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**MORPHO-BIOCHEMICAL REMODELING OF THE ENDOMETRY
OF COWS: DYNAMICS OF METABOLIC AND ENZYMATIC PROFILES
DURING THE ESTROUS CYCLE**

Abstract.

The current body of scientific literature, despite its depth, fails to provide a comprehensive and high-resolution mapping of the morphological and histological dynamics governing endometrial formation in cattle fetuses, a gap that significantly complicates the development of precision methods for physiological correction and metabolic stimulation during gestation and placental formation. This lack of systematic data is particularly critical for understanding the assembly of the maternal placenta during the estrus phase, where the structural integrity of the tissue determines the success of all subsequent reproductive stages.

The intricate process of gametogenesis and the concurrent maturation of reproductive organs are driven by intensive transamination reactions, characterized by a marked elevation in transferase activity where the cytoplasmic-mitochondrial fraction plays a functionally dominant role in maintaining cellular energy balance. It is analytically significant that the estrus stage acts as a metabolic catalyst, triggering a cascade of tissue enzyme activations and systemic metabolic surges, whereas the subsequent phases of inhibition and equilibration are defined by a compensatory reduction in these bioenergetic phenomena to restore basal homeostasis.

Contemporary reproductive biology has firmly established that the functional status of uterine glandular epithelial cells and the associated stromal components is characterized by profound heterogeneity throughout the estrous cycle, a state of "dynamic instability" that is paradoxically essential for maintaining long-term tissue homeostasis. Researchers hypothesize that this cellular heterogeneity serves as an evolutionary adaptation, allowing the endometrium to rapidly recruit reserve structural units in response to the volatile hormonal environment of the female organism.

In the immediate pre-ovulatory period, the morphometric thickness of the endometrium and the oviductal epithelium reaches its physiological zenith, driven by a precipitous surge in steroid hormone concentrations that stimulates the cervical epithelium to synthesize and discharge maximal volumes of low-viscosity mucus enriched with mucins, glycoproteins, and high-molecular-weight protein complexes.

Our rigorous empirical investigations confirm that the estrus phase is distinguished by a statistically significant ($p < 0.001$) escalation in total serum protein, which increases by 7.6% relative to metestrus and by 8.7% compared to proestrus, reflecting the massive hypertrophic and proliferative expansion of the myometrium and the functional endometrial layer.

Glandular epithelial units undergo a series of integrated structural-functional metamorphoses, where the proestrus phase is marked by aggressive proliferation and apical translocation of glycogen reserves within the uterine glands. The temporal duration of these cellular shifts is strictly governed by the maturation rate of the dominant ovarian follicle, ensuring a precise synchronization between ovulation and endometrial receptivity. The application of these localized metabolic findings on a macro-scale will facilitate a substantial increase in herd reproduction indices, thereby optimizing the economic efficiency of meat and dairy industries through a scientifically substantiated model of physiological remodeling.

Keywords: *endometrial remodeling, biochemical homeostasis, estrous cycle, bovine reproduction, metabolic status, enzymatic activity.*

Introduction

The critical issue of bovine reproduction, particularly concerning highly productive cows characterized by a complex physiological state of lactation dominance and prolonged reproductive cycles, remains a pivotal theme within the global landscape of veterinary morphology and endocrinology, as the maintenance of uterine physiological homeostasis directly dictates the economic viability of the entire livestock sector (Moore et al., 2019; Faria & Simões, 2015). Against the background of significantly diminished adaptive capabilities during the recovery period, even minor infractions in the conditions of maintenance and operational stressors can become excessive, subsequently precipitating unfavorable conditions that obstruct the manifestation of a complete and functional sexual cycle, as evidenced by the high risk of symptomatic and latent infertility in post-parturient animals (Bondarenko et al., 2023; Gray et al., 2002). [1, 4].

The systemic metabolism in the bovine organism serves as a comprehensive reflection of the underlying physiological processes, where a weakening of inherent

protective properties triggers the activation of compensatory and latent potential reserves whose expression is fundamentally contingent upon the stability of biochemical homeostasis (Fodor et al., 2019). Nutritional factors and the specificities of environmental maintenance exert a profound influence on these metabolic pathways, necessitating a detailed understanding of the morpho-biochemical remodeling of the endometrium—a tissue uniquely capable of cyclic destruction and total regeneration under the rigorous orchestration of ovarian steroid hormones (2023; Oznurlu et al., 2009). [2, 3].

For the clinical veterinarian, mastering the physiological features of the functional endometrial layer is paramount, as this internal environment of the uterus directly influences the integrity of the connective tissue skeleton and the underlying glandular apparatus through a series of complex structural-functional transformations (Filant & Spencer, 2013). While endometrial morphogenesis is dictated by a convergence of hereditary factors and fetal development conditions, there remains a critical lack of systematized data regarding the cattle-specific developmental stages of this tissue in the postnatal period, which justified the rigorous investigation of the morphological-biochemical remodeling of the bovine endometrium throughout the estral cycle (Moore et al., 2019). [4, 9].

The glandular epithelium undergoes integrated structural metamorphoses where, during the proestrus phase, uterine gland cells exhibit aggressive proliferation and growth, a period defined by the active translocation of glycogen from the basal to the apical region of the gland—a process strictly governed by the maturation rate of the dominant ovarian follicle (El-Gendy & Derbalah, 2021). Following ovulation, at the onset of the inhibitory stage, secretory transformation becomes manifest through the appearance of basal vacuoles, where the secretion of glycodein acts as a crucial local immunosuppressant to shield the developing embryo from the maternal immune response (Filant & Spencer, 2013; Gray et al., 2002). [3, 4].

Endometrial regeneration initiates during the diestrus phase against a landscape of low steroid concentrations, triggering a molecular interaction between local growth factors and specific cellular receptors that promotes fibroblast activity and the synthesis of collagen, elastin, and proteoglycans (Moore et al., 2019). This heterogeneity of the epithelial and stromal components, characterized by the influx of lymphocytes from blood vessels to regulate mitotic activity, ensures that the uterine mucosa remains a highly sensitive, hormone-dependent organ capable of dynamic responses to any homeostatic imbalance—from hyperplasia to atrophy (Bondarenko et al., 2023; Fodor et al., 2019). Consequently, in the current framework of intensive livestock farming, the application of targeted technological means to adjust reproductive function is essential to minimize the ecological footprint of production and maximize the output of high-quality food products (Moore et al., 2019). [2, 4].

Purpose and Methods. The primary objective of this study was to establish a rigorous morphofunctional and biochemical baseline for the cyclic remodeling of the bovine endometrium by quantifying systemic metabolic shifts across defined physiological stages of the estrous cycle.

To achieve this, a cohort of cows (n=50) aged 3 to 10 years was systematically screened and categorized into three distinct experimental groups based on synchronized clinical markers and endocrinological profiles. Group 1 (n=11) was composed of animals in the peak of the estrus phase, verified by the presence of a distinct standing reflex and the physiological zenith of the Luteinizing Hormone (LH) surge, which serves as the critical window for reproductive correction and artificial insemination.

Group 2 (n=20) represented the metestrus stage, specifically the 7th to 8th day post-ovulation, during which the corpus luteum reaches its maximal functional flourishing and secretes progesterone levels sufficient for pre-gestational endometrial preparation. Conversely, any identified low secretory activity of the corpus luteum during this period was analyzed as a precursor to the blockage of uterine myometrial receptors and the subsequent inhibition of glandular synthesis (Bondarenko et al., 2023; Moore et al., 2019).

Group 3 (n=19) consisted of cows in the proestrus phase (days 17–18), diagnosed via clinical evidence of hypertrophy and hyperemia of the internal reproductive tract, signaling the initiation of cellular proliferation under the stimulatory influence of hypothalamic liberins and Follicle-Stimulating Hormone (FSH). This phase is characterized by the gonadal production of estrogens—estrone, estriol, and estradiol—which directly target the endometrial glands to initiate the next wave of morphological remodeling. Furthermore, a specialized clinical subgroup was integrated into the study, consisting of healthy cows undergoing sexual cyclicity restoration following pathological complications, including endometritis (n=17) and retained placenta (n=14), who had remained anestrous for a period exceeding 30 days post-clinical recovery.

Blood samples were extracted via the jugular or tail vein under strict aseptic and antiseptic conditions in the morning prior to feeding to minimize exogenous metabolic interference.

The determination of total protein was executed using the biuret method, while total lipid concentrations were quantified utilizing the phosphovanillin reagent, and total cholesterol levels were measured via the Zlatkis-Zak method with specialized diagnostic kits (PE Danysh, Lviv, Ukraine) and a KFK-3 photoelectrocolorimeter.

To assess the intensity of cellular remodeling and tissue damage, the activity of aspartate aminotransferase (AsAT) and alanine aminotransferase (AlAT) in the blood serum was determined by the Reitman-Frankel method using standardized diagnostic kits from "Simko LTD" (Lviv, Ukraine). This integrated biochemical approach allows for the verification of the systemic metabolic status in relation to the localized morphological state of the uterine mucosa, ensuring a mathematically consistent and biologically representative dataset (Bondarenko et al., 2023).

Presentation of the main material of the study.

In contemporary veterinary medicine, the physiology of reproductive capacity remains a central challenge due to the insufficient systemic mapping of the structural-functional processes occurring within the bovine endometrium across distinct stages of the estrous cycle (Moore et al., 2019). The regenerative capacity of the uterine mucosa

post-parturition is highly variable; depending on the individual physiological status, endometrial restoration may extend over several months, thereby delaying subsequent conception and disrupting the economic cycle of the dairy enterprise. Empirical data indicate that within the first month post-calving, complete endometrial restoration and successful re-pregnancy are achieved in only 37% of cows aged three to six years, while approximately 75% of the herd exhibits irregular estrous cyclicity with periods ranging from 16 to 30 days. This variability is often attributed to a pathological lag in estrus manifestation, which is further exacerbated by adverse exogenous and endogenous stimuli encountered during intensive exploitation. [5,6,7].

During the manifestation of physiological estrous cyclicity, the cow's metabolism acquires significant intensity, making the organism highly susceptible to stress factors that disrupt steroidogenesis and hematopoiesis. These metabolic disturbances lead to profound morphological alterations in internal organs and the uterine mucosa, directly affecting the physiological sequence of structural transformations required for successful zygote nidation and stable pregnancy (Faria & Simões, 2015; Filant & Spencer, 2013). To mitigate these dysfunctional disorders, modern livestock management integrates preventive systems that combine balanced nutritional protocols with targeted veterinary interventions, such as the administration of antioxidants and uterine contractility stimulants to enhance regenerative capacity. The endometrium, as the primary hormone-sensitive lining, plays a decisive role in this process, providing the biophysical and energy support necessary for the embryo until the full establishment of the maternal placenta. [8].

The structural quality of the endometrium and its future receptivity are fundamentally established during the embryonic development of the heifer, long before the activation of other reproductive components. Scientific evidence confirms that full cellular proliferation and effective metabolism within the uterine mucosa require a precise stoichiometric ratio of estradiol to progesterone. Secretory transformation is achievable only through a cyclic surge in progesterone levels against a background of maintained local estradiol concentrations and optimized uterine blood flow. Any violation of this hormonal equilibrium triggers a cascade of dysfunction, leading to hyperplastic disorders or atrophy. Contemporary research has identified complex intercellular relationships where growth factors, cytokines, and the cellular immune system act as co-regulators of endometrial proliferative activity, maintaining a critical balance between cell growth and apoptosis to ensure tissue homeostasis (Moore et al., 2019). [9].

To establish the general metabolic status, a comprehensive quantification of protein-lipid metabolism and aminotransferase activity was conducted across the estrous stages. Total serum protein serves as a primary indicator of vital activity, acting as the plastic material for all tissue organs and a catalyst for biochemical signaling. Our results demonstrate that during estrus, the total blood protein significantly ($p < 0.001$) escalates to a peak, showing a mathematically verified increase of 7.6% compared to the metestrus phase and an 8.7% rise relative to proestrus. This surge reflects the massive mobilization of protein components required for the rapid proliferation of the

functional endometrial layer and the synthesis of protein-rich cervical mucus (mucin and glycoproteins). [2,4].

Lipid metabolism follows a similar upwards trajectory during the excitation stage; lipids and neutral fats are essential for the structural organization of cell membranes and the stabilization of antioxidant systems under stress. The concentration of total lipids during estrus significantly increased by 29.5% compared to metestrus ($p < 0.001$) and by 14.76% relative to proestrus ($p < 0.001$). Correspondingly, total cholesterol—the fundamental precursor for steroid hormone biosynthesis—rose by 46.6% during estrus compared to the flowering of the corpus luteum (metestrus) and by 23.9% relative to proestrus ($p < 0.001$). This intensive lipid accumulation within epithelial cells acts as a functional barrier, protecting the uterine environment during the heightened activity of the estrus phase. Conversely, in cows suffering from postpartum complications (endometritis or retained placenta), lipid levels remained significantly lower (by 14.19% to 32%), indicating a failure of the endometrial barrier function and a suppression of necessary proliferative processes. (Fig. 1).

The quantitative analysis of globulin fractions revealed a physiological trajectory congruent with the total protein dynamics, where all indicators remained within strictly defined homeostatic norms yet exhibited statistically significant inter-group variance. This differentiation reflects the adaptive redistribution of protein sub-fractions—specifically alpha-, beta-, and gamma-globulins—to support the humoral immune response and the transport of steroid ligands during the peak of the estrous cycle. Lipids, acting as complex ergomic compounds that encompass neutral fats and steroids, exert a profound regulatory influence on the renewal of uterine structures, the modulation of maternal-embryonic immune relationships, and the overall fertilization success (Moore et al., 2019).

Lipids play a multifaceted role in the structural-functional organization of endometrial cells, serving as both integral membrane components and precursors for secondary messengers in intracellular signaling pathways. Their participation in the regulation of membrane stability and the orchestration of inflammatory and immunological reactions is paramount for maintaining tissue integrity during the intensive remodeling phase of the estrous cycle. Under the influence of negative exogenous stressors, the bovine organism frequently undergoes oxidative stress, leading to a precipitous increase in reactive oxygen species (ROS). This triggers the mobilization of antioxidant defense systems and the activation of specialized lipid-mediated metabolic pathways designed to stabilize cell membranes and preserve the functionality of the secretory apparatus under extreme physiological conditions (Faria & Simões, 2015; El-Gendy & Derbalah, 2021).

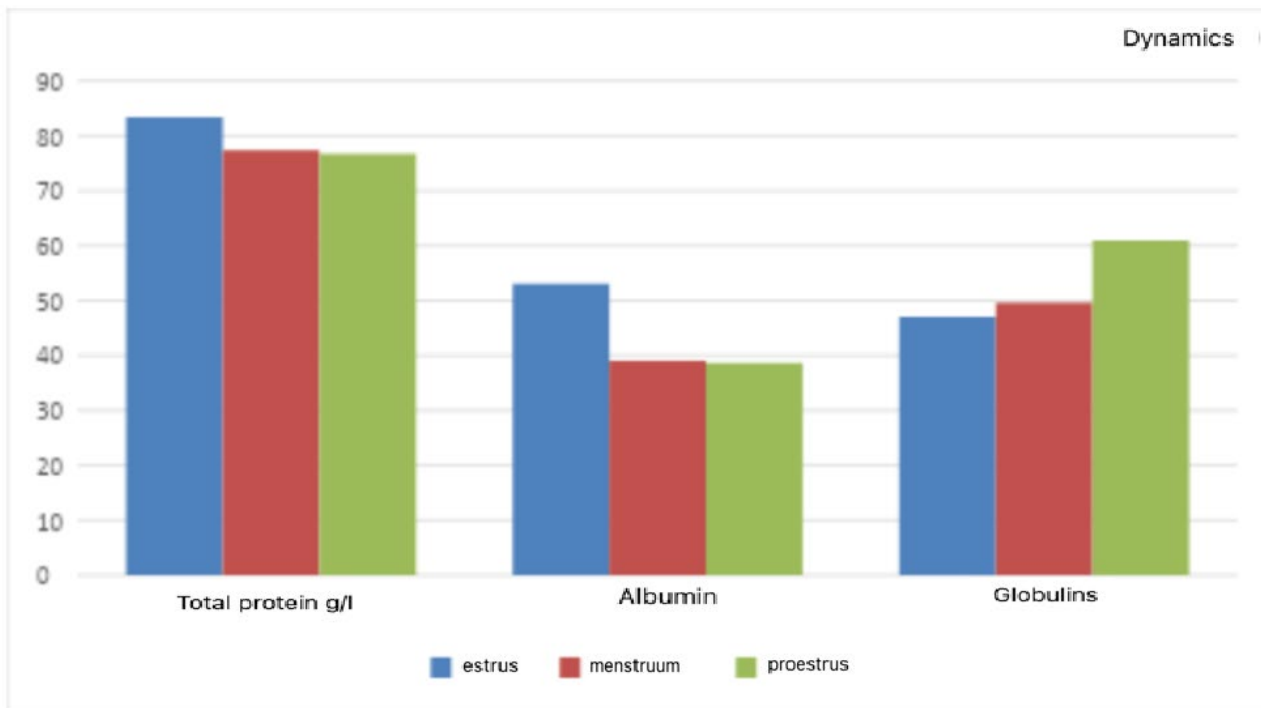


Fig. 1. Dynamics of protein metabolism in the body of cows during the estrous cycle

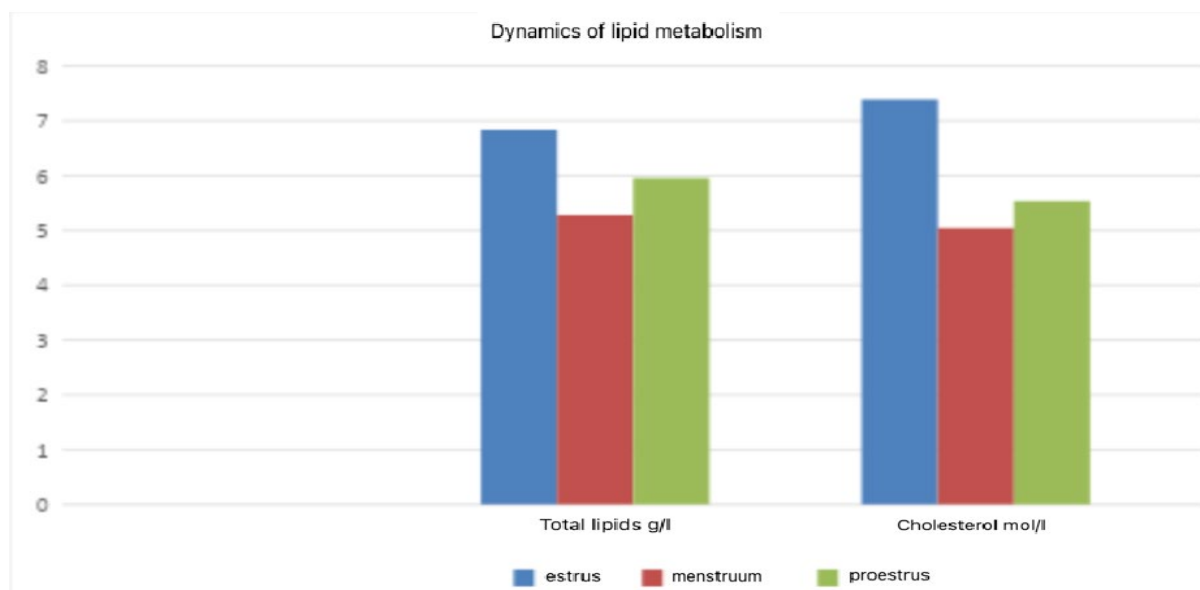


Fig. 2. Dynamics of lipid metabolism in the body of cows during the estrous cycle.

Steroid hormones, which dictate the rhythm of the sexual cycle, are fundamentally derived from cholesterol through a series of complex enzymatic transformations. Cholesterol is not merely a precursor but a vital structural element that determines the fluidity and permeability of all cellular membranes within the reproductive tract. A precise homeostatic balance between cholesterol concentrations and the unsaturated fatty acid profiles of phospholipids is mandatory for the preservation of membrane integrity. By directly modulating the physical state of the

phospholipid bilayer, cholesterol regulates the functional conformation of membrane-bound receptor proteins, including transferrin, nicotinic, and acetylcholine receptors, as well as the critically important oxytocin and rhodopsin receptors (Moore et al., 2019). This molecular architecture ensures that the endometrium remains highly responsive to the hormonal surges required for successful ovulation and subsequent nidation.

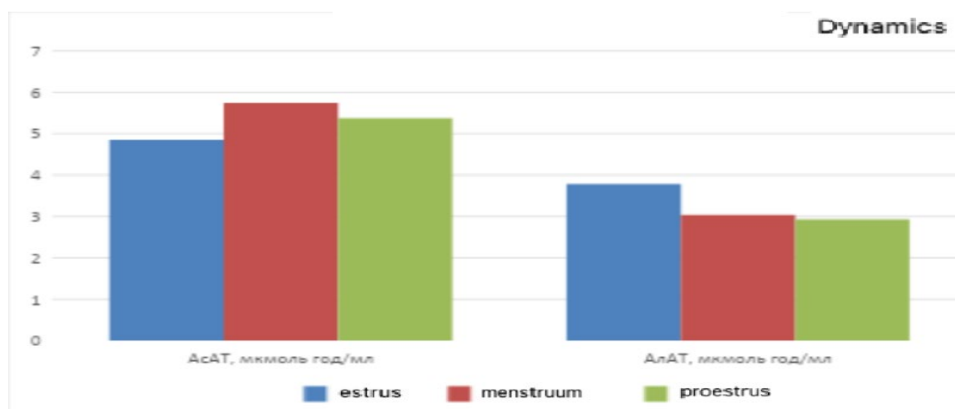


Fig. 3. Dynamics of aspartic and alanine transferase activity in the body of cows at different stages of the estrous cycle n=11-20. (M± m)

The quantitative evaluation of lipid metabolism during the estrous cycle reveals a statistically significant ($p < 0.001$) surge in total lipids during the estrus phase, exhibiting a mathematically verified increase of 29.5% compared to the metestrus period and 14.76% relative to proestrus. This accumulation is directly associated with the accelerated mitotic activity of endometrial epithelial cells, which function as a biological reservoir for neutral fats. Furthermore, the elevation in lipid concentrations serves as a critical component of the endometrial barrier function, providing the necessary structural stability for cells undergoing rapid proliferation. In contrast, the significant depletion of lipids observed during the flowering stage of the corpus luteum (metestrus) acts as a biochemical indicator of epithelial exposure and the initiation of localized tissue destruction.

A parallel and even more pronounced trajectory is observed in cholesterol dynamics, where levels during estrus escalate by 46.6% compared to metestrus and by 23.9% relative to proestrus ($p < 0.001$). This surge is physiologically indispensable for the heightened biosynthesis of steroid hormones required to sustain the phenomenon of heat (estrus) and ensure the receptivity of the uterine environment. Crucially, in cows experiencing delayed restoration of sexual cyclicity post-partum, cholesterol and lipid levels remain significantly lower (by 14.19% to 32%), indicating a profound suppression of the barrier function and hormonal signaling pathways caused by parturition-related trauma and subsequent metabolic failure (Bondarenko et al., 2023).

The activity of intracellular enzymes, specifically aspartate (AST) and alanine (ALT) aminotransferases, provides a window into the intensity of protein metabolism

and cellular remodeling. As catalysts for amino acid metabolism, these enzymes are mobilized to meet the increased energy and plastic needs of the reproductive tract. While AST is traditionally viewed as an indicator of cytoplasmic integrity, ALT—distributed across both cytoplasm and mitochondria—reflects the deeper metabolic intensity of the tissue. Our findings indicate a unique enzymatic inversion during estrus: AST activity significantly decreases by 15.5% compared to metestrus and by 9.8% relative to proestrus ($p < 0.001$), signaling a shift from catabolic cell destruction to constructive protein synthesis.

Conversely, ALT activity during estrus exhibits a sharp increase of 19.6% relative to metestrus and a 29.1% surge compared to proestrus ($p < 0.001$). This intensive activation of alanine aminotransferase facilitates the physiological increase in total proteins and lipids, creating the optimal metabolic substrate for follicular maturation and subsequent zygote implantation. The dominance of the cytoplasmic-mitochondrial enzymatic fraction during the excitation stage substantiates the transition of the endometrium into a state of maximal functional readiness. These enzymatic fluctuations, while remaining within physiological limits, act as sensitive biomarkers of the organism's adaptive capacity and the precision of the neurohumoral regulation of the reproductive cycle.

Conclusions and prospects for further research.

The clinical and biochemical manifestation of the estrus phase in cows is driven by a systemic activation of physiological and metabolic pathways, primarily mediated by the mobilization of key tissue enzymes and the redistribution of intracellular substrates. Our findings substantiate that the peak of the excitation stage is characterized by a statistically significant ($p < 0.001$) escalation in blood serum total protein, which increases by 7.6-8.7% as a direct consequence of the intensive proliferation of the myometrium and the functional endometrial layer. This anabolic surge is accompanied by a concurrent rise in total lipids (+29.5%) and cholesterol (+46.6%), reflecting the heightened demand for structural membrane components and steroid precursors necessary for effective ovulation and subsequent nidation. Furthermore, the dynamic shifts in aminotransferase activity—specifically the adaptive inversion of AsAT and AlAT—serve as precise indicators of the intensity of cellular remodeling and the organism's readiness for zygote implantation. This enzymatic profile, characterized by the dominance of the cytoplasmic-mitochondrial fraction, underscores the transition of the reproductive system from a state of post-ovulatory inhibition to maximal functional activity.

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МОРФО-БІОХІМІЧНЕ РЕМОДЕЛЮВАННЯ ЕНДОМЕТРІЇ КОРІВ: ДИНАМІКА МЕТАБОЛІЧНИХ ТА ФЕРМЕНТНИХ ПРОФІЛІВ ПРОТЯГОМ ТЕЧОВОГО ЦИКЛУ

Анотація

Сучасний корпус наукової літератури, незважаючи на його глибину, не забезпечує комплексного та високороздільного картування морфологічної та гістологічної динаміки, що регулює формування ендометрію у плодів великої рогатої худоби, що значно ускладнює розробку прецизійних методів фізіологічної корекції та метаболічної стимуляції під час вагітності та формування плаценти. Ця відсутність систематичних даних особливо важлива для розуміння формування материнської плаценти під час фази еструсу, де структурна цілісність тканини визначає успіх усіх наступних репродуктивних стадій.

Складний процес гаметогенезу та одночасного дозрівання репродуктивних органів зумовлений інтенсивними реакціями трансамінування, що характеризуються значним підвищенням трансферазної активності, де цитоплазматично-мітохондріальна фракція відіграє функціонально домінуючу роль у підтримці клітинного енергетичного балансу. Аналітично важливо, що стадія еструсу діє як метаболічний каталізатор, запускаючи каскад активації тканинних ферментів та системних метаболічних сплесків, тоді як наступні фази гальмування та врівноваження визначаються компенсаторним зниженням цих біоенергетичних явищ для відновлення базального гомеостазу.

Сучасна репродуктивна біологія твердо встановила, що функціональний стан залозистих епітеліальних клітин матки та пов'язаних з ними стромальних компонентів характеризується глибокою гетерогенністю протягом усього естрального циклу, станом «динамічної нестабільності», який парадоксально необхідний для підтримки довгострокового тканинного гомеостазу. Дослідники висувають гіпотезу, що ця клітинна гетерогенність слугує еволюційною адаптацією, дозволяючи ендометрію швидко набирати резервні структурні одиниці у відповідь на мінливе гормональне середовище жіночого організму.

Близько в передовуляторний період морфометрична товщина ендометрію та епітелію яйцепроводів досягає свого фізіологічного zenіту, що зумовлено різким зростанням концентрації стероїдних гормонів, що стимулює епітелій шийки матки синтезувати та виділяти максимальні об'єми низьков'язкого слизу, збагаченого муцинами, глікопротеїнами та високомолекулярними білковими комплексами.

Наші ретельні емпіричні дослідження підтверджують, що фаза еструсу характеризується статистично значущим ($p < 0,001$) зростанням загального білка сироватки крові, яке збільшується на 7,6% порівняно з метеструсом та на 8,7% порівняно з проеструсом, що відображає масивне гіпертрофічне та проліферативне розширення міометрія та функціонального шару ендометрію.

Залозисті епітеліальні одиниці зазнають серії інтегрованих структурно-функціональних метаморфоз, де фаза проеструсу характеризується агресивною проліферацією та апікальним переміщенням запасів глікогену в маткових залозах. Тривалість цих клітинних зрушень суворо регулюється швидкістю дозрівання домінантного фолікула яєчника, що забезпечує точну синхронізацію між овуляцією та рецептивністю ендометрію. Застосування цих локалізованих метаболічних даних на макрорівні сприятиме суттєвому збільшенню показників відтворення стада, тим самим оптимізуючи економічну ефективність м'ясної та молочної промисловості за допомогою науково обґрунтованої моделі фізіологічного ремодельовання.

Ключові слова: *ремодельовання ендометрію, біохімічний гомеостаз, естральний цикл, розмноження великої рогатої худоби, метаболічний статус, ферментативна активність.*

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