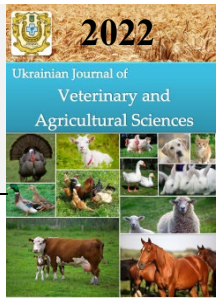
Ukrainian Journal of
Veterinary and Agricultural Sciences
http://ujvas.com.ua

The Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv

original article | UDC 619:612.174:612.1:636.4 | doi: 10.32718/ujvas5-2.06

Volume 5
Number 2



Peculiarities of physico-chemical condition of uterine vaginal mucus during estral cycle

H. V. Maksymyuk[1], V. M. Maksymyuk[2], H. M. Sedilo[2], O. I. Stadnytska[2], O. K. Onufrovych[1], Z. D. Vorobets[1], B. V. Gutyj[3]

[1]*Danylo Halytsky Lviv National Medical University, Pekarska Str., 69, Lviv, 79010, Ukraine*
[2]*Institute of Agriculture of the Carpathian region of the National Academy of Agrarian Sciences of Ukraine, Grushevskogo Str. 5, Obroshino, Pustomytovsky District, Lviv Region, 81115, Ukraine*
[3]*Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv, Pekarska Str., 50, Lviv, 79010, Ukraine*

Article info
Received 22.07.2022
Received in revised form 23.08.2022
Accepted 24.08.2022

Correspondence author
Olha Stadnytska
Tel.: +38-067-747-18-36
E-mail: stadnytskaolha@ukr.net

2022 Maksymyuk H. et al. This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Contents

Abstract

The primary aim of the work was to study the peculiarities of the influence of endogenous factors (products of inflammatory processes occurring in the tissues of female genitals) in the biological system of type "environment (uterine-vaginal mucus) – substance (water, organic and inorganic substances)" on the indicators of mass (g, mg) of its components. The results of the experiments indicate that their harmful effect changes the color and density of mucus released during estrus (UVM); reduces the parameters of the mass content of H_2O and inorganic substances (IS), but increases – organic (OS). Under these circumstances, the distribution of the parameters of the mass of the components of the dry residue (DR, dried at 105 °C) of UVM of cows of the experimental group is presented in a row where the mass of OS_1 (burn at 520–530 °C) >IS >OS_2 (burn at 650 °C), but for control is another as well as mass of IS>OS_1>OS_2. This means that the harmful effects of inflammatory products on the genital tissues of females negatively affect the distribution of the mass of OS and IS in the system "environment-substance".

Keywords: uterine-vaginal mucus, "environment-substance" system, mass parameters of organic and inorganic substances.

Citation:
Maksymyuk, H. V., Maksymyuk, V. M., Sedilo, H. M., Stadnytska, O. I., Onufrovych, O. K., Vorobets, Z. D., & Gutyj, B. V. (2022). Peculiarities of physico-chemical condition of uterine vaginal mucus during estral cycle. *Ukrainian Journal of Veterinary and Agricultural Sciences*, 5(2), 37–42.

1. Introduction

The study of the physicochemical state of any biological fluid of the human or animal body under the harmful effects of endogenous and exogenous factors requires an objective assessment of the dynamics of the mass parameters of its inorganic (IS) and organic (OS) substances (Duzu, 1980; Bagrov, 1984; Terekhov & Petrov, 1990; Bulychev, 1991; Socha-Banasiak et al., 2021; Cao et al., 2021; Mylostyvyi et al., 2021; Fischer et al., 2021; Rietjens et al., 2022).

In our case, this is due to the condition and composition of uterine-vaginal mucus (UVM), which creates dynamically optimal conditions for the normal functioning of sperm and ensures their efficient movement through the channels and ducts of the female reproductive system. It is probably also known that the level of intracellular and extracellular homeostasis of the parameters of the mass and content of OR and IS of sperm of males and UVM of females carries out the primary selection of full-fledged spermatozoa. The processes of chemo- and electrotaxis move the separated cells from the cervical canal into the fallopian tubes, the osmotically active environment which provides effective contact of sperm with the ovum (Roldan, 1998; Silva & Gadella, 2006; Denisenko et al., 2008; Vigodner, 2011; Kropp et al., 2014).

From our previous studies (Maksymiuk et al., 2012; Maksymyuk, 2013), the results of which were aimed at identifying peculiarities of the imbalance of alkaline metal ion homeostasis in sperm and aqueous extracts of bovine genital tissues, the existence of the direct and inverse rela-

tionship between concentration parameters Ca^{2+}, K^+, Na^+ and its ratios between pairs (Na^+: Ca^{2+}, K^+: Ca^{2+}, Na^+: K^+) of ions with indicators of sperm motility and fertility were revealed. Under the studied circumstances (breed and age of males, mating season of females), it was found that low (16–25 mM) and high (46–70 mM) concentration of K^+ samples of freshly obtained sperm and deconserved spermatozoa causes low (50–65 %) fertility of cows after the first insemination, while the average (26–45 mM) causes high one (65–75 %). A significant difference in the physical and chemical state of the UVM of cows during estrus was experimentally revealed, too (Maksymiuk et al., 2012; Maksymyuk, 2013; Maksymyuk & Vorobets, 2013; Maksymiuk et al., 2017).

Therefore, despite the above facts and the relationship between the limit of mass and content of components (IS, OS) of the UVM with the parameters of volume (cm^3) and signs (color, density) of its different physical and chemical states, the aim of the study was directed of the study of peculiarities of the dynamics of the mass parameters of the components of the biological system of the type "environment-substance" under the action of factors of harmful effects.

On the one hand, it will allow us to receive: parameters of an estimation of the degree of influence of a physical and chemical condition (harmful factors) of the UVM on the structure and functions of sperms; on the other hand, the possibility of substantiating its connection with the fertilization of eggs after insemination of females; to formulate new theoretical postulates and introductory provisions for the objective assessment of the peculiarities of the "reaction-response" of sperm to the harmful effects of products (endogenous factor) of inflammatory processes occurring in the tissues of the genital organs.

2. Materials and methods

Determination of the dynamics of the parameters of volume (cm^3), traits of color and density (%), and mass content (g, mg, %) of the components of utero-vaginal mucus were carried out by quantitative (gravimetric) and qualitative chemical analysis (Maksymyuk, 2013). Specially introduced formulas were used to establish the connection between the signs of UVM and the parameters of its physicochemical state (Δ changes or imbalance of homeostasis of a biological system of the "environment-substance" type). *($\Delta = x_0 \pm x_i$; $\sum \Delta m = \Delta m_1 + \Delta m_2 + ... \Delta m_i$)*. Indicators assessed the identified changes *($M \pm m$, σ, Cv, P, lim)* (Sopin & Vinogradova, 1975; Rokitsky, 1986; Avtandilov, 1990) Microsoft Excel computer program.

During the mating season (April – June 2021) of the first – third (1–72 hours) days of estrus, during the detected signs of hunting (behavioral arousal, swelling of the external genitalia, mucus secretion), samples of the UVM were taken from the vagina of cows of the Ukrainian black-spotted (Holsteinized) breed 3–9 years of age. The native state of the selected samples was assessed by signs of color (transparent, gray-white), densities (consistency – densely viscous, viscous, liquid), the presence of impurities (blood, pus), and parameters of specific volume indicators (cm^3).

The samples of the UVM selected for research were divided into control (n = 10) and experimental (n = 4) groups. The control group included samples, indicators, and signs which indicate normal function, and the experimental group included indicators and signs which show dysfunction of genital tissues.

Peculiarities of changes in the parameters of the mass of components (H_2O, total content of organic-inorganic substances) of the UVM were carried out by drying in a thermostat at 105 °C. Their registration was carried out until the fourth sign (0.0001) of the mass determined by microtaresis stopped changing after the comma. Constantly repeated parameters of unchanged mass of organic (OS_1, OS_2) and inorganic (IS) substances (Maksymiuk et al., 2012; 2017) were determined after burning the dry residue on an open flame burner (OS_1) and in a muffle furnace (OS_2 and IS). Relative indicators were determined by the composite proportional relationship between the absolute parameters of the mass of H_2O and OS and IS of the dry residue of the samples of the UVM.

3. Results and discussion

Assessment of the native state of uterine and vaginal mucus of cows

The analysis of specific indicators (Table 1) shows that the cows of the studied groups secrete an average of 14–20 cm^3 of uterine and vaginal mucus into the vagina. The fluidity of the mucus of the control group is very high; the consistency is thick-viscous, and the color is clear glass. No impurities.

Samples of mucus from the experimental group have entirely different properties: fluidity – low, consistency – liquefied, and the color is changed to milky white with off-yellow to green shades. Depending on the degree of development of inflammatory processes, the secretions of genital tissues, in some cases, have the appropriate amount of impurities of pus and blood.

The difference between the indicators of raw mucus is demonstrated by the identified and analyzed peculiarities of the dynamics of mass balance and mass content of constituent organic (OR) and inorganic (HP) substances in aqueous extracts of powdered samples of dry mucus residue. The defined limits of their averages indicate that the harmful effects of exo- (maintenance, care, feeding) and endogenous (inflammatory processes of genital tissues) factors lead to the destabilization of homeostasis of the components of the UVM.

Analysis of the obtained volume of the samples of the UVM of two groups of cows (control, experiment) shows that its average parameters have different values (20 against 14 cm^3).

Therefore, in this regard, it is worth emphasizing that the obtained limit (lim: min = 10 against 8; max = 40 against 21) of a specific volume is characterized by a very high index (43–37 %) of the coefficient of variation (Cv).

However, the difference in this indicator between cows' groups is insignificant and only 6%. This means that the high variability of the volume parameters of the UVM samples may be the reason for their low ($P > 0.2$) probable difference (Table 1).

Table 1
The native state of urogenital mucus samples

Statistical indicators	Indicators of samples UVM		
	V (см^3)	Color (%)	Density (%)
Control (n = 10)			
M ± m	20.20 ± 2.76	Transparent – 80 Gray and white – 20	Densely viscous – 30 Viscous and liquid – 70
Cv	43.21		
lim	10–40		
Experiment (n = 4)			
M ± m	14.12 ± 2.58		
Cv	36.60	Transparent – 50 Gray and white – 50	Viscous – 50 liquid – 50
lim	8–21		
P	> 0.2		

However, if the volume parameters are characterized by an incredible rate of change, the analysis of transparent color samples obtained from control (80 and 20 %) and experimental (50 %) groups of cows shows that the difference in transparent (80 against 50 %) and gray-white (20 against 50 %) color between groups of cows is quite significant, which is ±30 %.

The difference between the signs of mucus density (30 against 50 and 70 against 50 %) is 10 % less than the difference between the characteristics of its color. In this regard, it should also be noted that the experimental group of cows is characterized by less transparent samples (50 against 80 %) but more gray-white (50 against 20 %).

However, the density of mucus is characterized by slightly different peculiarities. Suppose the samples of UVM of dense-viscous consistency in the experimental group are absent. In that case, 70 % of viscous and liquid in the control group is represented by the same indicators of their viscous (50 %) and liquid (50 %) consistency in the experimental group.

Absolute indicators of mass distribution of the components of the UVM and its DR

After drying the samples of the control and experimental cows' cows, it was determined that the average mass of evaporated H_2O is 20 and 13 g, respectively, but the mass of the components of the obtained DR – 336 and 325 mg (Table 2). The probability of its changes between groups of cows is low (P =>0.2–0.5). However, in this regard, it should be noted that the coefficient of variation of H_2O and DR mass of both groups of cows is different, namely: if the variability of the mass parameters of samples of the control group is 48–61 %, the experimental samples is 38–39 %, which is 10–22 % less.

Table 2
Parameters of mass of components of uterine and vaginal mucus

Statistical indicators	H_2O and DR UVM mass parameters			
	absolute, g		relative, %	
	H_2O	C3	H_2O	C3
Control (n = 10)				
M ± m	20.01 ± 3.05	0.3344 ± 0.06	98.40 ± 0.08	1.60 ± 0.08
Cv	48.17	61.31	0.26	15.85
lim	9–42	0.1–0.8	98–99	1.2–1.9
Experiment (n = 4)				
M ± m	13.45 ± 2.55	0.3253 ± 0.06	97.64 ± 0.07	2.36 ± 0.07
Cv	37.99	38.77	5.35	6.25
lim	8–20	0.2–0.5	97–98	2–3
P	> 0.2	> 0.5	< 0.001	< 0.001

The absence of a potential difference in the data, represented by absolute (g, mg) indicators of the mass components, denies the statistical analysis data, represented by its relative (%) indicators.

Therefore, it should be noted that there are some differences for specific average mass parameters of H_2O and DR. Thus, if the *lim* of the relative indicator of evaporated water weight, which is determined for samples of the control group of cows, is 98–99 % the experimental is 97–98 %. The limit in the relative parameters changes of the mass of the components of the DR samples of the control group of cows is < 2 %, but the experimental is > 2 %.

To the above results of the analysis of statistical indicators, which are determined for the ratios of the mass of components of the UVM, it should be added that the coefficient of variation of evaporated H_2O parameters does not exceed 5 %. However, the sum of components of control DR (16 %) is 2.7 times greater than experimental (6 %). This leads to the probability of possible error in results being very low, but the relative difference between the parameters of the mass of H_2O and DR is more significant than 99.9 % (P < 0.001).

The distribution of absolute values of the total mass of the components (334 and 325 mg) after combustion of powdered samples of DR (Table 3) is as follows: a certain amount of OS_1 is 101 and 158, OS_2 – 40 and 46, IS – 193 and 121 mg. The above indicates that the control group's total mass of OS_1 and OS_2 (141 mg) is 1.4 times less than the experimental (204 mg). In addition, the control group's mass of IS of DR (193 mg) is 1.6 times higher than the experimental (121 mg). This means that the peculiarity of the mass content of the components of the DR of UVM is that the ratio of their mass in the samples of the studied groups of cows is characterized by inverse dependence.

Table 3
Absolute indicators of samples of the dry residue of uterine and vaginal mucus

Statistical indicators	The parameters of the mass of the components DR, g			∑ (m_1 +…m_3)
	OS_1 (m_1)	OS_2 (m_2)	IS (m_3)	
Control (n = 10)				
M ± m	0.1006 ± 0.02	0.0403 ± 0.01	0.1935 ± 0.04	0.3344 ± 0.6
Cv	76.90	76.22	59.93	61.31
lim	0.04–0.30	0.02–0.10	0.07–0.42	0.1–0.8
Experiment (n = 4)				
M ± m	0.1582 ± 0.03	0.0462 ± 0.02	0.1209 ± 0.02	0.3253 ± 0.06
Cv	40.59	48.09	40.67	38.77
lim	0.1–0.2	0.03–0.08	0.07–0.20	0.2–0.5
P	> 0.2	< 0.05	< 0.001	> 0.2

It should be added the following: even though the coefficients of variation of specific indicators of mass OS_2 (76 and 48 %) and IS (60 and 41 %) are pretty high, the probability of their difference between groups is 97 ($P < 0.05$) and 99 % ($P < 0.001$) respectively.

Relative indicators of the distribution of the mass of uterine-vaginal substances mucus

It was determined that changes in the relative parameters of the mass of H_2O and OS and IS of DR samples of the UVM for the control group of cows (Table 4). It indicates that under an open burner, 0.48% of the mass of substances of group OS_1 burn, and after burning, 0.20 % of substances of the OS_2 group.

The residual mass of IS, which did not burn under the conditions of an open burner fire, is 0.92 % of the total content of the components of the DR. The dynamics of the distribution of its components is characterized by a series in which the parameters of the relative mass of HP are represented (0.9 %) >OS_1 (0.5 %) > OS_2 (0.2 %). However, the order of placement of the parameters of the mass of the samples of the UVM of the experimental group represents an entirely different number of components of the DR, namely: OS_1 (1.2 %) > IS > (0.3 %) > OS_2 (0.9 %).

Table 4
Relative indicators of samples mass of dry residue of uterine and vaginal mucus

Statistical indicators	H_2O (m_0)	Component mass parameters of DR, %				∑ (m_1 + m_3)
		OS_1 (m_1)	OS_2 (m_2)	∑ (m_1 + m_2)	IS (m_3)	
Control (n = 10)						
M ± m	98.40 ± 0.08	0.48 ± 0.06	0.20 ± 0.02		0.92 ± 0.06	1.60 ± 0.08
Cv	0.26	38.08	36.56	0.68	19.29	15.85
Lim	98–99	0.2–0.8	0.01–0.3		0.6–1.3	1.2–1.9
Experiment (n = 4)						
M ± m	97.64 ± 0.07	1.15 ± 0.11	0.34 ± 0.05		0.87 ± 0.02	2.36 ± 0.07
Cv	5.35	19.72	26.98	1.49	4.98	6.25
Lim	97–98	1.0–1.5	0.2–0.4		0.8–0.9	2–3
P	< 0.001	< 0.001	< 0.05		< 0.001	< 0.001

It should be added that the difference in the limit of the variability of the mass components between the samples of the control (16–38 %) and experimental (5–20 %) groups is 3-2 times different. Nevertheless, the probability of their difference between the groups of components is 95 ($P < 0.05$) and 99 % ($P < 0.001$), respectively. However, if the analysis of mass distribution is carried out only for DR (Table 5), the order of placement of relative indicators of its components coincides, namely: in the control group samples, the mass index IS (52 %) > OS_1 (36 %) > OS_2 (13 %); experimental is OS_1 (48 %) > IS (37 %) > OS_2 (15 %).

However, it should be noted that the relative parameters of this indicator of the control and experimental groups of the UVM samples are expressed respectively 58–72–65 and 40–123–17 times by higher percentages. Under these circumstances, the indicator ∑ (m_1 + m_2), or the percentage of the mass of the DR components of UVM, is presented in the table 5, is 71 and 42 times higher than the percentage of the mass of the components of the raw samples of the UVM (Table 4). In addition, the parameters of the variability of the mass components between the samples of the control (30–61%) and experimental (7–39 %) groups are 1.4–2.0 times higher. However, the probability of their difference between the groups of components remains at a predetermined level, which is 95 ($P < 0.05$) and 99 % ($P < 0.001$), respectively.

Table 5
Relative indicators of dry residue mass

Statistical indicators	Mass of DR (m_0, г)	Component mass parameters C3, %			
		OS_1 (m_1)	OS_2 (m_2)	$\sum (m_1 + m_2)$	IS (m_3)
Control (n = 10)					
M ± m	0.3344 ± 0.6	35.64 ± 4.22	12.75 ± 1.27		51.61±4.93
Cv	61.31	37.41	31.65	48.39	30.19
lim	0.1–0.8	21–58	8–18		33–73
Experiment (n = 4)					
M ± m	0.3253 ± 0.06	48.32 ± 3.41	14.47 ± 2.04		37.21±1.39
Cv	38.77	14.11	28.14	62.79	7.47
lim	0.2–0.5	44–59	8–18		33–39
P	> 0.2	< 0.05	< 0.001		< 0.05

Analysis of peculiarities and mass distribution of components of the UVM and DR

The analysis of the mass of dried UVM shows that with the 1.5 times larger difference in the absolute parameters of the mass of H_2O, which was evaporated by samples of control and experimental groups of cows, the parameters of the mass of the components of the DR are almost the same. Under these circumstances, significant variability in the mass of samples of control (48–61 %) and experimental (38–39 %) groups of cows may be the reason for the low (P = > 0.2–0.5) probability of its changes. However, the lack of a potential difference in the absolute parameters of the mass of the components is denied by the statistical analysis data, which is represented by its relative indicators.

In this case, a slightly different feature was found: if the limit of changes in the relative parameters of the mass of the components of the DR samples of the control group is < 2 %, the experimental > 2 %. The coefficient of variation of the parameters of evaporated H_2O < 5 %; components of the DR of the control group (16 %) is 2.7 times greater than the experimental (6 %). This leads to the probability of possible error in the estimated results being very low (P < 0.001). That is, the determined difference between the parameters of the mass of H_2O and DR is more than 99.9 %.

Analysis of the distribution of absolute parameters of the total mass of the components of the burned powder samples of DR shows that the determined mass of the components of OS_1 samples of the experimental group, which burns at gas burner temperature, is 1.6 times higher than of the control. But the components of OS_2, the mass of which is burned in the muffle, are only 1.2 times bigger. Under these circumstances, the control group's mass index of IS of DR is 1.6 times higher than that of the experimental group. This means that the peculiarity of the mass content of the components of DR of UVM is that the mass parameters of OS and IS in the samples of DR of UVM of the studied groups of cows are characterized by inverse dependence. That is, inflammatory processes in the genital tissues of cows may be the reason that the secretions of their UVM contain a more significant mass of OS but less IS.

It should also be noted that for sufficiently high parameters of the coefficient of variation of the mass parameters of the components of OS_2 (76 and 48 %) and IS (60 and 41 %), the probability of their difference between groups is 97 (P < 0.05) and 99 % (P < 0.001) respectively.

The peculiarity of the dynamics of the distribution of the relative parameters of the mass of the components of the DR of UVM is also that the control group is characterized by a series in which the mass of IS >OS_1 >OS_2. However, the order of placement of the parameters of the mass of the samples of the DR of the UVM of the experimental group is an entirely different number of its components, namely: OS_1 > IS > OS_2. This feature of the mass of components is characterized by almost exact limits of its variability between samples of control and experimental groups. The probability of the difference between the groups of components is 95 % (P < 0.05) and 99% (P < 0.001), respectively. If the analysis of the mass distribution is carried out only for the components of the DR, the order of their placement coincides, namely: in the samples of the control group, the mass index is IS > OS_1> OS_2; the experimental group mass index is OS_1> IS > OS_2.

It should be noted that the relative parameters of this indicator of the control and experimental groups of samples of the UVM are expressed respectively in 58–72–65 and 40–123–17 times higher percentages. Under these circumstances, the indicator $\sum(m_1 + m_2)$ or the percentage by mass of the components of DR of UVM is between 71 and 42 times higher than the percentage by weight of the components of the raw samples of the UVM. In addition, the parameters of the variability of the mass of the components between the samples of the control group are 1.4–2.0 times greater than the experimental groups. However, the probability of their difference remains at a predetermined level, which is 95 (P < 0.05) and 99 % (P < 0.001), respectively.

4. Conclusions

With statistically insignificant changes in volume, the signs of color and density of uterine and vaginal mucus of the experimental and control groups of cows are different, namely: if 80 % of samples of the control group are transparent, 20 % are gray-white, 30 % are densely viscous, 70 % are viscous and liquid, then 50 % of the experimental – or transparent and gray-white and or viscous and liquid. Samples of the UVM of the experimental group of cows have a lower mass of water. The absolute parameters of the mass of his OS are more outstanding; IS are smaller. The order of distribution of the components of the DR of the UVM is presented in a row, where the mass of OS_1 > IS > OS_2, but the control is different, namely: the mass of IS> OS_1 > OS_2. Thus, the results of our experimental work mean that the unequal distribution of OS and IS in the system “environment-substance” is due to the intensity of their synthesis in the genital tissues of females (cows).

Conflict of interest

The authors declare that there is no conflict of interest.

References

Avtandilov, G. G. (1990). *Medical morphometry*. Guidance. Moscow: Medicine (in Russian).
[Google Scholar]

Bagrov, Yu Ya. (1984). *Water-salt homeostasis in circulatory failure*. Leningrad: Nauka (in Russian).
[Google Scholar]

Bulychev, A. G. (1991). *Cell segregation function.* Leningrad: Science. Leningr. Department (in Russian).
[Google Scholar]

Cao, Y., Li, B., Ismail, N., Smith, K., Li, T., Dai, R., & Deng, Y. (2021). Neurotoxicity and Underlying Mechanisms of Endogenous Neurotoxins. *International Journal of Molecular* Sciences, 22(23), 12805.
[Crossref] [Google Scholar]

Denisenko, S. V., Dariy, A. S., Kononenko, M. I., & Zerova-Lyubimova, T. Ye. (2008). Genetics of reproduction. Ed. Dr. Med. Sciences, Prof. T.I. Buzhievskaya. Kyiv (in Russian).
[Google Scholar]

Duzu, P. (1980). *Cryobiochemistry (introduction)*. Moscow: Mir (in Russian).
[Google Scholar]

Fischer, S., Nasyrov, E., Brosien, M., Preissner, K. T., Marti, H. H., & Kunze, R. (2021). Self-extracellular RNA promotes pro-inflammatory response of astrocytes to exogenous and endogenous danger signals. *Journal of Neuroinflammation*, 18(1), 252.
[Crossref] [Google Scholar]

Kropp, J. F., Penagaricano, S. M., Slih, S. M., & Khatib, H. (2014). Invited review: Genetic contributions underlying the development of preimplantotion bovine embryos. *Journal of Dairy Science,* 97(3), 1187–2001.
[Crossref] [Google Scholar]

Maksymyuk, G. V. (2013). Age aspect of the ratios of concentration of Ca^{2+}, K^{+}, $Na^{+}$of sperm plasma and sperm. *Bulletin of Problems of Biology and Medicine*, 2(100), 83–88 (in Ukrainian).
[Article] [Google Scholar]

Maksymyuk, G. V., & Vorobets, Z. D. (2013). The content and ratio of macronutrients in the organs and tissues of the male reproductive system. *World of Medicine and Biology*, 1, 133–136 (in Ukrainian).
[Article] [Google Scholar]

Maksymiuk, H. V., Vorobets, Z. D., Lapovets, L. Ye., Sanahurskyi, D. I., Sedilo, H. M., & Maksymiuk, V. M. (2012). Patent Ukrainy 69773. Kyiv: Derzhavne patentne vidomstvo Ukrainy (in Ukrainian).
[Patent]

Maksymiuk, H. V., Vorobets, Z. D., Lapovets, L. Ye., Fafula, R. V., Sedilo, H. M., Maksymiuk, V. M., & Sharan, M. M. (2017). Patent Ukrainy 119753. Kyiv: Derzhavne patentne vidomstvo Ukrainy (in Ukrainian).
[Patent]

Mylostyvyi, R., Sejian, V., Izhboldina, O., Kalinichenko, O., Karlova, L., Lesnovskay, O., Begma, N., Marenkov, O., Lykhach, V., Midyk, S., Cherniy, N., Gutyj, B., & Hoffmann, G. (2021). Changes in the Spectrum of Free Fatty Acids in Blood Serum of Dairy Cows during a Prolonged Summer Heat Wave. *Animals*, 11(12), 3391.
[Crossref] [Google Scholar]

Rietjens, I. M. C. M., Michael, A., Bolt, H.M., Siméon, B., Andrea, H., Nils, H., Christine, K., Angela, M., Gloria, P., Daniel, R., Natalie, T., & Gerhard, E. (2022). The role of endogenous versus exogenous sources in the exposome of putative genotoxins and consequences for risk assessment. *Archives of Toxicology*, 96(5), 1297–1352.
[Crossref] [Google Scholar]

Rokitsky, P. F. (1986). *Biological statistics*. Ed. 2, corrected. Minsk (in Russian).
[Google Scholar]

Roldan, E. R. S. (1998). Role of phospholipases during sperm acrosome exocytosis. *Frontiers in Bioscience*, 3, 1109–1119.
[Crossref] [Google Scholar]

Silva, P. F., & Gadella, B. M. (2006). Detection of danage in mammalian sperm cells. *Theriogenology*, 65(5), 958–978.
[Crossref] [Google Scholar]

Socha-Banasiak, A., Pawłowska, M., Czkwianianc, E., & Pierzynowska, K. (2021). From Intrauterine to Extrauterine Life-The Role of Endogenous and Exogenous Factors in the Regulation of the Intestinal Microbiota Community and Gut Maturation in Early Life. *Frontiers in Nutrition*, 8, 696966.
[Crossref] [Google Scholar]

Sopin, E. F., & Vinogradova, R. P. (1975). *Fundamentals of biochemical research methods*. Kyiv: High school (in Ukrainian).
[Google Scholar]

Terekhov, N. T., & Petrov, M. M. (1990). Effective transfusion erythrocyte media. Kyiv: Health (in Russian).
[Google Scholar]

Vigodner, M. (2011). Roles of small ubiquitin-related modifiers in male reproductive function. *International Review of Cell and Molecular Biology*, 288, 227–259.
[Crossref] [Google Scholar]