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Features of the Imbalance in the Mass of Components of the Uterine-Vaginal Mucus of Cows Due to the Harmful Effects of Endogenous Factors

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Abstract. The ever-increasing harmful effect of endo- (products of inflammatory processes of genital tissues) and exogenous factors (environmental conditions) on the body of women and animals (cows) leads to a substantial decrease in ova fertilisation during mating and artificial insemination. Therefore, the purpose of this study was to identify changes in the signs of physical condition (colour, consistency, fluidity, impurities, etc.) and ratios (homeostasis) of H_2O mass, organic (OS) and inorganic (IS) substances of uterine-vaginal mucus (UVM) should be used to assess their harmful effect on the fertilisation of cows after their first insemination. The following methods were used for this study: ocular estimate of the physical condition of freshly obtained mucus; gravimetric assessment of the features of changed mass parameters of its dry residue (DR) components; mathematical and statistical analysis of certain indicators. Determined by the gravimetric method, the features of the imbalance of mass (g, mg), its distribution (%) and ratios (Im:1, Ic:1) in the system of the "medium – substance" type due to the harmful effects of endogenous factors (products of inflammatory processes) indicate that the secretions of the genitals released during oestrus are characterised not only by changes in signs, but also ±2-4 times different from the norm (control) indicators of the mass of components. Under such circumstances, the distribution of substances in the DR samples of the experimental group is expressed by the series, where the mass OS_1>IS>OS_2, but in the control group – OS_1>IS>OS_2. This means that the products of inflammatory processes released by the genitals of cows change the norm of homeostasis of the mass (distribution of components) of OS and IS of the biological system of the "medium – substance" type. Indicators of the obtained mass ratios between component pairs H_2O: DR; H_2O: OS_2, H_2O: OS_1, H_2O: IS (control group – 63:1, 586:1, 231:1, 109:1; experiment group – 42:1, 310:1, 87:1, 112:1), except for the H_2O: IS pair ($P<0.05$), indicate the presence of probable changes in the homeostasis of OS and IS in the "H_2O – components" system. The high probability ($P<0.02$; <0.001) of the detected changes is inherent in the "DR – components" system, namely: if the average ratio indicators of pairs DR: OS_2; DR: OS_1; DR: IS samples of the control group are 9:1, 4:1, 2:1, then the same indicators in the experimental group are 7:1, 3:1, 2:1, respectively

Keywords: uterine-vaginal mucus of cows, dynamics and imbalance of homeostasis of the mass of organic and inorganic substances

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INTRODUCTION

Modern researchers associate the issue of reducing the fertilisation rate of ova under conditions of artificial insemination and natural mating of cows and women with an elevated level of microbiocoenosis in the environment of the secretions of their genitals (vagina, uterus) (Srinivasan *et al.*, 2021). The harmful effects of vaginal microflora and products of inflammatory processes (endogenous factors) accumulated by genital tissues (hypotrophy and atrophy of the vaginal mucosa, cervical neoplasia, acute and chronic endometritis) violate the norm of ratios of components of uterine-vaginal mucus (UVM), the parameters of which adversely affect the reproductive ability of females (Armengol & Fraile, 2015; Boby *et al.*, 2017; Emre *et al.*, 2021).

In this regard, it is known that the accumulated products of inflammatory processes increase the level of thiol/disulphide homeostasis, which is a sensitive indicator of oxidative stress in the body (Emre *et al.*, 2021); the pH level changes from acidic to alkaline (Berre *et al.*, 2021; Charonis & Larson, 2006); follicular fluid and UVM contain elevated concentrations of pro-inflammatory interleukins (Boby *et al.*, 2017; Brodzki *et al.*, 2015; Mounir *et al.*, 2018). This leads to changes in the hormonal and enzyme statuses of the body during puberty (Gonchar, 2014; Denisenko *et al.*, 2008), increased risk of miscarriage (Adnane & Chapwanya, 2022; Ki, *et al.*, 2014; Srinivasan *et al.*, 2021), a low-weight foetus is born (Hernandez-Castellano *et al.*, 2020). The level of fertilisation of cows also depends on the quantitative and qualitative composition of the UVM, which contributes to the active movement of sperm through the channels and ducts of the female genital tract towards the ovum (contact) (Maksymyuk *et al.*, 2021; Maksymyuk *et al.*, 2019).

However, even though the functional and biochemical aspects of the above issue are widely covered by the studies of Ukrainian (Gonchar, 2014; Denisenko *et al.*, 2008; Kraevsky *et al.*, 2020) and foreign scientists (Berre *et al.*, 2021; Rodder & Mathew, 2018; Sheldon *et al.*, 2020), there is still no particular information about the features of the correlation between changes in the colour and consistency of the UVM and the mass indicators of its components. Therefore, the task of this study was to assess the physical and chemical state of the samples of the UVM according to the obtained limits of volume parameters (8-40 cm^3), signs of colour (from transparent to grey-white), consistency (from fluid, thick-viscous to liquid), ratios of the content (or homeostasis of components) and their harmful effect on the fertilisation rate of cows after the first insemination.

MATERIALS AND METHODS

Experiments on animals were carried out according to the norms and principles of bioethics of conducting experiments and for other scientific purposes that require compliance with the requirements of directives of the European Parliament and the Council of the European Union (Directives of the European Parliament..., 2010); information of the Verkhovna Rada (IVR) on the protection of animals from ill-treatment (Law of Ukraine "On the Protection", 2021); regulations of the Bila Tserkva National Agrarian University on the treatment of animals in scientific research (Regulations of Bila Tserkva National Agrarian University..., 2018).

The features of volume dynamics (cm^3), colour and consistency characteristics (ocular estimate) and mass (g, mg,%) of the UVM components were established according to the requirements of quantitative (gravimetric) and qualitative analysis methods (Williams *et al.*, 2005; World Health Organisation, 2000). Specially introduced (Maksymyuk *et al.*, 2021; Maksymyuk *et al.*, 2019) formulas (1) and (2) were used to establish the correlation between the UVM signs and the indicators of its physical and chemical state (Δ changes, or an imbalance of homeostasis of a biological system of the "medium — substance" type):

$$\Delta = x_0 + x_i \quad (1)$$

$$\sum \Delta m = \Delta m_1 + \Delta m_2 + \Delta m_3 + \Delta m_i \quad (2)$$

where x_0 and Δm are parameters of the sum ($\sum$) of the masses of the UVM components; Δm_1 are the parameters of the mass of organic substances (OS_1), which burn on an open burner fire at 520-530°C; Δm_2 are the parameters of the mass of organic substances (OS_2), which are burned in a muffle furnace at 650°C; Δm_3 are the parameters of the ash mass, or heat-resistant inorganic substances (IS).

The detected changes were evaluated by indicators (M±m, Cv, P, lim) of the statistical method (Sharaf *et al.*, 1989) of the Microsoft Excel software.

During the mating season (April–June 2021) of the first-third (1-72 hours) days of oestrus, during the detected signs (behavioural arousal, swelling of the external genitalia, mucus discharge) of desire (Bugrov, 2013), UVM samples were taken by gloved hand from the vagina of Ukrainian black-spotted (Holsteinised) breeds of 3-9-year-old cows according to the method of (Williams *et al.*, 2005). The native state of the selected samples was evaluated by volume indicators (cm^3), signs of colour (transparent, grey-white) and consistency (thick-viscous, viscous, liquid), the presence of extrinsic impurities (protein plates, pus, blood, etc.).

Mucus samples selected according to these characteristics were divided into control (n=10) and experimental (n=4) groups. The control group included samples, the signs of which indicate normal function; the experimental group included samples with dysfunction of genital tissues.

Features of changes in the mass of components (N_2O, the total content of OS and IS) of the UVM was determined by drying in a thermostat at 105°C. Changes were recorded until the fourth decimal (0.0001) obtained by a microbalance ceased to change. OS_1 mass was determined after burning powdered samples of dry residue (DR) ground in a mortar on an open burner fire at 520-530°C; OS_2 and IS — in a muffle furnace at 650°C. Relative indicators were calculated by the composite proportion of absolute parameters of the H_2O mass, OS and IS (DSTU B B.2.1-16: 2009, 2010).

The designation of the features of the mass imbalance of the UVM components and its DR is given by non-systemic absolute (Im:1 — g, mg) and relative (Ic:1 —%) indicators. The methodology for determining and assessing the harmful effects of mass imbalance is represented by indicators of fractions of aqueous extracts of powdered samples of the DR of the UVM (Maksymyuk *et al*., 2021; Maksymyuk *et al*., 2019).

RESULTS AND DISCUSSION

Assessment of signs, indicators of the UVM volume, and the number of fertilised cows after the first insemination. Analysis of the parameters of the obtained UVM volume in both groups of cows (control, experimental) shows that its average indicators have different values (20 vs. 14 cm^3). In this regard, it is advisable to note that the volume limit (lim: min=10 vs. 8; max=40 vs. 21) is characterised by a high indicator (43-37%) of the coefficient of variation (Cv), but its difference between the groups is insignificant (6%). This means that with such variability in the UVM volume, the probability of its difference (P>0.2) between groups remains low (Table 1).

Table 1. *Status of UVM samples and results of insemination of cows in groups*

Stat. indicator	Sample volume, cm^3 (m_0)	Percentage of samples by signs: Colour, %	Percentage of samples by signs: Consistency, %	Percentage fertilised cows, %
Samples of the control group of cows (n=10)				
M±m	20.20±2.76	Transparent — 80 Grey-white — 20	Thick-viscous — 30 Viscous and liquid — 70	75
Cv	43			
Lim	10-40			
Samples of the experimental group of cows (n=4)				
M±m	14.12±2.58	Transparent — 50 Grey-white — 50	Viscous — 50 Liquid — 50	30
Cv	37			
Lim	8-21			
P	>0.2			

However, even though the difference in the parameters of the UVM volume of both groups of cows is insignificant, the difference in the signs of transparent and grey-white colour is substantial, namely: if the percentage of samples of transparent colour in the control group is 80%, and in the experimental group — 50%, then grey-white colour is 20% vs. 50%, respectively. Therefore, the difference in the characteristics of the mucus colour scheme between the samples of the control and experimental groups is ±30%.

The difference between thick-viscous and liquid samples of the control group (30% and 70%) and viscous and liquid samples of the experimental group (50% and 50%) is 10% less than the colour signs. Notably, there are no signs of a thick-viscous consistency in the samples of the experimental group. The presence of 70% of viscous and liquid samples in the control group is represented by the same percentage of viscous and liquid samples in the experimental group.

Thus, if considering the results of the first stage of assessment of the physical and chemical state of the UVM samples, different signs of their colour and consistency may be associated with a substantially higher percentage (75%) of fertilised cows of the control group after their first insemination.

Features of distribution of absolute mass parameters (g, mg) of the UVM components and its DR.

After three-day (72 hours) drying of control and experimental UVM samples in a thermostat at 105°C it is established that the distribution of absolute mass parameters H_2O (m_1) and the sum of the masses of the DR components (m_2), respectively, is 19.7 vs. 13.4 g and 329.4 and 325.3 mg, respectively (Fig. 1a, b). It should also be added that provided a sufficiently wide limit of variation (CV=39-61%) of the H_2O and DR mass parameters, the difference between them is unlikely (P>0.2).

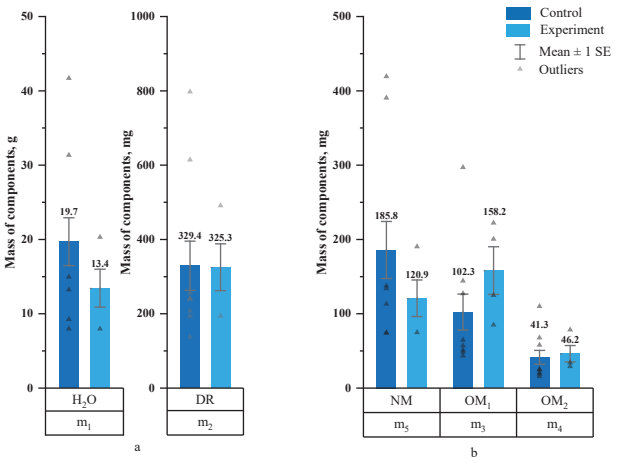

Figure 1*. Absolute mass parameters of the UVM components and its DR*

Note*: a) (m_1, m_2) are the UVM components' masses; b) (m_5, m_3, m_4) are the DR components' masses*

After burning powdered DR samples of the UVM on an open burner fire at 520-530°C and in a muffle furnace at 650°C, it turned out that the average mass index of IS (m_5) of the control group is 1.5 times larger than the experimental group, but for the OS_1 (m_3) and OS_2 (m_4) masses is 1.5 and 1.1 times lesser, respectively. If the mass of the components of the series (IS-OS_1-OS_2) of control samples is 186-102-41, then of experimental samples — 121-158-46 mg. This means that the correlation between the mass parameters of IS and OS of a biological system of the "medium-substance" type is expressed inversely. The harmful and/or protective effect of the products of inflammatory processes accumulated by the tissues of the genitals of cows of the experimental group is characterised by a high mass index of isolated IS, but low OS_1 and OS_2 in the control group. At the same time, even though the coefficients of variation of the mass indicators of OS_2 (76% and 48%) and IS (60 and 41%) are quite high, the probability of their difference between the groups is 97 (P<0.02) and 99% (p<0.001), respectively.

Features of distribution of relative mass parameters (%) of components of the UVM and its DR

Calculated relative mass indicators N_2O and OS and IS DR samples of the UVM (Fig. 2a, b) indicate that the remainder of the IS mass, which was not burned as a result of burning samples of the control group, is 0.92% of the total content of DR components. Under open fire conditions, 0.48% of the mass of OS_1 substances burns, and after combustion in the muffle — 0.20% of OS_2 substances.

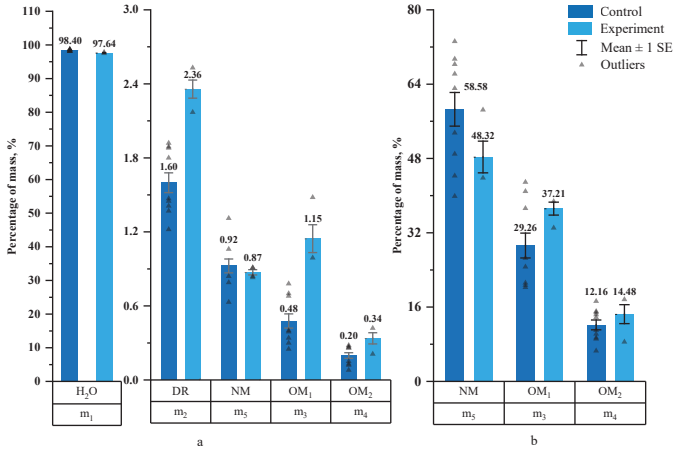

***Figure 2**. Relative mass parameters of the components of the UVM and its DR*
***Note:** a) (m_1, m_2, m_5, m_3, m_4) are the masses of UVM components; b) (m_5, m_3, m_4) are the masses of DR components*

The above means that the dynamics of the distribution of its components (IS – 0.92%>OS_1-0.48%>OS_2-0.20%) in the DR samples of the UVM of the control group of cows characterises a series where the percentage of the remaining mass of refractory IS is 1.8 times greater than the mass of burned OS_1 substances, and OS_1 – 2.5 times larger than the OS_2. However, the percentage of mass burned organic (OS_1, OS_2) and unburned refractory IS of the DR of the UVM of the experimental group are placed differently, namely: the mass index of the OS_1 (1.15%)>IS (0.87%)>OS_2 (0.34%).

The presented changes in the relative (%) parameters of the UVM mass under harmful endogenous factors of influence indicate that the products of inflammatory processes accumulated by the tissues of the genitals of cows of the experimental group do not substantially affect the mass indicators of IS (0.87 vs. 0.92%). However, the mass of OS_1 and OS_2 of the UVM of cows of the experimental group is 2.4 (1.15 vs. 0.48%) and 1.7 (0.34 vs. 0.20%) times greater than that of the control group.

The revealed difference is confirmed the by 3.8-1.4 times greater minimum (19 % vs. 5%) and maximum (38% vs. 27%) parameters of the mass variability limit (Cv=19-38% vs. 5-28%) of the UVM components of the control group relative to the experimental group. The probability of their difference is 95-99% (P<0.05; P<0.001). Notably, the order of placement of mass percentages of the UVM components (Fig. 2a) and its DR (Fig. 2b) in the control group (IS>OS_1>OS_2) and the experimental group (OS_1>IS>OS_2) is similar.

Features of mass ratios of the UVM components and its DR.

The features of the mass distribution of the UVM components illustrated (Fig. 2a, b) a high (P<0.001) probability of its difference between the samples of the control and experimental groups of cows. Therefore, to detail and specify the features of the correlation between the imbalance of the mass of the UVM components and the state of reproductive ability of females (cow) and the reaction of genital tissues to the harmful effects of endogenous factors (products of inflammatory processes), the above facts are supplemented by changes in the mass ratios of the components of the biological system of the "medium (H_2O UVM) — substance (OS, IS DR)".

The results presented in Figure 3a, b show that one part of the mass of substances of the DR of the UVM (63:1 vs. 42:1) of the control group of cows bonds 1.5 times the mass of evaporated H_2O, than the cows of the experimental group. This feature is also typical for the OS mass that burns at different temperatures. Thus, if the components of the control group (OS_1), which burn at 520-530°C, bond 2.6 times the mass of H_2O (231 vs. 87:1), then substances (OS_2) burned in the muffle at

650°C — only 1.9 times more (586 vs. 310:1). It is also advisable to emphasise that the high (P<0.001) ability of the OS_1 components to bond H_2O is accompanied by a low (P<0.05) ability of the components of OS_2 and IS. Under such circumstances, the limit of the coefficient of variation of certain mass ratios of substances in the control group is 16-48%; the experimental group is 5-32%, which is 3.0-1.5 times more.

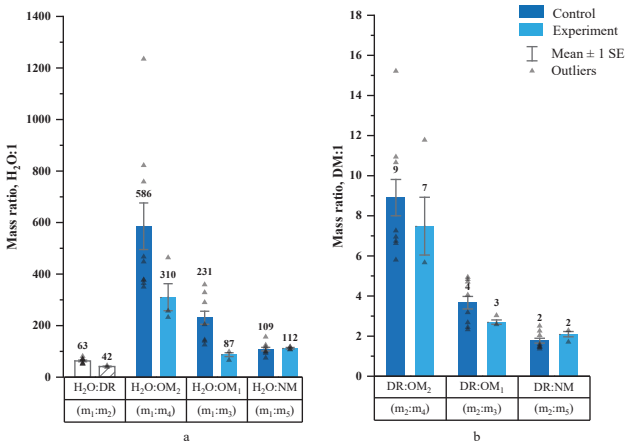

***Figure 3**. Mass ratio of the UVM components and its DR*

***Note:** a) H_2O:1 is the ratio of the mass of the evaporated H_2O (m_1) of the UVM to mass (m_2) of the DR substances that burn under different temperature conditions (OS – m_4, m_3), and to the remaining mass (IS – m_5) of refractory inorganic substances; b) DR:1 is the mass ratio of the DR (m_2) to mass (m_4, m_3) of the burnt organic and refractory inorganic substances (m_5)*

The identified features of the difference in the mass ratios of pairs DR: OS_1 and DR: OS_2 UVM in cows of the experimental and control groups complement the data presented in Figure 3b. The registered mass parameters of the DR of the UVM indicate that the sex glands of cows of the control group (OS_2 and OS_1) synthesise 1.3-2.0 times more mass of components (9 vs. 7 and 4 vs. 3) than the glands of cows of the experimental group. However, if the parameters of the ratios of the pairs DR: OS_1 (4 vs. 3:1) and DR: OS_2 (9 vs. 7:1) are compared with the pair DR: IS (2 vs. 2:1), it turns out that the obtained values indicate their inverse correlation. The limit of the coefficient of variation of the ratios of the mass of DR: OS_1 and OS_2 is 4-32%, DR: IS — 6-22%. Under these circumstances, the probability of differences in the mass ratios of DR substances remains high (P<0.02-0.001).

Analysis of the estimated features of raw (not dried) UVM samples of the groups under study indicates that with almost identical volume parameters (20-14 cm^3), its colour and consistency differ: 80% of the samples of the control group have a transparent colour, but 20% — grey-white; their consistency is thick-viscous (30%) or viscous and liquid (70%); 50% of the samples of the experimental group have a transparent or grey-white colour, the consistency is viscous and liquid.

The UVM samples of the experimental group have 0.76% less water. Absolute values of the total OS mass (158+46=204 mg) are 1.43 times higher (102+41=143 mg); IS mass of the control group (186:121 mg) is 1.5 times higher than that of the experimental one. The order of distribution of the mass of the constituent mucus samples of cows of the experimental group is presented in a series where the indicator OS_1>IS>OS_2, but is different in the control group, namely: IS>OS_1>OS_2. The unequal mass of components of the "medium — substance" type system, the change of which is expressed by absolute (Im) and relative (Ic) indicators of H_2O and OS and IS of the DR of the UVM leads to the fact that the correlation (Fig. 3a) H_2O:∑OS of the control group (586281=867:1) is 2.2 times larger than that of the experimental group (310+87= =397:1), while H_2O: IS correlations remained almost the same (109-112:1). Under such circumstances, the correlations (Fig. 3b) of the components (9+4:1 vs. 4+3:1) of the DR:∑OS are 1.9 times larger, but those of the DR: IS (2 vs. 2:1) are almost the same.

Summarising the obtained and analysed results of the experiment, it should be expected that an objective indicator of quantitative changes in the tissues of the genitals of women and animals (cows) under the endo- (products of inflammatory processes) and exogenous (feeding, maintenance, etc.) factors of harmful influence, in the future may be the limits of the parameters of the mass of components (H_2O, OS, IS), which indicate an imbalance of their homeostasis in the "medium — substance" system.

Without sources of scientific information on the Internet on certain features of the imbalance of parameters of H_2O content and the sum of masses of OS and IS of the UVM due to the harmful effects of endogenous factors on women and cows, their discussion is limited to analysis of statistical results.

CONCLUSIONS

1. In case of improbable (20 vs. 14 cm^3, P>0.2) changes in the UVM volume, the colour and consistency of samples of cows of the experimental group undergo substantial changes: if 50% of experimental samples are

transparent or grey-white with opaque viscous or liquid consistency, 80% of control group are transparent, and 20% are grey-white; 30% have a thick-viscous, and 70% — viscous or liquid consistency.

2. The effect of products of inflammatory processes (endogenous factor of harmful effects) on the functional state of the genitals is expressed by the inverse correlation of the parameters of the mass of OS to IS. This leads to the fact that the order of distribution of the mass of the components of the DR in the experimental group is presented in a row, where the mass indicators are OS_1>IS>OS_2, but in the control group these indicators are IS>OS_1>OS_2.

3. Changes in the homeostasis of the mass components of the UVM in the "medium — substance" system, which are expressed by external signs of its physical and chemical state (colour, consistency, impurities of pus and blood, etc.), is the reason that the percentage of fertilised cows in the experimental group (30%) after the first insemination is 2.5 times smaller than that in the control group (75%).

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Особливості дисбалансу маси складових матково-вагінального слизу корів за шкодочинного впливу ендогенних факторів

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Анотація. Постійно зростаюча шкодочинна дія ендо- (продукти запальних процесів тканин статевих органів) та екзогенних факторів (умови зовнішнього середовища) на організм жінок і тварин (корови) призводить до суттєвого зниження заплідненості яйцеклітин при паруванні та штучному осіменінні. Тому метою досліджень було виявлені зміни ознак фізичного стану (колір, консистенція, плинність, домішки тощо) та співвідношень (гомеостаз) маси H_2O, органічних (ОР) і неорганічних (НР) речовин матково-вагінального слизу (МВС) використати для оцінки їх шкодочинного впливу на рівень запліднюваності корів після їх першого осіменіння. Для досліджень застосували методи: окомірної оцінки фізичного стану свіжоотриманого слизу; гравіметричної оцінки особливостей змінених параметрів маси складових його сухого залишку (СЗ); математико-статистичного аналізу визначених показників. Визначені гравіметричним методом особливості дисбалансу маси (г, мг), її розподілу (%) та співвідношень (Im:1, Ic:1) у системі типу «середовище — речовина» за шкодочинної дії ендогенних факторів (продукти запальних процесів) свідчать, що виділеним під час тічки секретам статевих органів властиві не лише зміни ознак, але і в ±2—4 рази інші ніж норма (контроль) показники маси складових. За таких обставин розподіл речовин у зразках СЗ дослідної групи виражено рядом, де маса OP_1>HP>OP_2, але контрольної — HP>OP_1>OP_2. Це означає, що виділені статевими органами корів продукти запальних процесів змінюють норму гомеостазу маси (розподілу складових) ОР і НР біологічної системи типу «середовище — речовина». Показники отриманих співвідношень маси між парами складових H_2O: СЗ; H_2O: OP_2, H_2O: OP_1, H_2O: НР (контроль — 63:1, 586:1, 231:1, 109:1; дослід — 42:1, 310:1, 87:1, 112:1), за винятком пари H_2O: НР ($P<0,05$), свідчать про наявність вірогідних змін гомеостазу ОР і НР у системі «H_2O — складові». Висока ймовірність ($P<0,02$; $<0,001$) виявлених змін властива системі «СЗ – складові», а саме: якщо середні показники співвідношень пар СЗ: OP_2; СЗ: OP_1; СЗ: НР зразків контрольної групи становлять 9:1, 4:1, 2:1, то дослідної — 7:1, 3:1, 2:1

Ключові слова: матково-вагінальний слиз корів, динаміка і дисбаланс гомеостазу маси органічних і неорганічних речовин