# ANIMAL PATHOLOGY, MORPHOLOGY, PHYSIOLOGY, PHARMACOLOGY AND TOXICOLOGY

# ПАТОЛОГИЯ ЖИВОТНЫХ, МОРФОЛОГИЯ, ФИЗИОЛОГИЯ, ФАРМАКОЛОГИЯ И ТОКСИКОЛОГИЯ





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*Original Empirical Research*

## The Relationship between Caspase-3, -6 Expression and Morphological Changes in Kidneys of Cows

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**Abstract**

***Introduction.*** Cow kidneys are exposed to the impact of industrial factors, which is manifested in development of the various nephropathies with systemic effects on organism. The kidneys are the main organs responsible for homeostasis by regulating osmolarity, mineral balance, and acid-base balance in organism through the excretion of water and inorganic electrolytes. Renal pathology often has latent character and is diagnosed postmortem. Significant destructive changes, including cell death, are often detected in lactating cows due to high metabolic load they undergo. Caspase pathway is one of the pathways in the complex apoptotic signaling cascade. Unfortunately, the expression of caspases in bovine kidneys has not been yet described in the literature. The aim of the study is to describe the relationship between the expression of caspase-3 and caspase-6 and the pathological changes in the kidneys of high-yielding cows.

***Materials and Methods.*** A morphological study of renal samples from high-yielding cows (4–6 lactations, 7–8 years old, n=4) was conducted at the Ural Federal Agrarian Scientific Research Center, Ural Branch of the Russian Academy of Sciences in the period from 2023 to 2025. Standard histological techniques including hematoxylin and eosin staining and Mallory's trichrome staining were used. Immunohistochemical analysis of the samples using antibodies to caspase-3, and -6 was performed.

***Results.*** It was found that in the kidneys of 7–8-year-old cows there develop the destructive processes including renal failure, inflammatory reactions, and sclerotic and microcirculatory disorders. The observed conditions are associated with high expression of caspase-6 at the level of the renal tubules, which confirms damage to the renal parenchyma. Caspase-3 is expressed primarily in the renal tubules and in renal stroma elements, which indicates intensification of apoptotic processes across the entire organ.

***Discussion and Conclusion.*** The destructive processes revealed in the kidneys of high-yielding cows are likely to be associated not only with the age but also with the high metabolic load imposed on the organism during lactation. The research findings substantiate the need for further study of pro-apoptotic factors due to their influence on the development of various pathologies in cattle.

**Keywords:** caspase-3, caspase-6, apoptosis, cows, nephrosclerosis, polycystic kidney disease, morphological study

**Declaration on Compliance with the Principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes:** the authors declare that all the studies were conducted in compliance with the principles of Good Laboratory Practice.

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*Оригинальное эмпирическое исследование*

# Взаимосвязь экспрессии каспазы-3 и -6 с морфологическими изменениями в почках коров

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**Аннотация**

***Введение.*** Почки коров подвержены воздействию факторов промышленного содержания, что проявляется развитием различных нефропатий, оказывающих системное действие на организм. Почки — основной орган, обеспечивающий гомеостаз путем регуляции осмолярности, минерального и кислотно-основного состояния организма посредством экскреции воды и неорганических электролитов. Патология почек зачастую носит латентный характер и диагностируется постмортально. У лактирующих коров в связи с высокой метаболической нагрузкой часто выявляются значительные деструктивные изменения, проявляющиеся в том числе гибелью клеток. Одним из путей в сложном каскаде сигналинга апоптоза является каспазный. К сожалению, на сегодняшний день для крупного рогатого скота экспрессия ферментов-каспаз в почках не описана. Цель работы — описание взаимосвязи экспрессии каспазы-3 и каспазы-6 с патологическими изменениями в почках высокопродуктивных коров.

***Материалы и методы.*** Морфологическое исследование образцов почек высокопродуктивных коров (4–6 лактаций, возраст 7-8 лет, n=4) проведено в ФГБНУ УрФАНИЦ УрО РАН в период с 2023 по 2025 г. Использованы стандартные методы гистотехники с окрашиванием препаратов гематоксилином и эозином, а также по Маллори. Проведено иммуногистохимическое исследование образцов с антителами к каспазе-3 и -6.

***Результаты исследования.*** Выявлено, что у коров в возрасте 7-8 лет в почках развиваются деструктивные процессы в виде почечной недостаточности, воспалительных реакций, склеротических и микроциркуляторных изменений. Обнаруженные явления ассоциированы с высокой экспрессией каспазы-6 на уровне почечных канальцев, что подтверждает повреждение почечной паренхимы. Каспаза-3 экспрессируется преимущественно в канальцах почки, а также в элементах ее стромы, что указывает на интенсификацию апоптических процессов по всему органу.

***Обсуждение и заключение.*** Обнаруженные деструктивные процессы в почках высокопродуктивных коров ассоциированы, вероятно, не только с возрастом, но также с высокой метаболической нагрузкой на организм во время лактаций. Полученные данные обосновывают дальнейшее изучение проапоптических факторов в свете их влияния на развитие различных патологий у крупного рогатого скота.

**Ключевые слова:** каспаза-3, каспаза-6, апоптоз, коровы, нефросклероз, поликистоз, почки, морфологическое исследование

**Декларация о соблюдении принципов Европейской конвенции о защите позвоночных животных, используемых для экспериментов и других научных целей**: авторы заявляют, что все проведенные исследования соответствовали принципам конвенции и правилам надлежащей лабораторной практики.

**Финансирование.** Работа выполнена в рамках проекта Российского научного фонда 2023 г. «Пространственная характеристика транскриптомных и метаболомных особенностей преждевременной структурно-функциональной дегенерации тканей органов сельскохозяйственных животных и птицы» (№ 23-16-00117).

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**Introduction.** The organism of high-yielding dairy cows is susceptible to metabolic imbalances. One of the consequences of milk production stress is the development of acidotic syndrome, which negatively effects the kidneys [1]. Ketosis, which cows are particularly susceptible to during lactation, can also lead to various nephropathies [2]. The pathological processes developed in the kidneys inhibit organ functioning, have a systemic nature and affect the overall condition of an animal. In the scientific community the role of apoptosis in the pathogenesis of various kidney diseases has long been actively discussed [3]. For example, it has been established that programmed death of tubular epithelial cells, along with necrosis, are the basic signs of acute renal failure [4]. In addition to nephrocyte damage, renal vascular damage and endothelial cell apoptosis are important factors in the development of renal dysfunction. The loss of endothelial

cells by apoptosis is deemed to play a significant role in changing the microvascular network observed during renal damage [5]. Apoptosis has also been described in renal interstitial cells following ischemic renal injury [6].

Caspases are enzymes belonging to a group of proteases that are involved in cell apoptosis. 12 caspases have been described for humans and rodents, however for cattle, the exact number of enzymes from this group has to be determined yet. Caspases are divided into 3 groups: executioners, initiators and inflammatory caspases. Caspase-3 represents the executive caspase group, and caspase-6 — the initiator caspase group. Studies on the role of caspases in the development of various pathologies in the organs of cattle are extremely few. For example, the role of caspase-3 in the apoptosis of mammary gland cells in cows fed with concentrated feed has been described [7]. Data on the role of caspases in the development of renal pathologies in cattle are not available in the literature. However, caspases are known to be involved in inflammatory responses, apoptosis, vasoconstriction, and tubular necrosis in various kidney diseases in humans and rodents [8]. Furthermore, the important role of caspases in renal damage makes them a favourable target for therapy. For example, on the example of polycystic kidney disease in rats, it was demonstrated that caspase inhibition can reduce apoptosis in renal tubules and slow disease progression [9].

Therefore, the aim of the present study is to describe the relationship between caspase-3 and caspase-6 expression and morphological changes in the kidneys of high-yielding cows with pathologies.

**Materials and Methods.** The work was carried out at the Ural Federal Agrarian Scientific Research Center, Ural Branch of the Russian Academy of Sciences (Ekaterinburg) in the period from 2023 to 2025. To study the morphological changes in the kidneys of cows, the material was collected postmortem from animals aged 7–8 years old, that had 4–6 lactations (n=4). For histological examination, tissue specimens of no more than 0.5 cm thick were fixed in 10% Neutral Buffered Formalin (NBF) in a proportion of 1:20. After fixation, the specimens were rinsed in running water, dehydrated, then soaked in paraffin, and paraffin blocks were prepared. Paraffin sections of 3–4 μm thickness were made. Sections were deparaffinized and stained using hematoxylin and eosin and Mallory's trichrome staining techniques.

Immunohistochemical staining of the samples with antibodies to caspase-3 and caspase-6 was performed to assess the intensity of apoptosis in the organ. For immunohistochemical analysis, sections were placed on the adhesive slides (Superfrost), then placed on a slide drying bench (+60°C) until the paraffin was visibly melted. Immunohistochemical analysis was performed in compliance with the following protocol:

1. The sections were placed in a Tris-EDTA buffer solution heated in a water-bath up to 96°C for 12 min for heat-induced epitope retrieval (HIER, pH 6; "PrimeBio-Med", Russia).

2. After 12 minute incubation in a water-bath, the sections were left in the buffer solution to cool to indoor temperature and then rinsed with distilled water.

3. The sections were incubated in an $H_2O_2$ solution for 10 min, then placed in distilled water for 2 min.

4. The sections were dried and outlined with a hydrophobic pen.

5. The sections were soaked in TBST buffer solution for 1 min.

6. Primary antibodies were applied to the sections, which were then placed in the humidified incubator for 1 hour at 30°C.

7. Sections were rinsed three times with TBST buffer solution.

8. Secondary antibodies were applied to the sections, which were then placed in the humidified incubator for 30 minutes at 30°C.

9. Sections were rinsed three times with TBST buffer solution.

10. DAB chromogen was applied to the sections, which were incubated until darkening, approximately during 30–60 seconds.

11. Sections were rinsed in distilled water and then counterstained with hematoxylin for 10 seconds.

12. Sections were rinsed in distilled water, decolorized, and placed in balsam.

Antibodies to caspase-3 (PAA626Bo01, Cloud-Clone Corp., China) and caspase-6 (PAC340Bo02, Cloud-Clone Corp., China) were used as primary antibodies at a 1:100 dilution. Antibodies to rabbit IgG (SAA544Rb19, Cloud-Clone Corp., China) were used as secondary antibodies with DAB (Bio-Rad Laboratories, Inc., USA) as a horseradish peroxidase substrate in accordance with the manufacturer's instructions.

Histological description of the specimens was made, and the intensity of immunohistochemical staining was visually assessed.

**Research Results.** Examination of the cows' renal specimens has revealed significant changes in the organ. The dilation in Shumlyansky Bowman capsule of renal corpuscles was seen in the renal cortex. Endothelial proliferation was visible in the Juxtaglomerular apparatus, indicating the development of intracapillary glomerulonephritis (Fig. 1.1). Cystic cavities of varying sizes were found throughout the cortex, indicating renal failure (Fig. 1.2). Protein casts were deposited in the lumen of the proximal tubules, indicating a protein metabolism disorder. Nephrocytes with degenerative changes, and a number of cells exhibiting structural abnormalities were observed. The renal tubules demonstrated narrowing of the lumen, swelling of the nephrocytes, and changes in their structure (Figs. 1.1 and 1.2).

Mallory's trichrome staining has revealed increased connective tissue patterns in the Shumlyansky Bowman capsule and between the proximal convoluted tubules, indicating productive inflammation (Fig. 1.3). Foci of nephrosclerosis were found in renal interstitium between the tubules (Fig. 1.4). In the area of blood vessels, particularly near the perivascular zone of arcuate arteries, the pronounced sclerotic processes were observed — the extensive areas of connective tissue proliferation, stained in various shades of blue (Fig. 1.5). Within the vessels themselves, the active processes of disorganization and proliferation of vascular wall elements, and sharp narrowing of the arterial lumen were observed (Fig. 1.6).

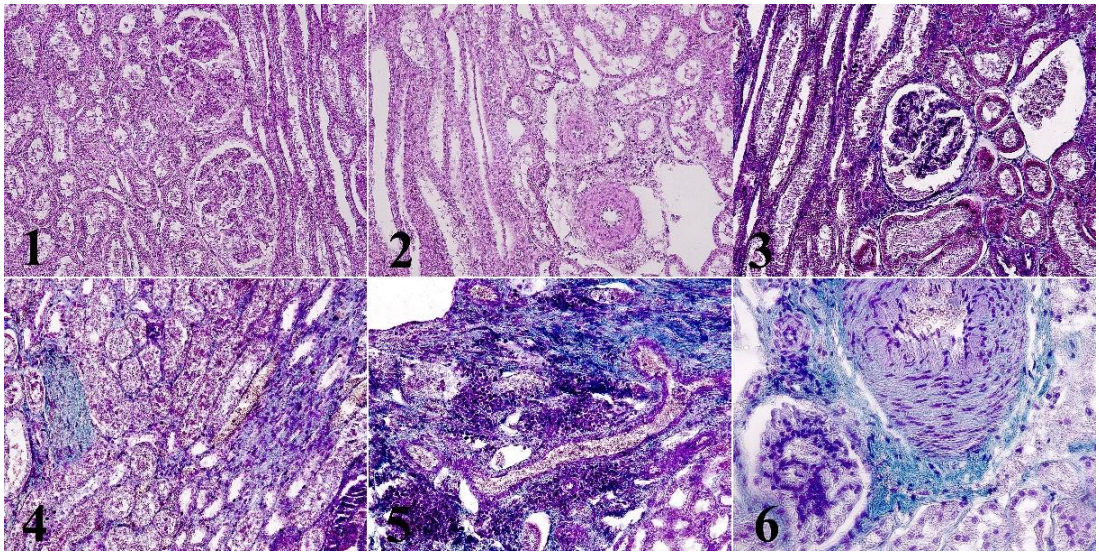


Fig. 1. Morphological structure of the kidneys of high-yielding cows aged 7–8 years:
1, 2 — hematoxylin and eosin staining, 200× magnification; 3–5 — Mallory's trichrome staining, 200× magnification;
6 — Mallory's trichrome staining, 400× magnification

Along with the morphological changes, expression of the proapoptotic proteins caspase-3 and caspase-6 was observed in the kidneys of the cows studied. Moderate expression of caspase-3 was revealed in the nephrocytes of the cortical tubules (Fig. 2.1), as well as in the cells of the parietal layer of the Shumlyansky-Bowman capsule of deformed glomeruli (Fig. 2.2). A large number of caspase-3-positive cells were also detected in the interstitium around the cystic cavities (Fig. 2.3). Nephrocytes in both the renal cortex and medulla tubules were intensely colored in brown, which indicates a high degree of caspase-6 expression in them (Fig. 2.4 and 2.5). At the same time, renal corpuscles that retained their structure were not expressing caspase-6, unlike the deformed glomeruli (Fig. 2.6). It is worth noting that not all the tubules had a caspase-positive reaction, which may be due to the possibility of cell death not only by apoptosis, but also by necrosis, and is discussed in the literature [4].

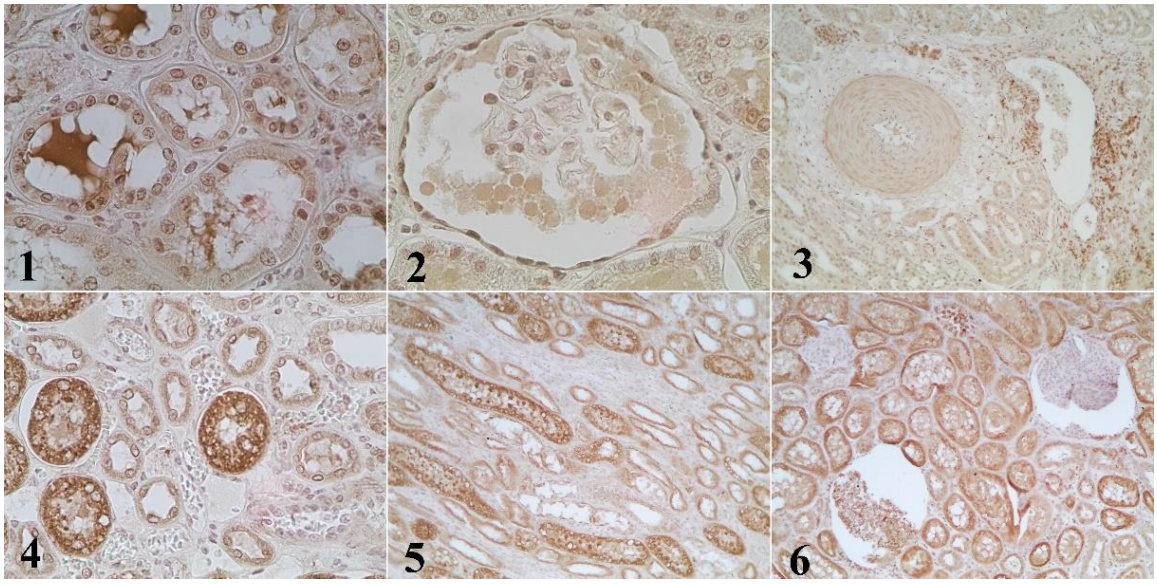


Fig. 2. Immunohistochemical detection of caspase-3 (1–3) and caspase-6 (4–6) in kidney tissues of high-yielding cows aged 7–8 years, hematoxylin counterstaining: 1, 2, 4, 5 – 400× magnification; 3 and 6 – 200× magnification

**Discussion and Conclusion.** Morphological examination of kidney tissues in cows aged from 7- to 8-year-old reveals the development of destructive processes in the organ, which are likely related not only to the age but also to the high metabolic load endured by the organism during lactation. Changes affect not only functioning of the organ parenchyma but also the stroma and microvasculature. The formation of cystic cavities, the presence of deformed glomeruli, and abnormalities in the structure of the Juxtaglomerular apparatus indicate the development of renal failure. The deposition of protein casts in the renal tubules and the death of nephrocytes indicate the development of degenerative processes. Foci of nephrosclerosis and significant microcirculatory disorders indicate the presence of inflammatory reactions and fibrotic changes in the organ.

The described changes are accompanied by the expression of apoptotic factors—caspase-3 and caspase-6. Caspase-6 is predominantly expressed by renal tubular cells, which, judging by morphological description, undergo degenerative processes caused by the protein metabolism disorder. Thus, the elevated caspase-6 expression is a marker of renal cell damage and pre-apoptotic state triggering the caspase cascade. Caspase-3 expression is observed not only in renal parenchyma functioning, but also in the stromal elements of the organ —in the interstitium and vascular wall cells. Thus, due to the executive role of caspase-3, the conclusion about extensive apoptotic processes occurring in various tissues of the organ can be drawn. The obtained data provide a profound basis for further in-depth study of pro-apoptotic factors in the context of their effect on the development of various pathologies in cattle.

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***Claimed Contributorship:***

**IM Petrova:** formulating the main concept, search for publications, analysis of the research results, formulating the conclusions

**MV Bytov:** development of research methodology, formal analysis, writing the manuscript.

**SL Khatsko:** conducting experiments and collecting data; preparing the initial draft of the manuscript.

**VD. Zubareva:** editing and refining the manuscript.

**LI Drozdova:** analysis and validation of the obtained results, preparing a draft of the manuscript.

**IA Shkuratova:** scientific supervision, developing the concept and methodology of research, preparing a draft of the manuscript.

***Conflict of Interest Statement:* the authors declare no conflict of interest.**

***All authors have read and approved the final manuscript.***

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