infection remains one of the two leading causes of foodborne toxicoinfections in terms of incidence and confirmed outbreaks during the period 2018-2022 [20, 21]. EU member states utilise standardised tools for data exchange, collection, and risk assessment when recording outbreaks and identifying contamination in food products as a reservoir or source of transmission for various infectious diseases, including salmonellosis [22, 23]. Given Ukraine's orientation toward European Union membership, an attempt was made to compare salmonellosis incidence across four adjacent regions of Poland and Ukraine, as well as to compare national incidence rates with those of the EU.

It was found that the average annual incidence of salmonellosis during 2014-2023 was 1.5 times higher in Poland (22.74 per 100,000 population) compared to Ukraine (15.02 per 100,000) ($p = 0.0025$). A similar pattern was observed in neighbouring cross-border regions: the incidence in Lublin Voivodeship (27.62) was 1.5 times higher than in the Volyn region (18.02), while the incidence in Podkarpackie Voivodeship (37.13) was 2.8 times higher than in the Lviv region (13.18) ($p < 0.001$). Notably, the COVID-19 pandemic led to a clear division in the epidemic dynamics of salmonellosis, creating two distinct subperiods: 2014-2019 and 2020-2023. In both Ukraine and Poland, incidence rates were consistently lower during the second subperiod. Specifically, incidence in Ukraine decreased by 2.3 times, and in Poland by 1.2 times. In the Volyn and Lviv regions, incidence declined by 3.1 and 2.2 times, respectively. In contrast, incidence rates in Podkarpackie and Lublin Voivodeships did not show a significant decrease. These findings are consistent with the results of studies

by M. Milczarek *et al*. [7, 8], which reported a marked reduction – exceeding 69% – in salmonellosis incidence in Poland during 2020 and 2021 compared to 2019. Similarly, N. Polishchuk *et al*. [10], in their study of the Zaporizhia region, observed a significant decline in incidence during 2020-2023 compared to 2018-2019.

An analysis of the seroprofile of *Salmonella* strains isolated from patients and carriers revealed that all identified serovars – 1,670 in the Lviv region and 2,081 in the Volyn region – belonged to the species *Salmonella enterica* subsp. *enterica*. When comparing serovars detected during epidemiological investigations of salmonellosis outbreaks (78 outbreaks in Lviv region and 26 in Volyn region), it was found that in all cases, the sole etiological agent was *S.* Enteritidis. A comparable analysis conducted in the Zaporizhia region by N. Polishchuk *et al*. [10] during 2018-2022 found that the most frequently isolated serovars were *S.* Enteritidis (40%), *S.* Blegdam (28%), and *S.* Typhimurium (18%) ($p < 0.05$). According to M. Milczarek *et al*. [8], an analysis of the seroprofile of dominant *Salmonella* serovars in 2021 in the voivodeships showed that in the Lublin Voivodeship, the following serovars prevailed: *S.* Enteritidis – 325 isolates; *S.* Typhimurium – 12; *S.* Infantis – 3; and *S.* Virchow and *S.* Derby – 1 each. In comparison, in the neighbouring Volyn region that same year, the dominant serovars were: *S.* Enteritidis – 67 isolates; *S.* Typhimurium – 14; and *S.* Java – 1. In the Podkarpackie Voivodeship in 2021, the following serovars were isolated: *S.* Enteritidis – 726; *S.* Typhimurium – 45; *S.* Infantis – 4; *S.* Virchow – 27; *S.* Schleissheim – 2; *S.* Virginia – 3; *S.* Agona – 2; and *S.* Kottbus and *S.* Hadar – 1 each. In the adjacent Lviv region, 21 *S.* Enteritidis isolates were recorded during the same year.

From the perspective of the evolutionary dynamics of *Salmonella* pathogenicity, the data confirm the continued dominance of specific serovars – primarily *S.* Enteritidis and *S.* Typhimurium – across the cross-border regions of Ukraine, central regions such as Zaporizhia, and in the studied areas of Poland, a trend that has persisted since the early 2000s [10, 24]. This observation aligns with the findings of M. Canning *et al*. [25] and H. Sun *et al*. [26]. With regard to reservoirs and transmission factors of *Salmonella* in the Volyn region, the largest proportion was attributed to eggs and egg products, followed by meat products, then culinary products, and subsequently fish and fish products alongside confectionery. In the Lviv region, the primary transmission sources over the study period were culinary products, meat products, and confectionery. *Salmonella* was isolated less frequently from eggs, milk, and dairy products. This pattern is consistent with the findings of P. Polański *et al*. [27], who reported that between 2018 and 2020 in Poland, the main categories of food products responsible for foodborne salmonellosis were eggs and egg-based products (36.01%), confectionery (18.6%), and meat and meat products (9.3%).

In previous studies conducted by the authors, an analysis was performed using official reporting data from laboratories affiliated with the State Sanitary and Epidemiological Service of Ukraine for the period 2012-2023 [5]. A significant volume of data was collected concerning the contamination of food products with *Salmonella*, as well as the serovars responsible. It was found that meat and meat products were the most frequently contaminated, accounting for 78.13% of all cases, followed by eggs and egg-based products (11.76%), dairy products (3.32%), and fish products (2.71%) ($p < 0.05$).

It is important to note certain structural differences in the vertical organisation of salmonellosis monitoring systems in Poland and Ukraine. In Poland, the State Sanitary and Epidemiological Service is subordinate to the Ministry of Health and operates regional departments in each voivodeship. Laboratory testing is conducted by the following accredited institutions: Regional branches of the National Research Institute "National Institute of Public Health – National Institute of Hygiene" (NIZP-PZH); The State Sanitary Inspectorate; The Sanitary Inspectorate of the Ministry of Internal Affairs and Administration; The Military Sanitary Inspectorate; as well as other accredited laboratories under agreements with these bodies [28]. This structure has been in continuous operation since 1985 [29]. The assessment of the epidemiological situation in Poland is based on data submitted by the aforementioned laboratories to the Department of Epidemiology of Infectious Diseases and monitored by sanitary-epidemiological stations. This data is processed using the System of Epidemiological Registration of Cases (SRWE) and the Register of Epidemic Outbreaks (ROE), and subsequently summarised in publicly available annual reports published online [16].

In Ukraine, a similar vertically integrated system existed from 1994 to 2017. However, following a governmental reform in 2017, the Cabinet of Ministers of Ukraine dissolved the State Sanitary and Epidemiological Service. Its responsibilities were redistributed among three entities: The State Service of Ukraine on Food Safety and Consumer Protection (subordinate to the Cabinet of Ministers since 2015 and to the Ministry of Agrarian Policy and Food since 2021); The Ministry of Health of Ukraine; The Ministry of Social Policy of Ukraine [30, 31]. Each of these bodies maintains its own network of authorised and accredited laboratories. However, unlike in Poland, Ukraine lacks a centralised institution for unified data collection and processing, such as Poland's National Institute of Public Health – National Institute of Hygiene (NIZP-PZH). Consequently, the present study relied solely on data collected by the regional Central Laboratory of the Ministry of Health and those gathered directly by the authors.

CONCLUSIONS

The intensity of the epidemic situation concerning salmonellosis in EU countries – particularly Poland – and in Ukraine, as measured by confirmed cases and incidence rates, has been characterised by overall stability and a downward trend over the period 2014-2023. At the regional level, the highest average annual incidence rates were recorded in the Podkarpackie (37.13 per 100,000) and Lublin (27.62) Voivodeships, while significantly lower rates were observed in the Volyn (18.02) and Lviv (13.18) regions. During and following the COVID-19 pandemic (2020-2023), incidence rates declined across all regions compared to the pre-pandemic period (2014-2019): by 1.2 times in Poland and by 2.3 times in Ukraine ($p = 0.0025$). Regionally, incidence decreased by 3.2 times in Volyn, 2.1 times in Lviv, 1.4 times in Lublin, and only slightly in Podkarpackie by 0.97 times ($p < 0.001$).

The dominant serovar in all outbreak cases and isolated salmonellosis cases in both the Volyn and Lviv regions was *Salmonella enterica* subsp. *enterica* Serovar Enteritidis, accounting for 87.51 and 77.90% of isolates, respectively. The Serovar *S.* Typhimurium was detected 8.7 and 4.2 times less frequently, comprising 10.04% of isolates in Volyn and 18.08% in Lviv. During the observation period, three isolates (0.18%) of *S.* Typhi were identified in the Lviv region, while no typhoid group serovars were detected in the Volyn region. The most common sources of *Salmonella* transmission in the Volyn region included eggs and egg products (29.51% of *Salmonella*-positive items), followed by meat and meat products (27.40%), culinary products (12.3%), and confectionery products (11.0%). In the Lviv region, the most frequently identified sources were culinary products (27.12%), meat products (17.53%), swab samples (14.79%), confectionery products (8.49%), and eggs (7.12%).

In Ukraine, research into salmonellosis is conducted by accredited laboratories under the Ministry of Health. However, a significant portion of food safety monitoring, particularly related to outbreaks, is carried out by authorised laboratories of the State Service for Food Safety and Consumer Protection, which is subordinate to a different governmental agency. The absence of unified systems or tools that facilitate the integration and comparison of data across agencies imposes limitations on the ability to utilise comprehensive datasets for analysis. A key prospect for further research involves the collection and processing of larger, unified datasets on salmonellosis in Ukraine. This would enable the development of effective risk assessment tools for the disease and enhance the availability of epidemiological data for the global scientific community.

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CONFLICT OF INTEREST

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REFERENCES

[1] Lee H, Yoon Y. Etiological agents implicated in foodborne illness world wide. Food Sci Anim Resour. 2021;41(1):1–7. DOI: 10.5851/kosfa.2020.e75
[2] Ehuwa O, Jaiswal AK, Jaiswal S. *Salmonella*, food safety and food handling practices. Foods. 2021;10(5):907. DOI: 10.3390/foods10050907
[3] Criscuolo A, Issenhuth-Jeanjean S, Didelot X, Thorell K, Hale J, Parkhill J, et al. The speciation and hybridization history of the genus *Salmonella*. Microb Genom. 2019;5(8):284. DOI: 10.1099/mgen.0.000284
[4] Konishi N, Obata H, Yokoyama K, Sadamasu K, Kai A. Comparison of the serovars and characteristics of *Salmonella* isolated from human feces and foods in the 1990s and 2010s in Tokyo. Jpn J Infect Dis. 2023;76(1):14–9. DOI: 10.7883/yoken.JJID.2022.174
[5] Boiko O, Garkavenko T, Musiiets I, Nedosekov V, Kozytska T. Salmonellosis in Ukraine: An analysis of food products contamination, *Salmonella* transmission, and serovar diversity during 2012-2023. Ger J Vet Res. 2024;4(2):65–74. DOI: 10.51585/gjvr.2024.2.0085
[6] Milczarek M, Sadkowska-Todys M, Czarkowski MP. Salmonellosis in Poland in 2018 and 2019. Epidemiol Rev. 2021;75(4):665–8. DOI: 10.32394/pe.75.62
[7] Milczarek M, Czarkowski MP, Sadkowska-Todys M. Salmonellosis in Poland in 2020. Epidemiol Rev. 2022;76(4):574–90. DOI: 10.32394/pe.76.53
[8] Milczarek M, Czarkowski MP, Sadkowska-Todys M. Salmonellosis in Poland in 2021. Epidemiol Rev. 2023;77(4):504–19. DOI: 10.32394/pe.77.41
[9] Zublenko O, Petrusevych T. Incidence of intestinal infections in Ukraine. In: Materials of the scientific and practical conference with international participation "Ecological and hygienic problems of the sphere of human life". Kyiv: Bogomolets National Medical University; 2021.
[10] Polishchuck N, Kolycheva N, Dehen A, Melnikova O, Kryazheva N. Epidemical process and aetiological structure of salmonelloses in the Zaporizhzhia region. Curr Issues Pharm Med Sci Pract. 2023;16(3):260–4. DOI: 10.14739/2409-2932.2023.3.285476
[11] Baharev E. The ecological structure of the zoogen tanks of *Salmonella* and their epidemic potential on the territory of the north-west Black Sea. Young Scientist. 2019;10(74):372–80. DOI: 10.32839/2304-5809/2019-10-74-81
[12] Provincial Sanitary and Epidemiological Station in Lublin. Assessment of the sanitary condition of the voivodeship [Internet]. [cited 2024 October 19]. Available from: https://www.gov.pl/web/wsse-lublin/ocena-stanu-sanitarnego-wojewodztwa
[13] Voivodship Sanitary and Epidemiological Station in Rzeszów. Sanitary and Hygienic Status of Podkarpackie Voivodeship [Internet]. [cited 2024 October 19]. Available from: https://www.gov.pl/web/wsse-rzeszow/stan-sanitarno---higieniczny-wojewodztwa-podkarpackiego
[14] Surveillance Atlas of Infectious Diseases [Internet]. [cited 2024 October 19]. Available from: https://atlas.ecdc.europa.eu/public/index.aspx
[15] Order of the Ministry of Health of Ukraine No. 132. On Approval of Sectoral Statistical Reporting Form N 40-health [Internet]. 2001 April 4 [cited 2024 October 19]. Available from: https://ips.ligazakon.net/document/MOZ1370

[16] Annual report “Infectious diseases and poisonings in Poland” [Internet]. 2023 [cited 2024 October 19]. Available from: https://wwwold.pzh.gov.pl/oldpage/epimeld/index_a.html
[17] Order of the Ministry of Health of Ukraine No. 190. On Improving the System of Operational Information of the Ministry of Health of Ukraine [Internet]. 2011 April 5 [cited 2024 October 19]. Available from: https://zakon.rada.gov.ua/rada/show/v0190282-11#Text
[18] Law of Ukraine No. 1645-III. On Protecting the Population from Infectious Diseases [Internet]. 2000 April 6 [cited 2024 October 19]. Available from: https://zakon.rada.gov.ua/laws/show/1645-14#Text
[19] Order of the Ministry of Health of Ukraine No. 425. On Approval of the Methodological Recommendations “Methods of *Salmonella* Isolation and Identification” [Internet]. 2013 May 24 [cited 2024 October 19]. Available from: https://zakononline.com.ua/documents/show/53036__53036
[20] European Food Safety Authority (EFSA), European Centre for Disease Prevention and Control (ECDC). The European Union one health 2022 zoonoses report. EFSA J. 2023;21(12):e8442. DOI: 10.2903/j.efsa.2023.8442
[21] Mkangara M. Prevention and control of human *Salmonella enterica* infections: An implication in food safety. Int J Food Sci. 2023;2023(1):8899596. DOI: 10.1155/2023/8899596
[22] Kasza G, Izsó T, Langsrud S, Vrbos D, Veflen N, Ueland Ø, et al. Institutional food safety risk communication – a self-evaluation tool and its interpretation. Trends Food Sci Technol. 2024;150:104594. DOI: 10.1016/j.tifs.2024.104594
[23] Teklemariam AD, Al-Hindi RR, Albiheyri RS, Alharbi MG, Alghamdi MA, Filimban AAR, et al. Human Salmonellosis: A continuous global threat in the farm-to-fork food safety continuum. Foods. 2023;12(9):1756. DOI: 10.3390/foods12091756
[24] European Food Safety Authority, European Centre for Disease Prevention and Control. The European Union one health 2019 zoonoses report. EFSA J. 2021;19(2):e06406. DOI: 10.2903/j.efsa.2021.6406
[25] Canning M, Birhane MG, Dewey-Mattia D, Lawinger H, Cote A, Gieraltowski L, et al. *Salmonella* outbreaks linked to beef, United States, 2012-2019. J Food Prot. 2023;86(5):100071. DOI: 10.1016/j.jfp.2023.100071
[26] Sun H, Wan Y, Du P, Bai L. The epidemiology of monophasic *Salmonella* Typhimurium. Foodborne Pathog Dis. 2020;17(2):87–97. DOI: 10.1089/fpd.2019.2676
[27] Polański P, Sadkowska-Todys M, Księżak E, Nowacka Z. Foodborne infections and intoxications in Poland in the years 2018-2020. Epidemiol Rev. 2022;76(4):626–40. DOI: 10.32394/pe.76.57
[28] Announcement by the Speaker of the Sejm of the Republic of Poland Dz. U. 2022 Item 1657. On the Announcement of the Uniform Text of the Act on the Prevention and Combating of Infections and Infectious Diseases in Humans [Internet]. 2022 June 6 [cited 2024 October 19]. Available from: https://isap.sejm.gov.pl/isap.nsf/download.xsp/WDU20220001657/T/D20221657L.pdf
[29] Act of the Sejm of the Republic of Poland Dz. U. 1985 No. 12 Item 49. On the State Sanitary Inspectorate. 1985 March 14 [cited 2024 October 19]. Available from: https://isap.sejm.gov.pl/isap.nsf/download.xsp/WDU19850120049/U/D19850049Lj.pdf
[30] Resolution of the Cabinet of Ministers of Ukraine No. 124. Some Issues of Central Executive Authorities. 2021 February 17 [cited 2024 October 19]. Available from: https://www.kmu.gov.ua/npas/deyaki-pitannya-diyalnosti-centralnih-organiv-vikonavchoyi-vladi-i170221-124
[31] Resolution of the Cabinet of Ministers of Ukraine No. 667. On Approval of the Regulation on the State Service of Ukraine on Food Safety and Consumer Protection. 2015 September 2 [cited 2024 October 19]. Available from: https://zakon.rada.gov.ua/laws/show/667-2015-%D0%BF#Text

Епідемічний процес сальмонельозу у транскордонних областях України та Польщі (2014-2023 рр.)

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Анотація. Епідеміологія сальмонельозу, незважаючи на тривалу історію вивчення, як один із аспектів забезпечення здоров'я, залишається актуальною. Метою досліджень було порівняти захворюваність сальмонельозу Польщі та України на національному рівні та на рівні деяких суміжних транскордонних областей обох країн протягом 2014-2023 рр. Середньорічні показники захворюваності людей на сальмонельоз за 10-річний період становили в Україні 15,02/100 тис., кількість підтверджених випадків сальмонельозу – 64108; у Польщі ці показники становили – 22,74/100 тис. та 86956 випадків відповідно ($p = 0,0025$). Кількість підтверджених випадків та захворюваність (на 100 тис.) у транскордонних областях становили: Волинська – 1879/18,02; Львівська – 3305/13,18; Люблінське воєводство – 6067/27,62; Підкарпатське – 7869/37,13 ($p < 0.001$). Вивчено тенденції поширеності та серологічний профіль сальмонел, ідентифікованих у людей і в харчових продуктах та інших об'єктах. Домінуючими серотипами під час спалахів та ідентифікованих випадках сальмонельозу та сальмонелоносійства у Волинській та у Львівській областях був вид *S. enterica* subsp. *enterica* var. Enteritidis (87,51 % та 77,90 % відповідно). Серовар *S.* Typhimurium, як етіологічний фактор, було ідентифіковано на Волині у 10,04 % випадках, на Львівщині – у 18,08 %. Встановлено, що найчастіше факторами передачі сальмонел у Волинській області були: яйця та яєчні продукти, які становили 29,51 % з усіх категорій сальмонела-позитивних об'єктів, м'ясо та м'ясні продукти – 27,40 %, кулінарні – 12,3 %, та кондитерські вироби – 11,0 %. У Львівській області найчастіше контамінованими виявлялися такі категорії: кулінарні вироби – 27,12 %; м'ясні продути – 17,53 %; кондитерські вироби – 8,49 %; яйця та яєчні продукти – 7,12 %. Результати цього дослідження можуть бути використані як основа доказової епідеміології, а також для реалізації спільних кроків щодо підвищення ефективності епідеміологічного нагляду і контролю за напруженістю епідемічної ситуації щодо сальмонельозу у транскордонних регіонах та в цілому у кожній країні

Ключові слова: серовари сальмонели; захворюваність; епідеміологічний аналіз; харчові продукти

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Use of mesenchymal stromal cells in the therapy of musculoskeletal disorders: A literature review

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Abstract. The rapid advancement of methods for obtaining multipotent progenitor cells, known as mesenchymal stromal/stem cells, from various human tissues and organs has driven progress in cellular therapy and regenerative medicine. This study aimed to review current scientific data on the use of mesenchymal stromal cells in the treatment of traumatic and orthopaedic disorders, providing clinicians with insights into the challenges and prospects of their clinical application. The sources of mesenchymal stromal cells, their characteristics, and their therapeutic effects on musculoskeletal disorders were analysed. These cells have been found to be applicable in both autologous and allogeneic forms due to their ability to differentiate into osteoblasts, chondrocytes, tenocytes, adipocytes, and other cell types, thereby promoting the regeneration of damaged tissues. Furthermore, mesenchymal stromal cells have been confirmed to exhibit paracrine activity by producing cytokines and growth factors, which contribute to their regenerative and regulatory effects both *in vitro* and *in vivo*. At the same time, it has been noted that despite their significant therapeutic potential, the clinical application of mesenchymal stromal cells is associated with several challenges, including immunocompatibility, stability, heterogeneity, and limited differentiation and migration capacities. The prospects for overcoming these limitations through cellfree approaches have been considered, particularly the use of exosomes secreted by mesenchymal stromal cells, which contain biologically active molecules such as mRNA, microRNA, proteins, and bioactive lipids. These components have been shown to promote cell proliferation, migration, regeneration, immunomodulation, and angiogenesis, making them a promising avenue in regenerative medicine. The findings of this study may contribute to the further development of effective therapeutic strategies in regenerative medicine, particularly in orthopaedics and traumatology. The practical significance of this research lies in the refinement of cellular therapy approaches aimed at restoring damaged tissues, which may serve as a foundation for future clinical trials and subsequent implementation in medical practice

Keywords: stem cells; exosomes; tissue regeneration; traumatology; orthopaedics

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INTRODUCTION

Mesenchymal stromal cells (MSCs) represent a promising avenue in regenerative medicine due to their unique biological properties. Current research highlights their pivotal role in regenerative processes, particularly in orthopaedics and traumatology. Damage to bone, cartilage, tendons, and ligaments is a common issue, especially among patients with degenerative musculoskeletal conditions and sports injuries. Existing treatment methods, including surgical interventions and pharmacological therapy, do not always provide full functional recovery of damaged tissues, driving a growing interest in cell-based technologies as an effective alternative.

Scientists are actively exploring the potential of MSCs as a source of regenerative therapy. A bibliometric analysis by Z. Deng *et al*. [1] demonstrated a significant increase in publications regarding their application in orthopaedics, specifically in the areas of osteogenic differentiation, cartilage regeneration, osteoarthritis treatment, and the use of biomaterials in combination with MSCs. MSCs can be obtained from various sources, including bone marrow, adipose tissue, synovial membrane, umbilical cord blood, dental pulp, and others. V.V. Maldonado *et al*. [2] note that each source has its own characteristics, which influence the cells' ability to differentiate and proliferate. For example, MSCs from adipose tissue are characterised by high availability and osteogenic potential.

MSCs are distinguished by their capacity for self-replication, self-division, and multipotent differentiation, forming various cell types: adipocytes, osteoblasts, chondrocytes, and myocytes. Scientists M. Sandonà *et al*. [3] have established that their migratory activity and interaction with the extracellular matrix contribute to tissue regeneration. According to reports by Y. Wang *et al*. [4], their proliferation and differentiation in *in vitro* conditions require biological signals formed through interactions with the extracellular matrix, cytokines, and growth factors.

J. Tang *et al*. [5] researched the role of exosomes secreted by MSCs in bone tissue regeneration processes. It was discovered that these extracellular vesicles contain growth factors and microRNAs, which stimulate osteogenesis and reduce inflammation in the affected area, opening up prospects for cell-free therapy. Despite their significant potential, the biological properties of MSCs depend on cultivation conditions, which can affect their clinical effectiveness. L.A. Costa *et al*. [6] emphasised the importance of the microenvironment for the functional activity of transplanted cells, highlighting the need to optimise methods for cultivating and administering MSCs.

MSCs are widely applied in the therapy of various diseases, including orthopaedic pathologies. Scientists Y. Zhang *et al*. [7] demonstrated that the use of MSCs accelerates the fusion of complex long bone fractures, stimulates osteogenesis, and improves the strength of newly formed bone tissue. Similar results were obtained by R. Ossendorff *et al*. [8], who proved that MSC injections significantly reduce bone tissue regeneration times, especially in cases of complicated healing.

In addition to fracture treatment, an important area of MSC application is the therapy of sports injuries. K.-I. Kim *et al*. [9] showed the effectiveness of their administration in meniscus and ligament ruptures, which promotes faster restoration of joint functional activity. Analogous results were obtained by J. Chen *et al*. [10] demonstrated that MSCs significantly improve tendon and ligament healing in professional athletes, reducing the risk of recurrence. Scientists O. Grabovyi *et al*. [11] established that the natural activation of MSCs in response to injuries contributes to their differentiation into fibroblasts, which form a connective scar tissue.

Particular attention is drawn to the potential of MSCs in cartilage tissue regeneration. Y. Jiang & R.S. Tuan [12] note that MSCs and their extracellular matrices are capable of activating chondrogenesis, promoting the repair of damaged cartilage in osteoarthritis and other degenerative joint diseases. The research by T. Frazier *et al*. [13], which focuses on the clinical application of MSCs in osteoarthritis treatment, is also of significant interest. The authors analysed the effectiveness of MSC transplantation in patients with knee joint pathology and found that cell therapy contributes to pain reduction, improved mobility, and slowed degenerative changes.

Thus, current research confirms the significant potential of mesenchymal stromal cells in the treatment of injuries and degenerative diseases of the musculoskeletal system. However, despite numerous studies, the use of MSCs in the therapy of orthopaedic diseases remains controversial. Achieving positive results requires consideration of several factors, including potential side effects. Therefore, it is important to systematise data on the positive and negative aspects of MSCs' impact on the treatment of musculoskeletal pathologies, predict development trends, and systematise literary information of the scientific base for further research in this field. This study aimed to analyse current scientific data on the use of MSCs in the treatment of orthopaedic and traumatological diseases in terms of their ability to differentiate and produce regulatory factors, as well as to highlight the challenges and prospects of their clinical use.

To achieve the stated aim, a systematic review of literature available in the MEDLINE/PubMed, Google Scholar, Scopus, and Web of Science databases, covering the period 2001-2024, was conducted; trends in publications for 2015-2024 were analysed separately. Review articles and described clinical cases related to the application of MSCs in traumatology and orthopaedics were considered. The study employed review-analytical and bibliosemantic methods to determine the current state of research on the problem. The literature search utilised various combinations of key terms, including "mesenchymal stem cells", "mesenchymal stromal cells", "*in vitro*", "*in vivo*", "differentiation", "regenerative medicine", "sports medicine", "clinical application", "treatment", "bone defect", "osteonecrosis", "fracture healing", "nonunion", "osteochondral defect", "cartilage repair", "cartilage defect", "osteoarthritis", "osteogenic differentiation", "knee", and "meniscus". All data were obtained from open sources; therefore, ethical approval or informed consent was not required.

INFORMATION SOURCES AND SEARCH STRATEGY

As shown in Figure 1, the database search in PubMed, Google Scholar, Web of Science, and Scopus yielded 1,057

literary sources in Ukrainian and English, which included studies utilising MSCs. After excluding duplicate articles based on titles and abstracts, 295 publications were selected, covering the use of MSCs in reconstructive and regenerative medicine. Following a review of the research results, examination of their full content with additional manual searching of references, and exclusion of articles unrelated to musculoskeletal disorders, 76 publications were selected that met the aim and criteria of the query. Approximately 65% of the studies focused on the treatment of knee joint diseases, predominantly osteoarthritis; about 30% concerned adipose tissue MSCs.

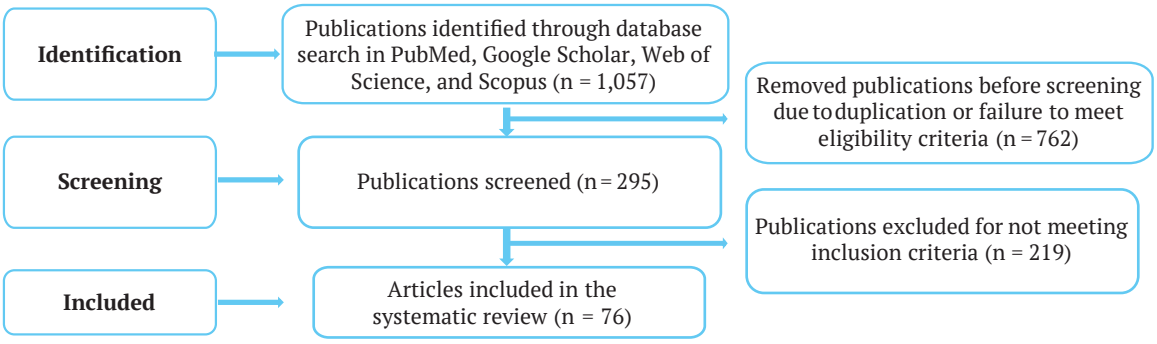

Figure 1. Flowchart of publication selection for the systematic review
Source: compiled by the authors

Scientific publications in this field are predominantly focused on fundamental research and clinical trials. As depicted in Figure 2, the analysis of the annual distribution of publications demonstrates a gradual increase in scientific activity within the field of orthopaedics from 2015 to 2024, except for a decrease in the number of studies in 2020. The authors of this article suggest that this reduction is likely related to the COVID-19 pandemic, during which an overall decline in scientific productivity was observed. The maximum number of publications (131) was recorded in 2019, while the minimum (77) occurred in 2015. The annual publication of over 80 articles confirms a sustained interest among researchers in the use of MSCs for treating orthopaedic diseases, fostering a robust research environment in this area. From the analysis of keyword cooccurrence across different topics in scientific publications (Fig. 3), it is evident that the primary research directions in traumatology include sources of stem cells, their differentiation into bone and cartilage tissue, exosomes, and regenerative medicine.

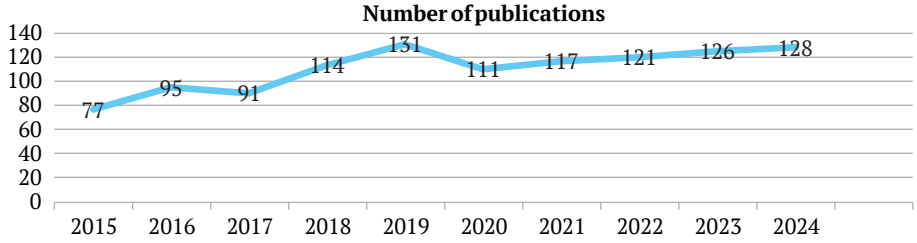

Figure 2. Dynamics of annual publication count and trends in MSC use for musculoskeletal regenerative medicine, 2015-2024
Source: compiled by the authors

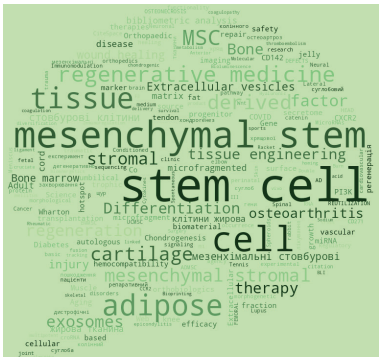

Figure 3. Cluster analysis of keywords
Source: compiled by the authors

Thus, the conducted literature analysis demonstrates a sustained interest within the scientific community in the application of MSCs in orthopaedic practice, particularly in the treatment of osteoarthritis and cartilage tissue regeneration. The identified trends confirm the increasing number of studies in this field, indicating the promising nature of MSC use in reconstructive medicine. Optimising MSC transplantation methods, studying their biological mechanisms, and conducting long-term evaluations of their clinical efficacy will contribute to the advancement of approaches for regenerating damaged tissues and the implementation of new therapeutic strategies.

SOURCES AND THERAPEUTIC POTENTIAL OF MSCS

The latest advancements in regenerative medicine have created new opportunities for the development of orthopaedics and traumatology, potentially transforming clinical practice approaches. Conditions that, as of 2025, are predominantly treated with surgical methods may in the future be effectively managed with less invasive approaches, such as MSC therapy. Research by C. Brown *et al*. [14] and L. da Silva Meirelles *et al*. [15] confirms that MSCs are capable of differentiating into cells of bone, cartilage, tendon, ligament, muscle, and other tissues. According to the classification by the International Society for Cellular Therapy, terms such as "mesenchymal stromal cells" are used for cells in the body (*in vivo*), and "multipotent stromal cells" are used for cells in culture (*in vitro*). The International Society for Cellular Therapy has defined basic criteria for MSC identification [16], including adherence to plastic under standard culture conditions; the presence of specific surface antigens CD73, CD90, CD105, and the absence of CD34, CD45, CD14/11b, CD79α/19, HLA-DR; and the ability to differentiate into osteoblasts, chondroblasts, and adipocytes *in vitro*.

The differentiation process is a crucial step in the transformation of MSCs into osteocytes and chondrocytes. This process is regulated by numerous signalling pathways, including BMP/Smad, Wnt/β-catenin, PI3K/AKT, MAPK, TGF-β, Notch, NF-kB, and others, which play a key role in regulating this process. For example, Q. Zhou *et al*. [17] demonstrated that the activation of BMP7/Smad5 promotes the selective differentiation of MSCs into chondrocytes. Furthermore, H.D. Fu *et al*. [18] established that BMP-7 can also accelerate the differentiation of MSCs into cartilage cells through the activation of the Wnt/β-catenin pathway. The PI3K/Akt pathway also plays an important role in bone regeneration: A. Yang *et al*. [19] showed that its activation involving IL-8 contributes to enhancing the therapeutic effect of MSCs in bone tissue repair. Research by U. Kozlowska *et al*. [20] indicated that MSCs obtained from different tissues share common characteristics, but their biological activity and markers vary depending on the source. It was found that prolonged cultivation affects the biological activity of MSCs; in particular, it can reduce their proliferative potential and alter the expression of certain markers. It has also been proven that bone marrow MSCs have the best capacity for multilineage differentiation. At the same time, adipose MSCs can serve as an alternative source due to their similar properties to bone marrow stromal cells.

Different sources of origin and differentiation ability allow MSCs to be classified into unipotent, multipotent, totipotent, and pluripotent cells, depending on their differentiation potential. Research by A. Vaish *et al*. [21] has shown that induced pluripotent MSCs, obtained by reprogramming somatic cells, retain the ability for multilineage differentiation, similar to embryonic stem cells, but have lower immunogenicity, which reduces the risk of rejection during transplantation. In another study by A. Vaish & R. Vaishya [22], the authors examined the molecular mechanisms that ensure MSC pluripotency. It was demonstrated that the transcription factors Sox2, Oct4, and Nanog play a key role in maintaining stem cell status and regulating the expression of genes associated with cell proliferation and self-renewal. The analysis also showed that the surrounding microenvironment (e.g., signals from growth factors and the extracellular matrix) can modulate the differentiation potential of MSCs, directing them towards a specific cellular phenotype.

Until 2008, bone marrow was the primary source of MSCs. However, as noted by S. Konovalov *et al*. [23, 24] and M. Tronko *et al*. [25], in recent years, cells obtained from perinatal and adipose tissues have been increasingly used. They demonstrate a high safety and haemocompatibility profile, allowing for their use in systemic infusion. Similar conclusions were drawn by G. Moll *et al*. [26, 27], who emphasise the need to improve the minimum safety criteria for MSCs. They analysed the intravascular application of MSCs and highlighted the importance of assessing tissue factors and haemocompatibility to enhance clinical efficacy. Adipose tissue-derived MSCs provide a higher cell yield and greater longevity compared to bone marrow stem cells, although they are less capable of differentiating into bone and cartilage tissue. Despite this, their effectiveness in treating osteoarthritis has been confirmed by long-term clinical results. As noted by U. Kozlowska *et al*. [20], in addition to multipotent differentiation, the paracrine effect of MSCs – the secretion of growth factors (BMP, TGF-β, VEGF, etc.) that promote angiogenesis, proliferation, and cell regeneration – plays a key role in tissue regeneration. Similar data are provided by H.H. Maniar *et al*. [28], highlighting the significant potential of stem cells in orthopaedic practice.

As of 2025, genetically modified MSCs, capable of releasing growth factors over extended periods, are being actively developed. In particular, research by G.E. Salazar-Noratto *et al*. [29] explores approaches to enhance the survival of transplanted MSCs in tissue engineering and regenerative medicine. However, the majority of transplanted stromal cells accumulate in internal organs (liver, spleen, lungs), which limits their effectiveness and indicates the need for the development of cell-free therapeutic approaches. As an alternative, the use of secretomes – bioactive substances produced by MSCs, including exosomes and microvesicles – is proposed. This approach has been actively researched by scientists M.D. Hade *et al*. [30] and M. Maqsood *et al*. [31]. They note that secretomes contain a wide range of growth factors, cytokines, and exosomes, which promote tissue regeneration and may serve as a promising therapeutic agent in regenerative medicine.

Exosomes are nanoscale vesicles (30-100 nm) with a lipid bilayer membrane, typically produced by MSCs and present in various biological fluids. M.H. Gerami *et al*. [32]

examine the role of MSCs and their exosomes in the treatment of musculoskeletal disorders, particularly bone and joint pathologies, and emphasise their significant potential for orthopaedic applications. Recent studies have shown that exosomes contain various biologically active molecules, such as mRNA, microRNA, proteins, and bioactive lipids [33, 34]. C. Porcu *et al*. [34] analysed their involvement in skeletal muscle regeneration, opening new perspectives for the therapy of musculoskeletal pathologies. It is known that exosomes are involved in the regulation of many key biological processes, including cell proliferation, migration, enhancement of regenerative properties, inhibition of apoptosis, immunomodulation, and stimulation of angiogenesis. D.H. Hoang *et al*. [33] confirm their effectiveness in improving tissue healing and repair processes. Current research focuses on studying the molecular mechanisms that influence stem cell differentiation, paracrine signalling, immunomodulatory properties, and tissue remodelling processes in the orthopaedic microenvironment. L.L. Bagno *et al*. [35] analysed the impact of exosome delivery methods on their biological activity and therapy effectiveness. In 2025, clinical trials are ongoing to assess the safety and efficacy of stem cell use in the treatment of orthopaedic diseases.

THE ROLE OF MSCS IN BONE DEFECT REGENERATION

Nonunion of long tubular bones remains a complex challenge in achieving effective bone osteogenesis [36]. MSC therapy may represent an innovative approach to treating such lesions. Z. Zhou *et al*. [37] focus on improving methods for utilising stem cells, growth factors, and integrating MSCs with biomaterials, which contribute to accelerating bone tissue regeneration. Y. Jiang & R.S. Tuan [12] were among the first to investigate the impact of MSCs in the field of orthopaedics. They established that cells isolated from adult human bone fragments are capable of differentiating into various cell types of mesenchymal origin *in vitro*. Similar results were obtained by R. Berebichez-Fridman & P.R. Montero-Olvera [38], noting that MSCs obtained from damaged muscle tissues have similar phenotypes to bone marrow MSCs and exhibit the ability to transform into osteoblasts, chondrocytes, and adipocytes. Furthermore, researchers R. BerebichezFridman *et al*. [39] found that bone morphogenetic protein (BMP-2) stimulates DNA synthesis, activates replication, and promotes the directed differentiation of MSCs into osteoblasts.

Most existing clinical studies focused on bone tissue regeneration involving stem cells are cohort studies. According to reports by A.M. Theodosaki *et al*. [40], the use of autologous, genetically unmodified MSCs *in vivo* for bone tissue regeneration demonstrates the safety and therapeutic potential of this treatment. E. Lucarelli *et al*. [41] were among the first to investigate the possibility of using autologous bone marrow stromal cells, cultured *ex vivo*, for the reconstruction of large bone defects. The authors demonstrated that the application of such cells in combination with biomaterials promotes the formation of new bone tissue and successful defect repair, which is confirmed by radiological and clinical observations. In a study by Y. Liu *et al*. [42], it was established that the transplantation of MSCs along with their secreted extracellular matrix ensures robust bone tissue formation both *in vitro* and *in vivo*. This occurs through endochondral ossification under the influence of chondrogenic and osteogenic signals.

Combining MSCs with scaffolds or growth factors significantly enhances the healing of traumatic bone defects and nonunion fractures. The study by D. Dufrane [43] demonstrated the effectiveness of using 3D-printed grafts made from autologous adipose tissue stem cells to repair significant long bone defects. In a systematic review conducted by A.M. Theodosaki *et al*. [40], out of 10,091 retrieved clinical studies, 14 of which met the inclusion criteria, 138 patients underwent treatment with MSCs cultured on scaffolds. In all cases, bone tissue regeneration was observed, with better results than standard treatments. For the treatment of avascular necrosis of the femoral head, stem cell-enriched bone marrow aspirate concentrate is widely used. Its effectiveness is confirmed in the research by N. Pawar *et al*. [44].

The studies described above confirm the safety of using autologous MSCs for bone tissue repair in segmental long bone defects. None of the studies reviewed in this section reported complications such as inflammatory processes or excessive tissue growth. However, according to G.-I. Im [45] and B. Lukomska *et al*. [46], extensive cultivation of stromal tissues (4-5 months) can lead to genomic instability, which poses a risk of malignant transformation. On the other hand, a shorter period of *in vitro* expansion (up to 8 weeks) may explain the absence of reports of malignancy in the conducted clinical studies. Despite the promising results of successful bone tissue regeneration, G.-I. Im [36] and B. Lukomska *et al*. [46] emphasise that it is premature to draw definitive conclusions about the efficacy and safety of MSC-based methods. This is due to the limited number of controlled studies and the need for further randomised clinical trials.

CARTILAGE REGENERATION USING MSCS

Articular cartilage, unlike bone tissue, has limited regenerative capabilities. Its damage often leads to the development of degenerative joint diseases, such as osteoarthritis, and loss of mobility [12, 45]. Research by H. Le *et al*. [47] notes that due to the absence of blood vessels in articular cartilage tissue, mature chondrocytes exhibit a low capacity for proliferation and extracellular matrix synthesis to repair defects. Current research focuses on the use of MSCs, particularly those derived from adipose tissue, as they demonstrate the ability to divide and differentiate into chondrocytes [13, 47, 48]. However, even MSC differentiation does not always ensure successful tissue regeneration [20]. The stages of chondrogenesis include cell condensation, matrix synthesis, and maintenance of tissue homeostasis. Each stage requires specific growth factors [12]. In cartilage tissue lesions, meniscus injuries, cruciate ligament ruptures, and other knee joint pathologies, stromal cells are considered one of the most promising components of regenerative medicine [48]. Significant research has been conducted to study cartilage regeneration processes. For example, S. Rodeo [49] made substantial contributions to the study of the fundamental principles and clinical use of MSCs in orthopaedic sports medicine, particularly in the repair of menisci and cartilage in knee joints, shoulder joint tendons, and more. Additionally, research by Y.-M. Pers *et al*. [50] focuses on the use of mesenchymal stem cells to control

inflammation in osteoarthritis, opening new perspectives for regenerative therapy.

In a clinical study by K.-Y. Saw *et al*. [51], patients with cartilage defects in the knee joint were administered MSCs or hyaluronic acid weekly for five weeks. The results showed that patients receiving MSC injections experienced hyaline cartilage regeneration, which was not observed in the hyaluronic acid group. Experimental studies by V. Fishchenko *et al*. [52] confirmed the positive impact of adipose tissue-derived MSCs on the restoration of articular cartilage structure and function in rats after traumatic injuries. The researchers demonstrated the effect of MSCs on accelerating reparative chondrogenesis, as well as the formation of orderly osteogenesis without premature excessive matrix ossification or dystrophic changes in chondrocytes and chondroblasts. V. Fishchenko & L. Mammadov [53] also conducted clinical studies that demonstrated the high effectiveness of MSCs administered intra-articularly for the treatment of distal femoral fractures (19 patients) and deforming osteoarthritis of the knee joint (15 patients), regardless of the type of injury and the stage of gonarthrosis, confirming the promising nature of this approach.

Other studies have also demonstrated the safety and improvement in clinical and radiological parameters following MSC injections in patients with osteoarthritis [9, 54, 55]. F. Davatchi *et al*. [54] observed three patients with knee osteoarthritis for five years after intraarticular MSC administration, noting long-term pain reduction and improved joint function. In a larger study, D. Screpis *et al*. [55] developed a minimally invasive approach for treating knee osteoarthritis. The trial involved 202 patients aged 18 to 70 years who received intra-articular injections of MSCs derived from micro-fragmented adipose tissue at various stages of the disease. After 24 months of follow-up, the positive impact of the injections on joint functional status was confirmed. In the research by K.-I. Kim *et al*. [9], it was demonstrated that intra-articular administration of autologous MSCs, derived from adipose tissue and stromal vascular fractions, without additional therapy, results in high clinical efficacy, treatment safety and stimulates cartilage repair in patients with knee osteoarthritis. When comparing allogeneic and autologous MSCs, both sources showed effectiveness in reducing pain after six months of follow-up, but after a year, autologous MSCs demonstrated superiority, particularly in improving functional outcomes. At the same time, their regenerative potential depends on the patient's comorbidities, such as cardiovascular pathologies, which is an important factor for elderly patients with osteoarthritis.

Y. Jiang & R.S. Tuan [12] analysed the bioactivity, specifically the chondrogenic properties, of multipotent stem cells and progenitor cells derived from autologous adipose tissue, autologous bone marrow, and autologous articular cartilage. They established that different cell types exhibit distinct effectiveness in cartilage repair *in vivo*: articular cartilage stem cells best form cartilage-like tissue; bone marrow cells create a favourable environment for chondrogenesis, promoting the deposition of aggrecan and matrix proteins; conversely, adipose tissue cells more rapidly form fibrous tissue filling, which is less similar to cartilage tissue. The authors attribute these differences to the fact that adipose tissue cells synthesise 20-60% more proteins than other cell types and form an extracellular matrix with high levels of fibronectin and type I collagen. Meanwhile, articular cartilage stem cells produce more type III collagen and deposit less fibronectin and type I collagen. These results underscore the importance of selecting the stem cell source for stimulating cartilage regeneration, as the activation of chondrogenic differentiation and matrix formation depends on several key growth factors specific to each cell type.

Studies by K. Čamernik *et al*. [56] have demonstrated that MSCs derived from skeletal muscles, when compared to MSCs derived from the bones of 21 osteoarthritis patients, exhibited higher clonogenicity, faster growth rates, and shorter cell doubling times, as well as showing good osteogenic and myogenic properties. Furthermore, a positive correlation was observed between CD271 expression and adipogenesis. In preclinical studies by A. Vadhan *et al*. [57], it was proven that exosomes secreted by stromal cells are effective in osteoarthritis due to their chondroprotective and anti-inflammatory properties. The scientists found that these vesicles are capable of reducing the synthesis of pro-inflammatory cytokines while stimulating the production of anti-inflammatory factors and also inhibiting the activity of matrix metalloproteinases, which degrade the cartilage extracellular matrix. Exosomes can enhance cartilage tissue regeneration by promoting the differentiation of chondrogenic cells into chondrocytes, as well as stimulating the synthesis of collagen and proteoglycans [32]. In clinical studies conducted by K. Kawata *et al*. [58], it was shown that intra-articular administration of MSC-derived extracellular vesicles promotes the repair of meniscus defects and also stimulates the growth and migration of chondrocytes and synovial stem cells. Transcriptome/RNA analysis by comprehensive sequencing confirmed that MSC-derived extracellular vesicles upregulate CXCL5 and CXCL6 in chondrocytes, activating their growth and migration through the CXCR2 axis. The effectiveness of MSC-derived exosomes in repairing damaged intervertebral discs was also demonstrated in a study by A. Hajiesmailpoor *et al*. [59].

Tissue engineering, based on the use of stem cells in combination with scaffolds such as tricalcium phosphate, hydroxyapatite, and bioactive glass, has demonstrated its effectiveness in repairing bone and cartilage tissue defects. A systematic review by N. Montemurro *et al*. [60] describes the latest biomaterials (Compact-bio BoneR) and growth factors used in neurosurgery, dentistry, and orthopaedics for bone tissue regeneration. The authors highlight the promising application of bioactive materials in combination with MSCs to enhance osteogenesis and chondrogenesis. Research by S. Nedunchezian *et al*. [61] is dedicated to the creation of bioprinted hydrogel scaffolds based on hyaluronic acid-containing adipose tissue stem cells. The use of a dual crosslinking method allowed for the production of a biomaterial with improved mechanical characteristics, which promoted effective chondrogenic differentiation of MSCs. In turn, Y. Zhang *et al*. [62] demonstrated that the combination of MSCs derived from the human umbilical cord and cell-free cartilage matrix significantly improves cartilage tissue regeneration in a goat model compared to the microfracture method. After a period of observation, animals that received the transplants showed the formation of hyaline-like cartilage with appropriate structural

organisation. In a study by G. Wang *et al*. [63], a bilayer bionic cartilage matrix (membrane) was created, mimicking the structure, chemical, and mechanical characteristics of mature articular cartilage. The upper layer of the membrane consisted of a combination of collagen, sodium hyaluronate, and chitosan, while the transition layer included collagen, silk fibroin, and chitosan. The membrane was implanted into a cartilage defect in the knee joint of rabbits with osteoarthritis. Studies showed that this bionic membrane promotes MSC proliferation and differentiation, providing effective articular cartilage repair. In turn, Z. Wang *et al*. [64] used coral scaffolds with differentiated osteoblasts from rabbit adipose tissue stem cells and transplanted them into bone defects in mice. After 8 weeks, a significant acceleration in new bone tissue formation was recorded. MSCs are a promising tool for treating cartilage tissue pathologies. The selection of stem cell sources and usage protocols remains an important factor that requires further research to optimise treatment strategies.

THE ROLE OF MSCS IN TENDON AND LIGAMENT REPAIR: CHALLENGES AND PROSPECTS FOR CLINICAL APPLICATION

Tendons are tissues with limited blood supply and low cellular density, which complicates their ability to self-repair. According to a review by L. Jiang *et al.* [65], the tendon healing process involves three phases: inflammatory, proliferative, and remodelling, during which scar tissue often forms. The authors note that MSCs play a significant role in tendon and ligament repair due to their multidirectional differentiation potential. In tendon injuries, MSCs contribute to their repair through four main mechanisms: reducing inflammation, neovascularisation, cell proliferation, and differentiation into target cells. Stromal cells are capable of differentiating into tendon cells under specific *in vivo* or *in vitro* induction conditions, which promotes the stimulation of regeneration. Due to their pronounced paracrine effect, they secrete cytokines, growth factors, and chemokines, stimulating angiogenesis and cell proliferation in damaged tissue. Y. Wang *et al*. [66] in their study on a rat model of Achilles tendinopathy showed that the administration of exosomes derived from tendon stem cells (TSCs) significantly reduces the expression of matrix metalloproteinases (MMP)3, increases the expression of tissue inhibitor of metalloproteinases-3 (TIMP-3) and Col1a1, which improves tendon biomechanical properties and promotes their regeneration. The obtained results confirm that TSC-derived exosomes may be a promising therapeutic strategy in repairing damaged tendons by balancing extracellular matrix remodelling and stimulating tenogenesis. Concurrently, according to L. Jiang *et al.* [65], MSCs also contribute to the reorganisation of the extracellular matrix by activating collagen synthesis and stimulating the transformation of type III collagen into type I collagen, which is a crucial aspect of tendon structural remodelling after injury.

MSCs can differentiate into fibroblasts, which comprise the main structural component of tendons and ligaments, thereby promoting their regeneration. Since the anterior cruciate ligament has low regenerative potential, its reconstruction is typically performed using autologous tendons. However, research on resident stem cells in tissues opens new possibilities for the repair of this structure. In a study by Y. Ogata *et al*. [67], it was established that MSCs isolated from the anterior cruciate ligament express CD90 and CD73 markers and have a high capacity for differentiation into fibroblasts.

A pilot study conducted by M. Khoury *et al*. [68] demonstrated significant clinical improvement and structural repair of tendons after the administration of autologous adipose tissue MSCs in 18 patients with chronic lateral epicondylitis of the elbow, resistant to conservative therapy. J.L. Ellera Gomes *et al*. [69] used mononuclear stem cells, isolated from iliac crest bone marrow aspirate, to treat 14 patients with rotator cuff tears. After 12 months of follow-up, clinical examinations and magnetic resonance imaging confirmed the complete healing of all 14 tears. Some MSC-based drugs have already undergone clinical trials. For example, the drug CARTISTEM, created from human umbilical cord blood MSCs, received approval from the Korean FDA in 2012 [70]. In December 2024, the FDA approved the drug Ryoncil (remestemcel-L-rknd), developed from allogeneic bone marrow mesenchymal stromal cells and intended for the treatment of steroidresistant acute graft-versus-host disease. These achievements indicate the growing interest and progress in the clinical application of mesenchymal stem cell-based drugs in various therapeutic areas.

Despite the significant potential of MSCs in the therapy of musculoskeletal disorders, their clinical application faces several challenges. For example, J. Xue & Y. Liu [70] note that, despite the pronounced immunomodulatory potential of MSCs, which affects innate and adaptive immunity through the production of immunosuppressive and immunomodulatory substances, their survival after infusion is quite low. This is due to the immediate inflammatory response that develops right after cell administration. U. Kozlowska *et al*. [20] emphasise that the rapid destruction of MSCs after administration is caused by the activation of the local immune response. Optimising transplantation conditions, including the use of anti-inflammatory agents or special carriers, can significantly improve treatment outcomes. V. Alonso-Goulart *et al*. [71] explored the possibility of using bioactive materials to increase MSC survival after implantation outside the bloodstream. The combination of MSCs with biomaterials allows for the creation of a favourable microenvironment, which reduces the risk of their destruction and improves integration into the recipient's tissues. Scientists J. Xue & Y. Liu [70] also draw attention to the effectiveness of biomaterial-carrier combinations based on bioceramics, hydrogels, and cell sheets, which improve cell survival after implantation and promote their effective functioning.

C. Tran & M.S. Damaser [72] found that MSCs are capable of synthesising a wide range of bioactive molecules, including cytokines, chemokines, angiogenesis and growth factors, which act in an autocrine and paracrine manner, regulating several physiological processes: recruitment of progenitor cells to damaged tissues, inhibition of apoptosis, scar formation, and tissue remodelling. J. He *et al*. [73] examined the features of using MSC spheroids in combination with hydrogels and confirmed their effectiveness in reducing the inflammatory response and improving cell survival. I. Mastrolia *et al*. [74] confirmed the ability of MSCs to actively migrate to injury sites, which promotes regeneration processes. The scientists

emphasise that the effectiveness of this mechanism can be increased using cytokines, chemical modifications, or bioparticles. It has been proven that MSCs integrated with biomaterials have a higher survival rate compared to cells after cryopreservation, which opens up wide prospects for the development of innovative approaches in regenerative medicine [26, 27].

G.E. Salazar-Noratto *et al*. [29] consider MSC transplantation as a safe and promising cell therapy strategy. However, the authors note that the effectiveness of this approach is characterised by significant variability, which is due to the complexity of the recipient's microenvironment. The study analysed various mechanisms that affect the survival of transplanted cells and proposed ways to optimise them to improve therapeutic efficacy. The use of vector materials in combination with MSCs has both advantages and limitations. In *in vitro* and *in vivo* studies, these materials enhanced the paracrine and autocrine effects of stem cells, as well as increased their therapeutic potential [70]. T. Zhou *et al*. [75] analyse the main challenges of MSC clinical application, including immunocompatibility, stability, heterogeneity, as well as differences in differentiation capacity between MSCs from different sources (bone marrow, umbilical cord, adipose tissue, muscles). They note that these factors can significantly affect the effectiveness of therapy and require an individual approach to the selection of cell sources for transplantation.

B. Lukomska *et al*. [46] investigated the issue of immunocompatibility between donor and recipient, which depended on environmental inflammatory molecules. Controlling these molecules is a key factor in reducing the risk of transplanted cell rejection. The authors also noted that inflammatory factors could stimulate MHC-II expression on MSCs, affecting their immune interaction. Y. Wang *et al.* [4] explored the immune response to stromal cell administration and their immunomodulatory properties in the orthopaedic microenvironment, emphasising the importance of understanding these mechanisms to reduce rejection risks and improve therapeutic outcomes.

A. Blandinières *et al*. [76] note that despite the confirmed multifunctional differentiation and paracrine capabilities of MSCs in *in vitro* studies, the mechanisms of their *in vivo* functioning remain poorly understood. The lack of standardised methods for isolating, characterising, and applying these cells complicates standardisation, which is critical for result reproducibility and regulatory compliance. This remains one of the key barriers to the widespread clinical application of MSCs.

Thus, the application of MSCs in tendon and ligament regeneration is a promising direction that requires further research. Optimising transplantation strategies, increasing cell survival, and minimising risks are key tasks for future studies. The use of bioengineering approaches and the combination of MSCs with biomaterials can significantly enhance the effectiveness of therapeutic strategies.

CONCLUSIONS

Thus, the conducted systematic review has summarised important information and analysed global research trends regarding the use of stem cells in regenerative medicine. A significant increase in the number of relevant publications has been noted, indicating the promising development of MSCs in the treatment of injuries and degenerative-dystrophic lesions of cartilage tissue. The therapeutic effect of MSCs is realised through mechanisms of migration, engraftment, and subsequent differentiation into target cells, which opens up broad possibilities for the treatment of musculoskeletal disorders. Along with this, there is a gradual transition of MSC research from the fundamental level to its clinical application. However, as of 2025, there is a limited amount of highvalue evidence regarding the routine use of MSCs for the repair and treatment of musculoskeletal injuries in clinical practice. For the successful clinical implementation of MSCs, large-scale trials with a larger patient sample and a long follow-up period are necessary. Standardising protocols for MSC isolation, cultivation, and transplantation is also important, ensuring their safety, effectiveness, and predictability of results. Special attention should be paid to the analysis of possible side effects and long-term consequences of MSC use. Given the promising nature of the field, it is important to continue interdisciplinary research to integrate regenerative medicine into routine clinical practice. This will open up new horizons for the personalised treatment of patients with musculoskeletal disorders, improve quality of life, and expand the possibilities for applying innovative approaches. In the future, active research is expected on combining MSCs with other biological components (PRP therapy, MFAT therapy), the use of stromal cells in tissue engineering, and the development of innovative drugs based on MSCs for the treatment of orthopaedic pathologies. The significant potential of this field requires further comprehensive study for effective implementation in clinical practice.

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REFERENCES

[1] Deng Z, Luo F, Lin Y, Luo J, Ke D, Song C, et al. Research trends of mesenchymal stem cells application in orthopedics: A bibliometric analysis of the past 2 decades. Front Public Health. 2022;10:1021818. DOI: 10.3389/fpubh.2022.1021818

[2] Maldonado VV, Patel NH, Smith EE, Barnes CL, Gustafson MP, Rao RR, et al. Clinical utility of mesenchymal stem/stromal cells in regenerative medicine and cellular therapy. J Biol Eng. 2023;17(1):44. DOI: 10.1186/s13036-023-00361-9

[3] Sandonà M, Di Pietro L, Esposito F, Ventura A, Silini AR, Parolini O, et al. Mesenchymal stromal cells and their secretome: New therapeutic perspectives for skeletal muscle regeneration. Front Bioeng Biotechnol. 2021;9:652970. DOI: 10.3389/fbioe.2021.652970

[4] Wang Y, Fang J, Liu B, Shao C, Shi Y. Reciprocal regulation of mesenchymal stem cells and immune responses. Cell Stem Cell. 2022;29(11):1515–30. DOI: 10.1016/j.stem.2022.10.001
[5] Tang J, Wang X, Lin X, Wu C. Mesenchymal stem cell-derived extracellular vesicles: A regulator and carrier for targeting bone-related diseases. Cell Death Discov. 2024;10(1):212. DOI: 10.1038/s41420-024-01973-w
[6] Costa LA, Eiro N, Fraile M, Gonzalez LO, Saá J, Garcia-Portabella P, et al. Functional heterogeneity of mesenchymal stem cells from natural niches to culture conditions: Implications for further clinical uses. Cell Mol Life Sci. 2021;78(2):447–67. DOI: 10.1007/s00018-020-03600-0
[7] Zhang Y, Fan M, Zhang Y. Revolutionizing bone defect healing: The power of mesenchymal stem cells as seeds. Front Bioeng Biotechnol. 2024;12:1421674. DOI: 10.3389/fbioe.2024.1421674
[8] Ossendorff R, Menon A, Schildberg FA, Randelli PS, Scheidt S, Burger C, et al. A worldwide analysis of adipose-derived stem cells and stromal vascular fraction in orthopedics: Current evidence and applications. J Clin Med. 2023;12(14):4719. DOI: 10.3390/jcm12144719
[9] Kim KI, Kim MS, Kim JH. Intra-articular injection of autologous adipose-derived stem cells or stromal vascular fractions: Are they effective for patients with knee osteoarthritis? A systematic review with meta-analysis of randomized controlled trials. Am J Sports Med. 2022;51(3):837–48. DOI: 10.1177/03635465211053893
[10] Chen J, Jiang C, Yin L, Liu Y, He Y, Li S, et al. A review of the role of tendon stem cells in tendon-bone regeneration. Med Sci Monit. 2023;29:e940805. DOI: 10.12659/MSM.940805
[11] Grabovyi O, Nevmerzhytska N, Yaremenko L, Kostynskyi H, Demydchuk A, Kondaurova H. Mesenchymal stem cells: Diversity. Pathologia. 2023;20(1):76–84. DOI: 10.14739/2310-1237.2023.1.272938
[12] Jiang Y, Tuan RS. Bioactivity of human adult stem cells and functional relevance of stem cell-derived extracellular matrix in chondrogenesis. Stem Cell Res Ther. 2023;14:160. DOI: 10.1186/s13287-023-03392-7
[13] Frazier T, March K, Garza JR, Bunnell BA, Darr KF, Rogers E, et al. Non-homologous use of adipose-derived cell and tissue therapies: Osteoarthritis as a case study. Bone Rep. 2022;17:101601. DOI: 10.1016/j.bonr.2022.101601
[14] Brown C, McKee C, Bakshi S, Walker K, Hakman E, Halassy S, et al. Mesenchymal stem cells: Cell therapy and regeneration potential. J Tissue Eng Regen Med. 2019;13(9):1738–55. DOI: 10.1002/term.2914
[15] Da Silva Meirelles L, Bieback K, Bolontrade MF. Editorial: Current progress in mesenchymal stem/stromal cell research. Front Cell Dev Biol. 2021;9:658903. DOI: 10.3389/fcell.2021.658903
[16] Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini FC, Krause DS, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006;8(4):315–7. DOI: 10.1080/14653240600855905
[17] Zhou Q, Zhang JH, Yuan S, Shao JH, Cai ZY, Chen S, et al. A new insight of Kartogenin induced the mesenchymal stem cells (MSCs) selectively differentiate into chondrocytes by activating the bone morphogenetic protein 7 (BMP-7)/Smad5 pathway. Med Sci Monit. 2019;25:4960–7. DOI: 10.12659/MSM.916696
[18] Fu HD, Wang HR, Li DH. BMP-7 accelerates the differentiation of rabbit mesenchymal stem cells into cartilage through the Wnt/β-catenin pathway. Exp Ther Med. 2017;14(6):5424–8. DOI: 10.3892/etm.2017.5210
[19] Yang A, Lu Y, Xing J, Li Z, Yin X, Dou C, et al. IL-8 enhances therapeutic effects of BMSCs on bone regeneration via CXCR2-mediated PI3k/Akt signaling pathway. Cell Physiol Biochem. 2018;48(1):361–70. DOI: 10.1159/000491742
[20] Kozlowska U, Krawczenko A, Futoma K, Jurek T, Rorat M, Patrzalek D, et al. Similarities and differences between mesenchymal stem/progenitor cells derived from various human tissues. World J Stem Cells. 2019;11(6):347–74. DOI: 10.4252/wjsc.v11.i6.347
[21] Vaish A, Murrell W, Vaishya R. History of regenerative medicine in the field of orthopedics. J Arthrosc Surg Sport Med. 2020;1(1):154–8. DOI: 10.25259/JASSM_12_2020
[22] Vaish A, Vaishya R. Stem cells in orthopaedics and sports injuries: A comprehensive review and future research directions. J Ortho Rep. 2024;3(4):100344. DOI: 10.1016/j.jorep.2024.100344
[23] Konovalov S, Moroz V, Yoltukhivskyi M, Gadzhula N, Deryabina O, Kordium V. Comparative effects of mesenchymal stromal cells of various origins and sources on biochemical parameters in the hippocampus of rats during cerebral ischemia-reperfusion. Cell Organ Transpl. 2024;12(2):118–25. DOI: 10.22494/cot.v12i2.169
[24] Konovalov S, Moroz V, Yoltukhivskyy M, Gadzhula N, Gusakova I, Deryabina O, et al. Therapeutic potential of mesenchymal stromal cells on morphological parameters in the hippocampus of rats with brain ischemia-reperfusion modeling. Rep Morphol. 2024;30(3):52–62. DOI: 10.31393/morphology-journal-2024-30(3)-06
[25] Tronko M, Pushkarev V, Kovzun O, Sokolova L, Pushkarev V. Mesenchymal stem cells – the main resource of cell therapy. Use for diabetes mellitus treatment. Endokrynologia. 2022:27(3):214–35. DOI: 10.31793/1680-1466.2022.27-3.214
[26] Moll G, Ankrum JA, Kamhieh-Milz J, Bieback K, Ringdén O, Volk HD, et al. Intravascular mesenchymal stromal/stem cell therapy product diversification: Time for new clinical guidelines. Trends Mol Med. 2019;25(2):149–63. DOI: 10.1016/j.molmed.2018.12.006
[27] Moll G, Ankrum JA, Olson SD, Nolta JA. Improved MSC minimal criteria to maximize patient safety: A call to embrace tissue factor and hemocompatibility assessment of MSC products. Stem Cells Transl Med. 2022;11(1):2–13. DOI: 10.1093/stcltm/szab005
[28] Maniar HH, Tawari AA, Suk M, Horwitz DS. The current role of stem cells in orthopaedic surgery. Malays Orthop J. 2015;9(3). DOI: 10.5704/MOJ.1511.016

[29] Salazar-Noratto GE, Luo G, Denoeud C, Padrona M, Moya A, Bensidhoum M, et al. Understanding and leveraging cell metabolism to enhance mesenchymal stem cell transplantation survival in tissue engineering and regenerative medicine applications. Stem Cells. 2020;38(1):22–33. DOI: 10.1002/stem.3079
[30] Hade MD, Suire CN, Suo Z. Mesenchymal stem cell-derived exosomes: Applications in regenerative medicine. Cells. 2021;10(8):1959. DOI: 10.3390/cells10081959
[31] Maqsood M, Kang M, Wu X, Chen J, Teng L, Qiu L. Adult mesenchymal stem cells and their exosomes: Sources, characteristics, and application in regenerative medicine. Life Sci. 2020;256:118002. DOI: 10.1016/j.lfs.2020.118002
[32] Gerami MH, Khorram R, Rasoolzadegan S, Mardpour S, Nakhaei P, Hashemi S, et al. Emerging role of mesenchymal stem/stromal cells (MSCs) and MSCs-derived exosomes in bone- and joint-associated musculoskeletal disorders: A new frontier. Eur J Med Res. 2023;28:86. DOI: 10.1186/s40001-023-01034-5
[33] Hoang DH, Nguyen TD, Nguyen HP, Nguyen XH, Do PTX, Dang VD, et al. Differential wound healing capacity of mesenchymal stem cell-derived exosomes originated from bone marrow, adipose tissue and umbilical cord under serum- and xeno-free condition. Front Mol Biosci. 2020;7:119. DOI: 10.3389/fmolb.2020.00119
[34] Porcu C, Dobrowolny G, Scicchitano BM. Exploring the role of extracellular vesicles in skeletal muscle regeneration. Int J Mol Sci. 2024;25(11):5811. DOI: 10.3390/ijms25115811
[35] Bagno LL, Salerno AG, Balkan W, Hare JM. Mechanism of action of mesenchymal stem cells (MSCs): Impact of delivery method. Expet Opin Biol Ther. 2022;22(4):449–63. DOI: 10.1080/14712598.2022.2016695
[36] Im GI. Clinical use of stem cells in orthopaedics. Eur Cell Mater. 2017;33:183–96. DOI: 10.22203/eCM.v033a14
[37] Zhou Z, Guo C, Sun X, Ren Z, Tao J. Extracellular vesicles secreted by TGF-β1-treated mesenchymal stem cells promote fracture healing by SCD1-regulated transference of LRP5. Stem Cell Int. 2023;2023:4980871. DOI: 10.1155/2023/4980871
[38] Berebichez-Fridman R, Montero-Olvera PR. Sources and clinical applications of mesenchymal stem cells: State-of-the-art review. Sultan Qaboos Univ Med J. 2018;18(3):e264–77. DOI: 10.18295/squmj.2018.18.03.002
[39] Berebichez-Fridman R, Gómez-García R, Granados-Montiel J, Berebichez-Fastlicht E, Olivos-Meza A, Granados J, et al. The holy grail of orthopedic surgery: Mesenchymal stem cells-their current uses and potential applications. Stem Cells Int. 2017;2017:2638305. DOI: 10.1155/2017/2638305
[40] Theodosaki AM, Tzemi M, Galanis N, Bakopoulou A, Kotsiomiti E, Aggelidou E, et al. Bone regeneration with mesenchymal stem cells in scaffolds: Systematic review of human clinical trials. Stem Cell Rev Rep. 2024;20(4):938–66. DOI: 10.1007/s12015-024-10696-5
[41] Lucarelli E, Donati D, Cenacchi A, Fornasari PM. Bone reconstruction of large defects using bone marrow derived autologous stem cells. Transfus Apher Sci. 2004;30(2):169–74. DOI: 10.1016/j.transci.2003.11.008
[42] Liu Y, Kuang B, Rothrauff BB, Tuan RS, Lin H. Robust bone regeneration through endochondral ossification of human mesenchymal stem cells within their own extracellular matrix. Biomaterials. 2019;218:119336. DOI: 10.1016/j.biomaterials.2019.119336
[43] Dufrane D. Impact of age on human adipose stem cells for bone tissue engineering. Cell Transplant. 2017;26(9):1496–504. DOI: 10.1177/0963689717721203
[44] Pawar N, Vaish A, Vaishya R. Core decompression and bone marrow aspirate concentrate injection for Avascular Necrosis (AVN) of the femoral head: A scoping review. J Clin Orthop Trauma. 2021;24:101691. DOI: 10.1016/j.jcot.2021.101691
[45] Im GI. Stem cells for reutilization in bone regeneration. J Cell Biochem. 2015;116(4):487–93. DOI: 10.1002/jcb.25027
[46] Lukomska B, Stanaszek L, ZubaSurma E, Legosz P, Sarzynska S, Drela K. Challenges and controversies in human mesenchymal stem cell therapy. Stem Cells Int. 2019;2019:9628536. DOI: 10.1155/2019/9628536
[47] Le H, Xu W, Zhuang X, Chang F, Wang Y, Ding J. Mesenchymal stem cells for cartilage regeneration. J Tissue Eng. 2020;11. DOI: 10.1177/2041731420943839
[48] Xia D, Wu J, Zhou F, Wang S, Zhang Z, Zhou P, et al. Mapping thematic trends and analysing hotspots concerning the use of stem cells for cartilage regeneration: A bibliometric analysis from 2010 to 2020. Front Pharmacol. 2021;12:737939. DOI: 10.3389/fphar.2021.737939
[49] Rodeo S. Stem cells 101. Amer J Sports Med. 2021;49(6):1417–20. DOI: 10.1177/03635465211011082
[50] Pers YM, Ruiz M, Noël D, Jorgensen C. Mesenchymal stem cells for the management of inflammation in osteoarthritis: State of the art and perspectives. Osteoarthr Cartil. 2015;23(11):2027–35. DOI: 10.1016/j.joca.2015.07.004
[51] Saw KY, Anz A, Merican S, Tay YG, Ragavanaidu K, Jee CSY, et al. Articular cartilage regeneration with autologous peripheral blood progenitor cells and hyaluronic acid after arthroscopic subchondral drilling: A report of 5 cases with histology. Arthroscopy. 2011;27(4):493–506. DOI: 10.1016/j.arthro.2010.11.054
[52] Fishchenko V, Gavrilyuk A, Rosha L, Botsul O, Bevz V, Sukhan D, et al. Reparative chondrogenesis at rats shinbone proximal epiphyphis traumatic injury with the use of Warton drugs star cells mezenchenichemis in the experiment. World Med Biol. 2021;3(77):249–54. DOI: 10.26724/2079-8334-2021-3-77-249-254
[53] Fishchenko O, Mammadov L. Possibilities of using adipose tissue-derived stem cells in deforming osteoarthritis of the knee joint. Bull Probl Biol Med. 2022;1(163):167–71. DOI: 10.29254/2077-4214-2022-1-163-167-171
[54] Davatchi F, Sadeghi Abdollahi B, Mohyeddin M, Nikbin B. Mesenchymal stem cell therapy for knee osteoarthritis: 5 years follow-up of three patients. Int J Rheum Dis. 2016;19(3):219–25. DOI: 10.1111/1756-185X.12670

[55] Screpis D, Natali S, Farinelli L, Piovan G, Iacono V, de Girolamo L, et al. Autologous microfragmented adipose tissue for the treatment of knee osteoarthritis: Real-world data at two years follow-up. J Clin Med. 2022;11(5):1268. DOI: 10.3390/jcm11051268
[56] Čamernik K, Mihelič A, Mihalič R, Marolt Presen D, Janež A, Trebše R, et al. Skeletal-muscle-derived mesenchymal stem/stromal cells from patients with osteoarthritis show superior biological properties compared to bone-derived cells. Stem Cell Res. 2019;38:101465. DOI: 10.1016/j.scr.2019.101465
[57] Vadhan A, Gupta T, Hsu WL. Mesenchymal stem cell-derived exosomes as a treatment option for osteoarthritis. Int J Mol Sci. 2024;25(17):9149. DOI: 10.3390/ijms25179149
[58] Kawata K, Koga H, Tsuji K, Miyatake K, Nakagawa Y, Yokota T, et al. Extracellular vesicles derived from mesenchymal stromal cells mediate endogenous cell growth and migration via the CXCL5 and CXCL6/CXCR2 axes and repair menisci. Stem Cell Res Ther. 2021;12(1):414. DOI: 10.1186/s13287-021-02481-9
[59] Hajiesmailpoor A, Mohamadi O, Farzanegan G, Emami P, Ghorbani M. Overview of stem cell therapy in intervertebral disc disease: Clinical perspective. Curr Stem Cell Res Ther. 2023;18(5):595–607. DOI: 10.2174/1574888X17666220628123912
[60] Montemurro N, Pierozzi E, Inchingolo AM, Pahwa B, De Carlo A, Palermo A, et al. New biograft solution, growth factors and bone regenerative approaches in neurosurgery, dentistry, and orthopedics: A review. Eur Rev Med Pharmacol Sci. 2023;27(16):7653–64. DOI: 10.26355/eurrev_202308_33419
[61] Nedunchezian S, Banerjee P, Lee CY, Lee SS, Lin CW, Wu CW, et al. Generating adipose stem cell-laden hyaluronic acid-based scaffolds using 3D bioprinting via the double crosslinked strategy for chondrogenesis. Mater Sci Eng C Mater Biol Appl. 2021;124:112072. DOI: 10.1016/j.msec.2021.112072
[62] Zhang Y, Liu S, Guo W, Wang M, Hao C, Gao S, et al. Human umbilical cord Wharton's jelly mesenchymal stem cells combined with an acellular cartilage extracellular matrix scaffold improve cartilage repair compared with microfracture in a caprine model. Osteoarthr Cartil. 2018;26(7):954–65. DOI: 10.1016/j.joca.2018.01.019
[63] Wang G, Cao K, Liu K, Xue Y, Roberts AI, Li F, et al. Kynurenic acid, an IDO metabolite, controls TSG-6-mediated immunosuppression of human mesenchymal stem cells. Cell Death Differ. 2018;25(7):1209–23. DOI: 10.1038/s41418-017-0006-2
[64] Wang Z, Han L, Sun T, Wang W, Li X, Wu B. Construction of tissue-engineered bone with differentiated osteoblasts from adipose-derived stem cell and coral scaffolds at an ectopic site. Br J Oral Maxillofac Surg. 2021;59(1):46–51. DOI: 10.1016/j.bjoms.2020.07.006
[65] Jiang L, Lu J, Chen Y, Lyu K, Long L, Wang X, et al. Mesenchymal stem cells: An efficient cell therapy for tendon repair (review). Int J Mol Med. 2023;52(2):70. DOI: 10.3892/ijmm.2023.5273
[66] Wang Y, He G, Guo Y, Tang H, Shi Y, Bian X, et al. Exosomes from tendon stem cells promote injury tendon healing through balancing synthesis and degradation of the tendon extracellular matrix. J Cell Mol Med. 2019;23(8):5475–85. DOI: 10.1111/jcmm.14430
[67] Ogata Y, Mabuchi Y, Shinoda K, Horiike Y, Mizuno M, Otabe K, et al. Anterior cruciate ligament-derived mesenchymal stromal cells have a propensity to differentiate into the ligament lineage. Regen Ther. 2018;8:20–8. DOI: 10.1016/j.reth.2017.12.001
[68] Khoury M, Tabben M, Rolón AU, Levi L, Chamari K, D'Hooghe P. Promising improvement of chronic lateral elbow tendinopathy by using adipose derived mesenchymal stromal cells: A pilot study. J Exp Orthop. 2021;8(1):6. DOI: 10.1186/s40634-020-00320-z
[69] Ellera Gomes JL, da Silva RC, Silla LMR, Abreu MR, Pellanda R. Conventional rotator cuff repair complemented by the aid of mononuclear autologous stem cells. Knee Surg Sports Traumatol Arthrosc. 2012;20(2):373–7. DOI: 10.1007/s00167-011-1607-9
[70] Xue J, Liu Y. Mesenchymal stromal/stem cell (MSC)-based vector biomaterials for clinical tissue engineering and inflammation research: A narrative mini review. J Inflamm Res. 2023;16:257–67. DOI: 10.2147/JIR.S396064
[71] AlonsoGoulart V, Carvalho LN, Marinho ALG, de Oliveira Souza BL, de Aquino Pinto Palis G, Lage HGD, et al. Biomaterials and adiposederived mesenchymal stem cells for regenerative medicine: A systematic review. Materials. 2021;14(16):4641. DOI: 10.3390/ma14164641
[72] Tran C, Damaser MS. Stem cells as drug delivery methods: Application of stem cell secretome for regeneration. Adv Drug Deliv Rev. 2015;82–83:1–11. DOI: 10.1016/j.addr.2014.10.007
[73] He J, Zhang N, Zhu Y, Jin R, Wu F. MSC spheroids-loaded collagen hydrogels simultaneously promote neuronal differentiation and suppress inflammatory reaction through PI3K-Akt signaling pathway. Biomaterials. 2021;265:120448. DOI: 10.1016/j.biomaterials.2020.120448
[74] Mastrolia I, Foppiani EM, Murgia A, Candini O, Samarelli AV, Grisendi G, et al. Challenges in clinical development of mesenchymal stromal/stem cells: Concise review. Stem Cells Transl Med. 2019;8(11):1135–48. DOI: 10.1002/sctm.19-0044
[75] Zhou T, Yuan Z, Weng J, Pei D, Du X, He C, et al. Challenges and advances in clinical applications of mesenchymal stromal cells. J Hematol Oncol. 2021;14(1):24. DOI: 10.1186/s1304502101037x
[76] Blandinières A, Randi AM, Paschalaki KE, Guerin CL, Melero-Martin JM, Smadja DM. Results of an international survey about methods used to isolate human endothelial colony-forming cells: Guidance from the SSC on Vascular Biology of the ISTH. J Thromb Haemostasis. 2023;21(9):2611–9. DOI: 10.1016/j.jtha.2023.06.014

Використання мезенхімальних стромальних клітин в терапії захворювань опорно-рухового апарату: огляд літератури

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Анотація. Швидкий розвиток методів отримання мультипотентних клітин-попередників, відомих як мезенхімальні стромальні/стовбурові клітини, з різноманітних тканин та органів людини сприяв прогресу у сфері клітинної терапії та регенеративної медицини. Метою цього дослідження став огляд сучасних наукових даних щодо використання мезенхімальних стромальних клітин у лікуванні травматичних і ортопедичних захворювань, а також ознайомлення клініцистів із викликами та перспективами їх клінічного застосування. У ході дослідження було проаналізовано джерела отримання мезенхімальних стромальних клітин, їхні характеристики та терапевтичний вплив при захворюваннях опорно-рухового апарату. Було встановлено, що ці клітини можуть використовуватися як в аутологічній, так і в алогенній формах завдяки їх здатності до диференціювання в остеобласти, хондроцити, теноцити, адипоцити та інші типи клітин, що сприяє регенерації ушкоджених тканин. Було підтверджено, що мезенхімальні стромальні клітини володіють паракринною активністю, продукуючи цитокіни та фактори росту, що забезпечує їх регенеративний і регуляторний вплив як *in vitro*, так і *in vivo*. Водночас було зазначено, що, попри значний терапевтичний потенціал, їх клінічне застосування пов'язане з низкою викликів, зокрема, питаннями імуносумісності, стабільності, гетерогенності, а також обмеженою здатністю до диференціювання та міграції. Було розглянуто перспективи подолання цих обмежень за допомогою безклітинних підходів, зокрема застосування екзосом, що виділяються мезенхімальними стромальними клітинами, та містять біологічно активні молекули мРНК, мікроРНК, білки та біоактивні ліпіди. Було відзначено, що ці компоненти сприяють клітинній проліферації, міграції, регенерації, імуномодуляції та ангіогенезу, що є перспективним напрямом у регенеративній медицині. Отримані результати можуть бути використані для подальшої розробки ефективних терапевтичних стратегій у регенеративній медицині, зокрема в ортопедії та травматології. Практичне значення дослідження полягає в удосконаленні підходів до клітинної терапії, спрямованих на відновлення уражених тканин, що може слугувати основою для майбутніх клінічних випробувань і подальшого впровадження в медичну практику

Ключові слова: стовбурові клітини; екзосоми; регенерація тканин; травматологія; ортопедія

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