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IMBALANCE OF SUBSTANCES IN UTERINE-VAGINAL MUCUS IN THE EARLY DAYS OF THE ESTROUS CYCLE

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Background. The macroscopic evaluation of the color and consistency of cow uterine-vaginal mucus (UVM) samples revealed that during days 1–3 of the estrous cycle, 45.4 % of the animals in the studied sample (n = 11) had a clear-glassy color, 27.3 % had blood impurities, 18.2 %, and 9.1 % – altered coloring. Additionally, 36.4 % of the samples had thick-viscous, liquid consistency, while 63.6 % had liquid texture.

Materials and methods. Mucus was collected from experimental animals at 7–8 a.m. on days 1–3 of the estrous cycle. The volume (cm^3) was measured and the color and consistency of the secretion were evaluated. Indicators of mass (g) and content (%) of water (H_2O), organic substances (OS), and inorganic substances (IS) were determined gravimetrically.

Results. The analysis of the volume and mass indicators of native UVM samples indicates a high (>95 %) error rate in the registered changes of H_2O. However, the error in the sum of the masses OS and IS samples of dry residue (DR) components is completely different. This indicator tends to change on days 1 and 2 ($P < 0.05$), but its stable probability is detected on days 2 and 3, as well as 1 and 3 ($P < 0.001$). Stable changes in the mass OS were detected on days 2 and 3 ($P < 0.01$), while the masses of IS changed on days 1 and 2 ($P < 0.01$) and 2 and 3 ($P < 0.001$). In all other cases, the error exceeds 95 %.

The mass percentages of H_2O, OS, and IS indicate a tendency ($P < 0.05$) for changes in H_2O and OS content on days 1 and 3; IS content changes on days 1 and 2, as well as 2 and 3, but the changes on days 1 and 3 are highly stable ($P < 0.001$). Stable changes in the mass of DR components were only observed on days 2 and 3.

The analysis of homeostasis changes in the mass of native samples indicates that the limit of mean values of UVM:H_2O (1.02–1.04:1) < H_2O:OS (67–77:1) < H_2O:IS (82–439:1), but the OS:IS ratios (1.2–5.5:1) of UVM sample align with the OS:IS ratios (1.2–5.5:1) of DR samples.

The limit of C_v for UVM:H_2O, H_2O:OS, H_2O:IS ratio is 0.8–5.3; 19–27; 46–93 % respectively. The OS:IS ratios of native UVM and its DR samples are the same (49; 63; 31 %). The indicators of UVM:H_2O, H_2O:IS, and OS:IS ratios suggest a tendency for change ($P < 0.05$) on days 1 and 3. Their stable changes ($P < 0.01$–0.001) were observed on days 1 and 2, as well as 2 and 3 for H_2O:IS and OS:IS ratios.

Conclusion. Given the above, there is a need for further thorough investigation of the characteristics of the imbalance in the components' mass of UVM during days 1–3 of the estrous cycle, as well as the response of secretory cells of the mucous membrane of the reproductive organs to the effects of pathological factors. This research will be applied in the development of new methods of prevention, diagnosis, and treatment of reproductive tract diseases in female animals.

Keywords: uterine-vaginal mucus, mass of water, organic and inorganic substances

INTRODUCTION

The indicator of the ratio between the mass of organic molecules (proteins, nucleic acids) and inorganic ions (K^+, Na^+, P^{2-5+}) (as a „trigger") was used for the first time by the Swiss scientist Miescher in Basel in 1874 to justify the initiation of the process of fertilizing salmon eggs (Lamm *et al.,* 2020).

The Estrous cycle duration and reproductive health is known to be associated with absolute and relative quantitative ratios of inflammatory biomarkers (α1-acid glycoprotein (AGP), immunoglobulin A, lactoferrin, lysozyme, pro- (IL-8) and anti-inflammatory (IL-10) cytokines (Adnane *et al.*, 2018b; Sheldon *et al.*, 2019).

Despite this, the specific imbalances in the equilibrium state of the components' mass (H_2O, OS, IS) of the UVM, which are observed during the period from the 1st to the 3rd day under the influence of pathological factors (inflammatory products), still require significant clarification and detailed investigation.

Therefore, the aim of our study was to determine changes in color, consistency, and mass indicators of H_2O, OS, and IS in samples of the UVM during the first three days of the estrous cycle in cows under the influence of certain pathological factors.

MATERIALS AND METHODS

Experiment details. Uterine-vaginal mucus from Ukrainian Black-and-White dairy cows aged 3 to 9 years (n = 11) was analyzed during the first three days of estrous cycle. Mucus samples were collected between 7 and 8 a.m. when estrus signs, including behavioral excitement, swelling of the external genital organs, and mucus discharge

were observed in the cows (Lim *et al*., 2014) The analysis included assessing the color and consistency of the secretion, as well as measuring the mass (g) and content (%) of water (H_2O), organic substances (OS), and inorganic substances (IS), along with their ratio (Icm:1). The obtained secretion was placed in Petri dishes, and the color and consistency were recorded.

Absolute mass of H_2O, OS, and IS was determined using quantitative gravimetric method. Percentages of content and ratio indices of its pairs were calculated using mathematical calculations, namely: UVM*:H_2O; H_2O:OS; H_2O:IS; OS:IS. (Maksym'yuk *et al.*, 2021; Maksymyuk *et al*., 2022).

The investigation of changes was conducted in three stages. During the first stage, color and consistency changes were recorded, as well as absolute mass indicators of components in native samples of UVM and its dehydrated DR. The second stage involved determining the content mass, and the third stage involved calculating the content mass ratios index. All procedures were conducted in accordance with the general ethical principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and in compliance with the Law of Ukraine On the Protection of Animals from Cruelty (No 3447-IV, dated 21.02.2006). Permission to conduct the research was obtained from the Bioethics committee of the Institute of Agricultural Sciences of the Carpathian Region NAAS (protocol No 1 of May 11, 2023).

Statistical analysis. The statistical analysis of the results was performed using Microsoft Excel version 16.56 and expressed as Mean ± Standard Deviation (M ± SD). One way ANOVA was used to assess the significance of variances between the groups, followed by Tukey–Kramer test. Significance levels were determined as follows: * for $P \leq 0.05$, ** for $P \leq 0.01$, *** for $P \leq 0.001$. A significance level of $P \leq 0.05$ was considered indicative of a statistically significant difference. To describe the data in the article and create graphs, Origin Pro 2019 b was used.

Connection of the publication with planned research works. The publication is related to ongoing scientific research projects. The experimental study was conducted within the framework of the research project „Investigation of the homeostasis of organic and inorganic components in amniotic fluid of cows and its correlation with the physical condition of the newborn calf" (State registration No 0121U100404) by the Institute of Agriculture of the Carpathian Region of the National Academy of Sciences of Ukraine and „Development of prognostic and diagnostic immuno-biochemical criteria under the influence of various extreme factors on the organism" (State registration No 021U100163) by Danylo Halytsky Lviv National Medical University.

RESULTS AND DISCUSSION

Step 1. The macroscopic assessment of the native state of UVM samples indicates that 5 cows (consistently, for three consecutive days, without noticeable changes), or 45 % of the total number of animals in the studied sample (n = 11), secreted a clear-glassy mucus; 27 % of the collected samples had traces of blood; 18 % were milky white; 9 % had a dirty-yellow coloration 36 % had a thick-viscous consistency, and 64 % – liquid consistency (**Fig. 1**).

The registered dynamics of the volume indicators of native mucus samples, their mass, and the mass of evaporated water (**Fig. 2**) indicate that in all cases ($P_{1:2;\ 2:3;\ 1:3}$)

during the 72-hour period from the 1st to the 3rd day, the probability (P) of their difference is greater than 0.05.

The average volume indicator of the obtained samples on the 1st day was 16.2 m^3, on the 2nd day it was 1.2 times smaller (13.9 cm^3), and on the 3rd it increased to 14.6 cm^3. However, it should be noted that its portion is only 1.1 times smaller than on the 1st day. Under these circumstances, their coefficient of variation (C_v) was 24, 10, and 23 % respectively, with a limit (lim) of minimum (min) and maximum (max) deviations of 11–24; 12–16 and 12–20 cm^3, respectively.

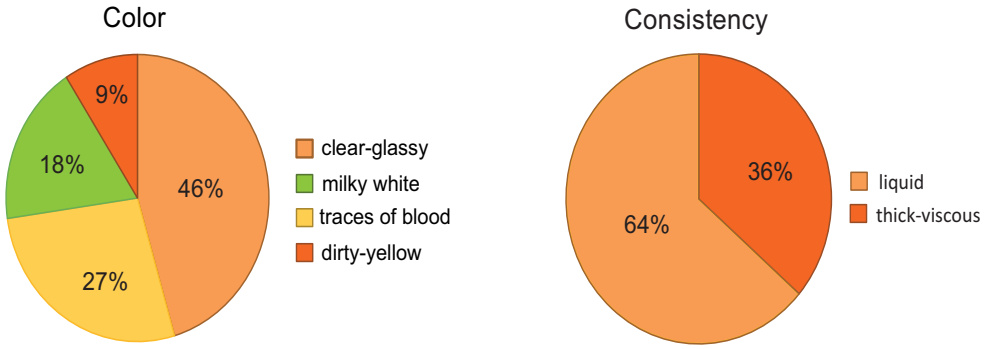

Fig. 1. Macroscopic examination of uterine-vaginal mucus color and consistency on days 1–3 of the estrous cycle in cows (n = 11)

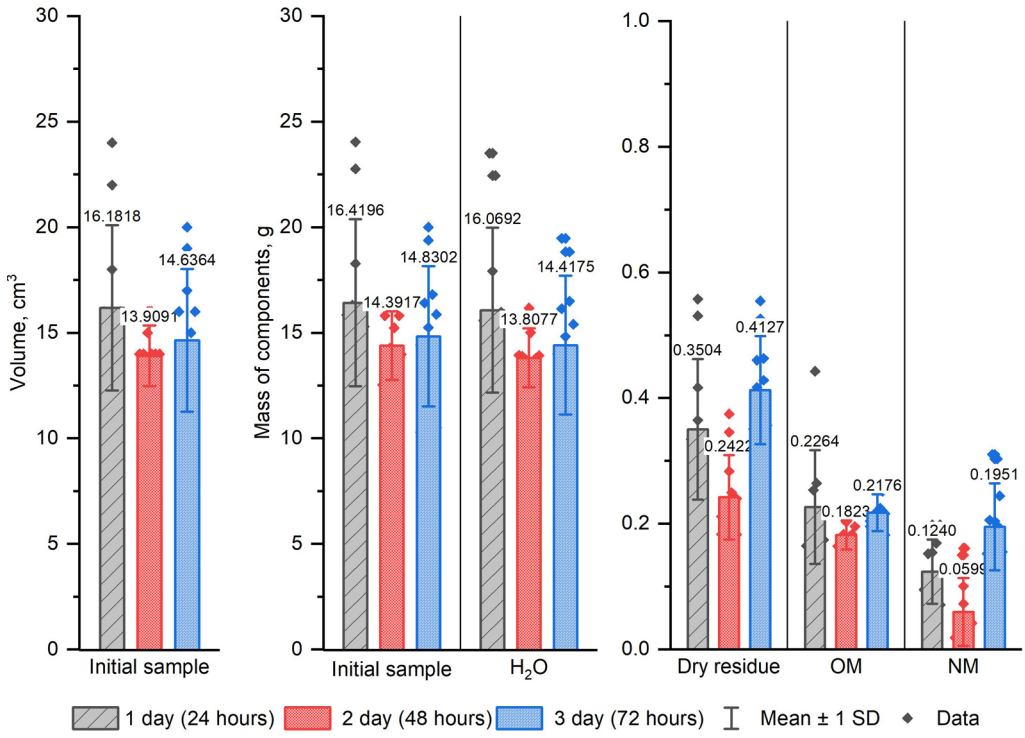

Fig. 2. Absolute indicators of the mass of constituent native mucus samples and their dry residue (M±SD, n = 11)

A similar situation was observed for the mass of raw mucus samples (16.4196–14.3917–14.8319 g), and the evaporation of H_2O from them (16.0692–13.8077–14.4175 g), namely: the volume indicators (16.18–13.91–14.63 cm^3) were 1.01–1.03–1.01 times smaller than their mass; the masses of OS and IS were 1.02–1.04–1.03 times smaller than the mass of H_2O.

The probability of error for the absolute indicators of volume and mass of native UVM samples in all cases is >0.05. However, the error in the parameters of mass of OS and IS samples of fine-dispersed powder of DR, namely the mass of OS and IS of 1st and 2nd ($P_{1:2}$) and 1st and 3rd ($P_{1:3}$) is <0.05. In all other cases, except for the sum of the masses of OS and IS of DR on the 1st and the 2nd day ($P_{1:2}$ <0.05), the probability of changes remains consistently high (P <0.01–0.001).

The results of the statistical analysis of the dynamics of the mass parameters of the components of DR confirm a relatively narrow limit of C_v for the sum of the masses of components (21–32) of DR and its OS (13–40), but a wide limit for IS (35–90 %). Under these circumstances, the limit of their mass is 0.2–0.6, 0.1–0.3, and 0.02–0.3 g, respectively. This means that the absolute parameters of the mass of OS are significantly more stable than the mass of IS during the period from the 1st to the 3rd day.

Step 2. The average parameters of the mass content of evaporated H_2O in native samples varied for each day as follows: on the first day – 98 %; on the second day – 96 %; on the third – 97 % (**Fig. 3**).

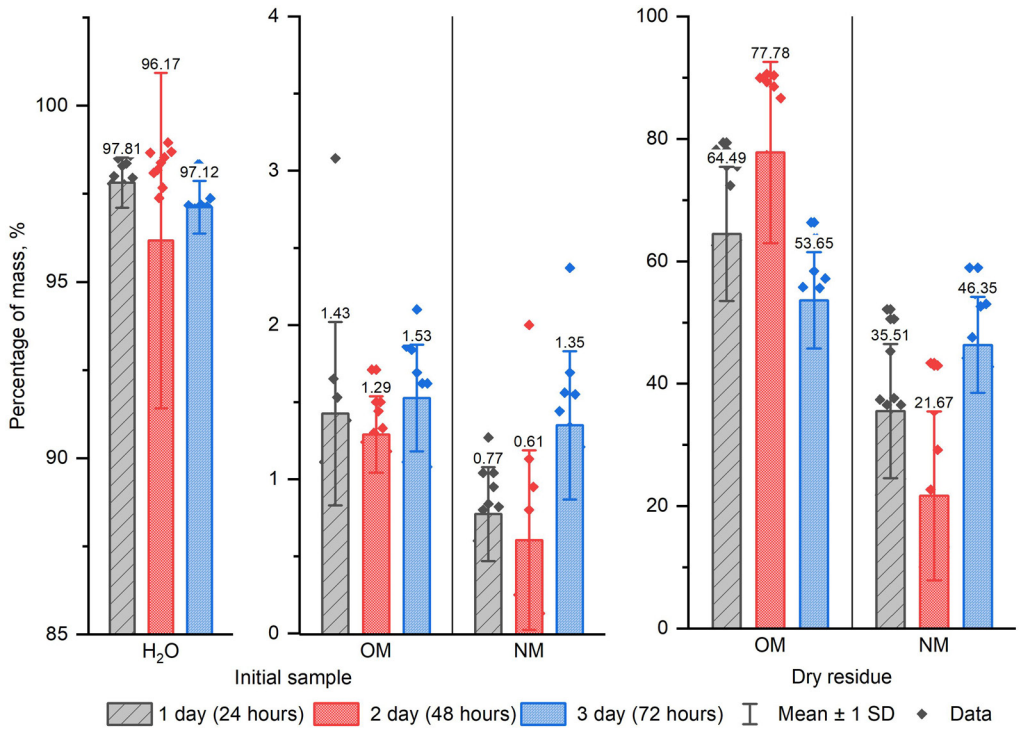

Fig. 3. Mass content of constituents of native mucus samples and its dry residue (M±SD, n = 11)

The rest of the mass content of the DR consists of organic (1.4–1.3–1.5 %) and inorganic (0.8–0.4–1.3 %) components. It is important to emphasize that the variation in the mass content of H_2O was low daily, with changes ranging from 0.8 % to 5.0 %. However, depending on the day, the content of OS varied from 19 % to 42 %, while the content of IS – from 39 % to 89 %.

When comparing the absolute indicators of the mass of constituents of native UVM samples with the percentages content, it was found that the error of volume and mass for the ratio $P_{1:2;2:3,1:3}$ is greater than 0.05. As for the content of H_2O and OS ($P_{1:3}$) and IS, there is a tendency for their difference (1:2, 2:3 ratio) to be less than 0.05, but for IS, the difference remains consistently high ($P_{1:3}$ <0.001).

The observed changes in the mass content of constituents in dehydrated mucus samples indicate that the average percentage of OS for the 1st day is 64 %; it increases to 78 % for the 2nd day and decreases to 54 % for the 3rd. The mass content of IS for the 1st day is 35 %; it decreases to 22 % for the 2nd day and increases to 46 % for the 3rd day. The coefficient of variation for the content of OS, calculated from the sum of the constituent masses, for the 1st (0.3504) – 2nd (0.2351) and 3rd days (0.4126 g) does not exceed the limit of 15–18 %. However, the changes in the content of IS show a wide variation range (9–59 %). The probability of error for the 1st and 2nd days ($P_{1:2}$) and the 1st and 3rd days ($P_{1:3}$) <0.05, while for the 2nd and 3rd days ($P_{2:3}$) <0.001.

Step 3. To assess the peculiarities of homeostasis or equilibrium changes in the components of a biological system, specifically the „medium (water) – organic or inorganic substances," a non-systemic unit was used, referred to as the mass content ratio index (Icm:1). It was applied to the ratio of mass content components in pairs of native samples (UVM*:H_2O; H_2O:OS; H_2O:IS; OS:IS) and dehydrated samples (OS:IS) of mucus (**Fig. 4**).

It was determined that during the period from the 1st to the 3rd day, the equilibrium index of the mass of UVM:H_2O was 1.02–1.04–1.03:1, respectively. However, despite the small limit of deviations (C_v) in its indicators (min – 0.8; max – 5.3 %), a tendency ($P_{1:3}$ <0.05) towards probable changes was observed only on the 3rd day.

A similar situation, but with significantly higher indicators (65–75 times), is observed for the index of the H_2O:OS pair. If the average index on the 1st day is 153:1, it increases to 439:1 on the 2nd day, and then decreases to 82:1 on the 3rd day. Under these circumstances, the C_v indicator varies from 46 to 69 %. The likelihood of error in the results is significant for the comparison between the 1st and 2nd days ($P_{1:2}$ <0.01) and 2nd and 3rd days (P2:3 <0.001). However, a marginally significant difference is observed between the 1st and 3rd days ($P_{1:3}$ <0.05).

The magnitude of the ratios between the absolute and relative indicators of the equilibrium state of the mass of the native UVM OS and IS, as well as its dehydrated DR, remains the same for the period from the 1st to the 3rd day. However, while it is on averages 2:1 on the 1st day, it increases to 5:1 on the 2nd day and then decreases to 1:1 on the 3rd day. The coefficient of their variation changes from 49 to 63 and then decreases to 31 %, respectively. The probability of error is consistent with the ratios of the mass components of the H_2O:OS pair, namely: $P_{1:2}$ – <0.01; $P_{2:3}$ – <0.001; $P_{1:3}$ – <0.05.

If we consider that the condition of physical indicators (color, viscosity, density, consistency, fluidity, crystallization, impedance, etc.) of the UVM depends not only on the degree of adverse effects of inflammatory processes but also on the duration of the first (prooestrus), the second (oestrum), and the third (metoestrum) stages of the sexual (estrous) cycle (Yablonskyi *et al.,* 2006), then, as confirmed by the results and conclusions by M. Adnane *et al*. (2018a) and T. Siregar *et al*. (2019), the viscosity, density, and crystallization of the mucus are associated with the time of ovulation and the probability of animal fertilization.

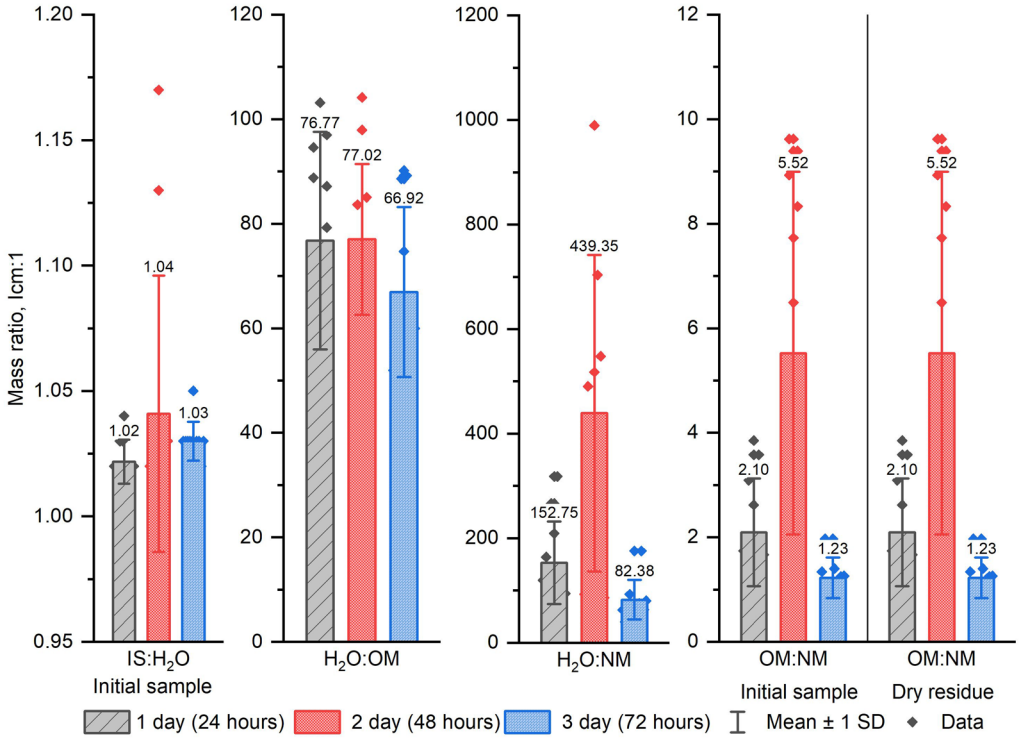

Fig. 4. Indices of ratios of mass content of constituent native mucus samples and its dry residue (M±SD, n = 11)

The relationship between impedance, viscosity, volume, and color of mucus during the second and third stages of the estrous cycle with the frequency of cows' fertilization is illustrated by the results reported by A. Gonchar (2014) and M. Srinivasan *et al*. (2021). At the beginning of estrus, the impedance value is 41 Ohms, while during metestrus, it is 61 Ohms. Researchers maintain that the frequency of fertilization of the egg cell is higher with low impedance values (25–34 Ohm) and high viscosity and volume (Mellado *et al*., 2015; Agbugba *et al*., 2020).

On the one hand, this means that the presented results and formulated conclusions regarding the changes in color and consistency are consistent with the harmful effect of the of inflammatory products (Maksym'yuk *et al*., 2022; Maksymyuk *et al*., 2022) which

changes the transparent-glassy color of UVM samples from the estrous cycle to milky-white and dirty-yellow with traces of blood, and their thick consistency to liquid. On the other hand, it should be noted that throughout the 1–3 days of estrous cycle, the macroscopic condition of mucus samples remains consistently the same.

Recent research (Galvão *et al.*, 2019) suggests that the altered proportion in the balance of components or homeostasis in biological system involving „medium – substance" interaction results from the influence of the products of pathological processes. These changes increase the risk of polycystic ovary syndrome in sterile animals and cause miscarriages in dairy cows (Boby *et al.*, 2017; Adnane *et al.*, 2018a).

M. Adnane *et al.* (2018a) assert that in cases of endometritis, the secretion of the UVM of cows has an increased level of cytokines and glycoproteins. However, the results and conclusions of studies by A. Kraevsky *et al.* (2020) indicate that under the same or similar circumstances, the level of lipid peroxidation, vitamin concentrations, the activity of glutathione peroxidase, alkaline phosphatase, and aspartate aminotransferase decreases. Depending on various factors, the parameters of total protein concentration, glucose, cholesterol, zinc, magnesium, calcium, potassium, and sodium are also variable (Dadarwal *et al.*, 2017; Agbugba *et al.*, 2020).

Therefore, based on the fact that 64 % of samples of UVM secretions in our experiment exhibited altered color and consistency, it is reasonable to assume that the significant deviation we observed ($P > 0.05$) and the consistent probabilities ($P < 0.01$ and 0.001) of increased or decreased percentages of H_2O, OS, and IS content, as well as the index of their mass ratios are associated with inflammatory processes occurring in the tissues of the reproductive organs of the cows under study.

CONCLUSIONS

1. During the period from the 1st to the 3rd day (72 hours) of estrous cycle, the color and consistency of UVM vary depending on the functional state of the cows' reproductive tissue. The secretion can be either clear and viscous (36 %) or milky-white with a dirty yellow shade, include blood impurities, and have a liquid consistency (64 %).
2. In mucus samples with varying consistencies and colors, water content averages between 96 % and 98 %, while organic and inorganic substances typically range from 1.3% to 1.5 % and from 0.4 % to 1.3 %, respectively. The ratios of pairs for mucus components are as follows: H_2O:OS (67:1 to 77:1), H_2O:IS (83:1 to 438:1), and OS:NS (1:1 to 5:1).
3. The detected changes in the macroscopic features of the mucus samples and the defined mass limits and ratios of its components in the „medium-substance" system indicate that the products of inflammatory processes accumulated in the tissues of the genital organs can have a negative effect on the fertilization of the ovum during mating and artificial insemination of cows.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: no special funding was received, and no conflict of interest was reported.

Human Rights: the article does not involve experiments on humans.

Animal Rights: all international, national and institutional guidelines for the care and maintenance of animals were followed.

AUTHORS' CONTRIBUTION

Conceptualization [V.M.]; methodology [V.M.]; an investigation [O.S., M.V.]; resources [V.M., G.S.]; data curation [V.M.]; visualization [O.S., M.M.]; writing – preparation of the original draft [V.M., O.S.]; writing – reviewing and editing [H.M., V.M.]; supervision [V.M.]; project administration [V.M., G.S.], receiving funding [–].

All authors have read and approved the published version of the manuscript.

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ОСОБЛИВОСТІ ДИСБАЛАНСУ МАСИ СКЛАДОВИХ МАТКОВО-ВАГІНАЛЬНОГО СЛИЗУ 1–3-Ї ДОБИ ЕСТРАЛЬНОГО ЦИКЛУ

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Вступ. Макроскопічна оцінка кольору та консистенції зразків матково-вагінального слизу (МВС) корів показала, що впродовж 1–3-ї доби естрального циклу 45,4 % тварин дослідженої вибірки (n = 11) мають прозоро-скляний колір, 27,3 % – домішки крові, 18,2 % та 9,1 % – змінене забарвлення; 36,4 % – густо-в'язку, текучу консистенцію, а 63,6 % – рідку.

Матеріали та методи. У період 7–8-ї год ранку 1–3-ї доби естрального циклу від дослідних тварин відбирали слиз і вимірювали об'єм (см^3), оцінювали забарвлення й консистенцію секрету. Показники маси (г) та вмісту (%) води (H_2O), органічних (ОР) і неорганічних речовин (НР) визначали гравіметрично.

Результати. Аналіз показників об'єму та маси H_2O нативних зразків МВС вказує на високу (>95 %) похибку зареєстрованих змін. Однак похибка суми мас ОР і НР зразків складових сухого залишку (СЗ) – цілком інша. Цей показник на 1-шу і 2-гу добу має тенденцію (P <0,05) до змін, але на 2-гу і 3-тю й 1-шу і 3-тю добу – їхню стійку вірогідність (P <0,001). Стійкі зміни маси ОР виявлено на 2-гу і 3-тю (P <0,01); маси НР – на 1-шу і 2-гу (P <0,01) та на 2-гу і 3-тю доби (P <0,001). У всіх інших випадках похибка більша за 95 %.

Відсотки маси H_2O, ОР і НР вказують на тенденцію (P <0,05) до змін вмісту H_2O та ОР на 1-шу і 3-тю доби; НР – на 1-шу і 2-гу та 2-гу і 3-тю, але зміни на 1-шу і 3-тю доби – дуже стійкі (P <0,001). Стійкі зміни маси складових СЗ виявлено лише на 2-гу і 3-тю доби.

Аналіз змін гомеостазу маси нативних зразків свідчить, що межа середніх значень Icm:1 пари МВС:H_2O (1,02–1,04:1) < H_2O:ОР (67–77:1) < H_2O:НР (82–439:1), але пари ОР:НР (1,2–5,5:1) збігається з ОР:НР (1,2–5,5:1) зразків СЗ. Межа C_v пар МВС:H_2O, H_2O:ОР, H_2O:НР становить 0,8–5,3; 19–27; 46–93 %; ОР:НР зразків

нативного МВС та його СЗ – однакова (49; 63; 31 %). На тенденцію до змін (P <0,05) на 1-шу і 3-тю доби вказують показники пар МВС:H_2O, H_2O:НР і ОР:НР. Їхні стійкі зміни (P <0,01–0,001) на 1-шу і 2-гу та 2-гу і 3-тю доби встановлено для пар H_2O:НР і ОР:НР.

Висновки. З огляду на зазначене вище постає потреба в подальшому ретельному вивченні особливостей дисбалансу маси складових МВС 1–3-ї доби естрального циклу та реакції секреторних клітин слизової оболонки статевих органів на дію шкодочинних факторів, що буде застосовано для розробки нових методів профілактики, діагностики й лікування хвороб репродуктивного тракту самок тварин.

Ключові слова: матково-вагінальний слиз, маса води, органічні та неорганічні речовини

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