https://doi.org/10.29326/2304-196X-2024-13-1-87-94

# Flow cytometry study of DNA transformation dynamics in BHK-21/SUSP/ARRIAH cell culture during rabies virus reproduction

**Marina N. Guseva, Maksim I. Doronin, Maxim A. Shevchenko, Dmitry V. Mikhalishin, Alexey V. Borisov, Yulia S. El'kina, Tatyana V. Okovytaya, Valery M. Zakharov, Valery V. Mikhalishin**
Federal Centre for Animal Health, Yur'evets, Vladimir 600901, Russia

## ABSTRACT

The study examines the DNA transformation dynamics of BHK-21/SUSP/ARRIAH subline cells during rabies virus reproduction. Cells infected with the virus and control intact cells were cultivated under similar conditions. The identification of dependence of the virus infectivity on reproduction time revealed that the virus infectivity titre increased from (3.2 ± 0.2) lg $CCID_{50}/cm^3$ at the time of inoculation to (7.63 ± 0.3) lg $CCID_{50}/cm^3$ after 48 hours of reproduction, with the most intensive increase having been observed within the first 24 hours. The cell concentration changed from 0.5 to 1.9 million/$cm^3$, i.e. increased by a factor of 3.8. After 24 hours, the cell growth rate slowed down. Findings from the examination of cell cycle phases during rabies virus reproduction in the host cell allowed for the estimation of duration and predominance of G1, S, G2 + M phases at different stages of cultivation. The dynamics of changes in the apoptotic cell population in the control and test samples was similar within 36 hours of cultivation. After the said period, the proportion of apoptotic infected cells was 28–42% higher than that of apoptotic control cells. After 9 hours, the proportion of cells undergoing G1 phase increased by 11.7% in the test samples, whereas it decreased by 16.6% in the control samples. Subsequently, the number of G1 phase cells in the control and test samples changed in the same way: a 40% decrease was observed after 15–18 hours, it was followed by a 45–46% growth jump, then again a 39–40% decrease and an increase were observed. After 33 hours of reproduction and till the end of cultivation, the proportion of infected cells undergoing G1 phase was significantly higher (by 12–21%) as compared with control cells. The percentage of S phase cells in the test and control samples was the same during the first day of the virus reproduction, with sharp jump-like 3.4- and 2.4-fold increases having been observed after 15 and 24 hours, respectively. After 24 hours, the infected and control cells began to demonstrate differences, which gradually increased from 8 to 137% by the end of reproduction. After 30 hours of reproduction, the proportion of test sample cells undergoing G2 + M phase began to decrease by 17–28% as compared with the control cells. The cell switch-over to the synthesis of complete rabies virus particles occurred after 24 hours of reproduction. This is indicated by changes in the host cell cycle phases, as well as by the slowing down of BHK-21/SUSP/ARRIAH cell population growth.

**Keywords:** flow cytometry, cell cycle phases, BHK-21 cell suspension, rabies virus

**Acknowledgements:** This work was funded by the Federal Centre for Animal Health within the scope of research activities "Veterinary Welfare".

**For citation:** Guseva M. N., Doronin M. I., Shevchenko M. A., Mikhalishin D. V., Borisov A. V., El'kina Yu. S., Okovytaya T. V., Zakharov V. M., Mikhalishin V. V. Flow cytometry study of DNA transformation dynamics in BHK-21/SUSP/ARRIAH cell culture during rabies virus reproduction. *Veterinary Science Today*. 2024; 13 (1): 87–94. https://doi.org/10.29326/2304-196X-2024-13-1-87-94

**Conflict of interests:** The authors declare no conflict of interests.

**For correspondence:** Marina N. Guseva, Cand. Sci. (Biology), Senior Researcher, Laboratory for FMD Prevention, Federal Centre for Animal Health, Yur'evets, Vladimir 600901, Russia, *e-mail: guseva_mn@arriah.ru*

УДК 619:578.824.11:57.082.26

# Исследование методом проточной цитометрии динамики трансформации ДНК в культуре клеток BHK-21/SUSP/ARRIAH при репродукции вируса бешенства

**М. Н. Гусева, М. И. Доронин, М. А. Шевченко, Д. В. Михалишин, А. В. Борисов, Ю. С. Елькина, Т. В. Оковытая, В. М. Захаров, В. В. Михалишин**
ФГБУ «Федеральный центр охраны здоровья животных» (ФГБУ «ВНИИЗЖ»), мкр. Юрьевец, г. Владимир, 600901, Россия

## РЕЗЮМЕ

Исследование посвящено изучению динамики трансформации ДНК клеток сублинии BHK-21/SUSP/ARRIAH при репродукции в них вируса бешенства. Инфицированные возбудителем и контрольные интактные клетки культивировались в аналогичных условиях. При выявлении зависимости

инфекционности вируса от времени репродукции установили, что титр его инфекционной активности увеличивался от (3,2 ± 0,2) lg $ККИД_{50}$/$см^3$ при инокуляции до (7,63 ± 0,3) lg $ККИД_{50}$/$см^3$ через 48 ч репродукции, но более интенсивно он возрастал в первые 24 ч. Концентрация клеток при этом изменилась с 0,5 до 1,9 млн/$см^3$, то есть выросла в 3,8 раза. Спустя 24 ч кратность прироста клеток замедлялась. В процессе изучения фаз клеточного цикла при репродукции в клетке-хозяине вируса бешенства получены результаты, позволяющие оценить продолжительность и доминирование фаз G1, S, G2 + M на разных этапах культивирования. Динамика изменений популяции клеток, находящихся в апоптозе, в контроле и опыте была одинаковой в течение 36 ч культивирования. По прошествии данного времени инфицированных клеток в стадии апоптоза было на 28–42% больше по сравнению с таковыми в контроле. Доля клеток, находящихся в стадии фазы G1, через 9 ч в опытных образцах увеличилась на 11,7%, в контрольных, наоборот, уменьшилась на 16,6%. В дальнейшем количество клеток в G1-фазе и в контроле, и в опыте изменялось одинаково: через 15–18 ч наблюдали уменьшение на 40%, далее – ростовой скачок на 45–46%, потом опять снижение на 39–40%, затем вновь увеличение. После 33 ч репродукции и до окончания культивирования доля инфицированных клеток, находящихся в фазе G1, была значительно больше (на 12–21%), чем контрольных. Количество клеток в фазе S в опыте и контроле в первые сутки репродукции вируса было одинаковым, при этом наблюдали резкое скачкообразное увеличение через 15 и 24 ч в 3,4 и 2,4 раза соответственно. Через 24 ч между инфицированными и контрольными клетками начали проявляться различия, которые постепенно возрастали с 8 до 137% к окончанию репродукции. В фазе G2 + M через 30 ч репродукции количество клеток опытных образцов начинало уменьшаться на 17–28% по сравнению с контрольными. Перестройка клетки на синтез полных частиц вируса бешенства наступала через 24 ч репродукции. Об этом говорят изменения в фазах клеточного цикла клетки-хозяина и замедление прироста самой популяции клеток линии ВНК-21/SUSP/ARRIAH.

**Ключевые слова:** проточная цитометрия, фазы клеточного цикла, суспензия клеток ВНК-21, вирус бешенства

**Благодарности:** Исследование проведено за счет средств ФГБУ «ВНИИЗЖ» в рамках научно-исследовательских работ по теме «Ветеринарное благополучие».

**Для цитирования:** Гусева М. Н., Доронин М. И., Шевченко М. А., Михалишин Д. В., Борисов А. В., Елькина Ю. С., Оковытая Т. В., Захаров В. М., Михалишин В. В. Исследование методом проточной цитометрии динамики трансформации ДНК в культуре клеток ВНК-21/SUSP/ARRIAH при репродукции вируса бешенства. *Ветеринария сегодня*. 2024; 13 (1): 87–94. https://doi.org/10.29326/2304-196X-2024-13-1-87-94

**Конфликт интересов:** Авторы заявляют об отсутствии конфликта интересов.

**Для корреспонденции:** Гусева Марина Николаевна, канд. биол. наук, старший научный сотрудник лаборатории профилактики ящура ФГБУ «ВНИИЗЖ», мкр. Юрьевец, г. Владимир, 600901, Россия, *e-mail: guseva_mn@arriah.ru*

## INTRODUCTION

Rabies is an acute disease of warm-blooded animals that affects the central nervous system. All domestic and wild animal species, as well as human beings are susceptible to the disease [1, 2, 3, 4, 5].

The virus reproduction cycle includes its adsorption onto the cell surface, penetration into the cell, uncoating, transcription, translation, replication, assembly and budding.

Primary transcription does not require structural protein synthesis. There are 2 types of rabies virus transcription: 1) at first, leader RNA is transcribed with genomic RNA, then 5 monocistronic mRNAs are sequentially transcribed, from which N, P, M, L proteins are translated in the cytoplasm, and G gene mRNA is translated in polysomes associated with the Golgi apparatus membrane; 2) transcription of antigenomic (positive-sense) RNAs, which serve as templates for the synthesis of new generations of negative-sense genomic RNAs, with genomic RNA. Unlike primary transcription, genomic RNA replication by the described mechanism can occur only simultaneously with translation and synthesis of structural proteins, especially N and P ones, necessary for RNA encapsidation by nucleocapsid structures. L, N, and P proteins mainly control RNA transcription and replication, whereas M protein occupies an intermediate position between the nucleocapsid and the virion shell and participates in RNA condensation. Besides, M protein plays a key role in RNA synthesis regulation and budding of the virus [6, 7]. At the final stage of assembly and budding, nucleocapsids "put on" the viral envelope represented by G protein integrated into the cell membrane. Glycoprotein determines the neurovirulence and neuroinvasiveness of rabies virus.

The place of viral particle formation depends on the virus and host cells. For representatives of the genus *Lyssavirus* of the family *Rhabdoviridae*, to which rabies virus belongs, the synthesis and assembly of nucleocapsids occur in the cytoplasm, and budding occurs through the plasma membrane of the cell, from which the virion acquires an additional lipid layer.

Rabies virus does not cause characteristic cytopathic changes in cells. All transcription and replication events occur in the cytoplasm inside Negri bodies. These structures are typical for rabies and, thus, can be used as a pathognomonic symptom of the infection [8].

Flow cytometry is a modern technology that ensures rapid, high-quality and multiparametric analysis of cells. It is widely used in such fields of medicine as immunology, pharmacology, cytology, oncology, hematology, genetics, infectology [9, 10, 11, 12, 13, 14, 15].

Flow cytometry makes it possible to collect a variety of data: to determine DNA and RNA content in a cell, the total number of proteins and the number of specific proteins recognized by monoclonal antibodies, to investigate cell metabolism, to study the transport of calcium ions and the kinetics of enzymatic reactions [15, 16, 17, 18, 19, 20].

Each cell has its life cycle from the moment of its formation by mother cell division to mitosis or death. It is called a cell cycle. The cell cycle consists of two periods: 1) cell growth period (interphase); 2) cell division period called M phase (from the Greek word "mitos", meaning "thread"). In its turn, each of the said periods has several phases. Usually, the interphase takes at least 90% of the time of the entire cell cycle. Most of cell components are synthesized throughout the interphase, and this makes it difficult to identify individual stages within it.

The interphase is divided into G1, S and G2 subphases. A period within the interphase, during which cell nucleus DNA is replicated, is called "S phase" (from the word "synthesis"). It should be noted that not only DNA replication, but also the basic biosynthesis of structural and functional proteins of the cell occurs during the interphase (mainly in S phase). A period between M phase and the beginning of S phase is called G1 phase (from the word "gap"), and a period between the end of S phase and subsequent M phase is called G2 phase [21].

The use of flow cytometers in the studies made it possible to identify important data on the reproduction cycle phases of cells, as well as to obtain the results that allowed for the estimation of duration and predominance of the corresponding cell cycle G1, S, G2 + M phases at different stages of cultivation [18, 19, 20, 22, 23]. In the light of the above, it can be concluded that the nature of changes in the cell cycle during virus replication in a cell is important for understanding the process of virus reproduction, but is understudied.

The aim of the study was to examine the dynamics of DNA transformation in BHK-21/SUSP/ARRIAH cell culture during rabies virus reproduction using flow cytometry.

## MATERIALS AND METHODS

*Cell line*. Suspension continuous culture of newborn Syrian hamster kidney cells BHK-21/SUSP/ARRIAH was used in the work [24]. The cells were grown in metal bioreactors with a working capacity of up to 1,800 $dm^3$ in accordance with the "Master formula record for production of vaccine against foot-and-mouth disease of different types" approved by the Director of the Federal Centre for Animal Health.

*Rabies virus.* Production "ARRIAH" strain of rabies virus was used to infect the cells.

*Nutrient medium used to grow cells*. Eagle's medium supplemented with 5% of fetal bovine serum (Serana, Germany) and 0.25% blood protein hydrolysate (Russia) was used.

*Rabies virus cultivation*. Rabies virus reproduction was carried out during 48 hours in 0.5 $dm^3$ flasks using Minisart® RC25 Syringe Filters 17764-ACK Ø 25 mm (Sartorius, Germany).

*Growth rate* was calculated as the ratio of the final (after 48 hours) and initial cell concentrations within one passage.

*Cell infection with rabies virus*. Suspension BHK-21/SUSP/ARRIAH cell culture was inoculated with culture rabies virus at a dose of 0.1 $CCID_{50}$/cell. Samples containing infected cells were designated as test samples; cells not inoculated with the virus served as control and were cultivated under similar conditions.

*The identification of cell cycle stages* was carried out using an Accuri™ C6 flow cytometer and a BD Cycletest™ kit for working with a cytometer, as well as a reagent kit for quantitative DNA content analysis in cells C6 Flow Cytometer Fluid Kit (Becton Dickinson and Company, USA).

*The determination of rabies virus infectivity titre* was carried out in accordance with "Methodical guidelines for indirect determination of infectivity titre of culture rabies virus "ARRIAH" strain in vaccine production seed with real-time reverse transcription polymerase chain reaction (real-time RT-PCR)"[1].

Samples were collected every 3 hours throughout the entire time of rabies virus reproduction. The concentration of BHK-21/SUSP/ARRIAH cells in the suspension was determined using a Goryaev chamber for counting blood cells, dA0.000.851, compliant with TU 64-1-816-84. The cell suspension in a volume of 1 $cm^3$ was supplemented with an equal volume of 0.2% trypan blue solution, thoroughly mixed and used to fill the chamber. The number of cells in 1 $cm^3$ of the suspension was calculated using the formula [21]:

$$X = \frac{A \times B \times 4{,}000}{3{,}600} \times 1{,}000,$$

where $X$ is the number of cells in 1 $cm^3$; $A$ is the total number of cells in the chamber; $B$ is the suspension dilution.

The counting was performed using a microscope at 10× magnification.

*Statistical data processing.* Numerical data were statistically processed with generally accepted methods of variation statistics, using a personal computer and Microsoft Excel software.

## RESULTS AND DISCUSSION

The virus reproduction dynamics was assessed based on the following indicators:

– dependence of the virus infectivity titre on reproduction time;

– changes in apoptosis and debris;

– changes in cell cycle stages (G1, S, G2 + M).

During cultivation, the hydrogen ion concentration (pH) of the suspension was checked every 3 hours.

Figure 1 shows the dynamics of rabies virus infectivity during its reproduction in BHK-21/SUSP/ARRIAH cell culture.

It was found that the virus infectivity titre increased from (3.2 ± 0.2) lg $CCID_{50}/cm^3$ at the time of inoculation to (7.63 ± 0.3) lg $CCID_{50}/cm^3$ after 48 hours of reproduction, with the most intensive increase having been observed within the first 24 hours (up to 6.76 $CCID_{50}/cm^3$).

Within 48 hours, the cell concentration changed from 0.5 to 1.9 million/$cm^3$, i.e. increased by a factor of 3.8 (Fig. 2).

At the next stage, experiments were conducted to study changes in rabies virus infectivity titre in BHK-21/SUSP/ARRIAH cells with different initial concentrations: (0.77 ± 0.1); (3.0 ± 0.2) and (1.1 ± 0.1) million/$cm^3$. It was found that the accumulation of complete viral particles in the infected cells with different concentrations occurred within the first 12 hours of reproduction (Fig. 3).

[1] Doronin M. I., Mikhalishin D. V., Borisov A. V., Balashov A. N., Mudrak N. S., Zakharov V. M. Methodical guidelines for indirect determination of infectivity titre of culture rabies virus "ARRIAH" strain in vaccine production seed with real-time reverse transcription polymerase chain reaction (real-time RT-PCR): approved by FGBI "ARRIAH" 23.12.2021 No. 66–21. Vladimir; 2021. 59 p.

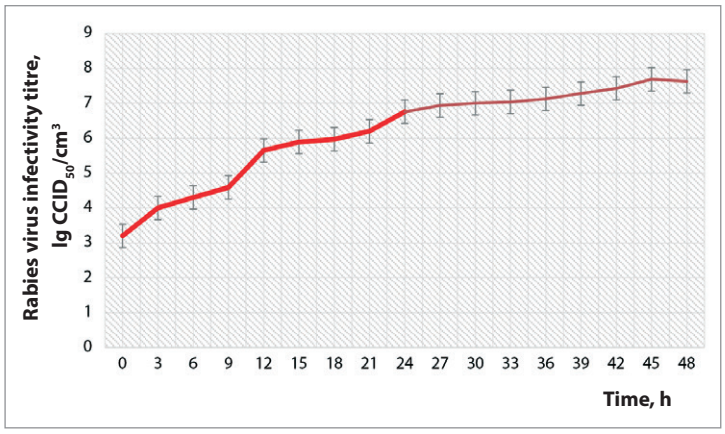


*Fig. 1. Rabies virus infectivity dynamics during its reproduction in cell culture (n = 3, p < 0.05)*

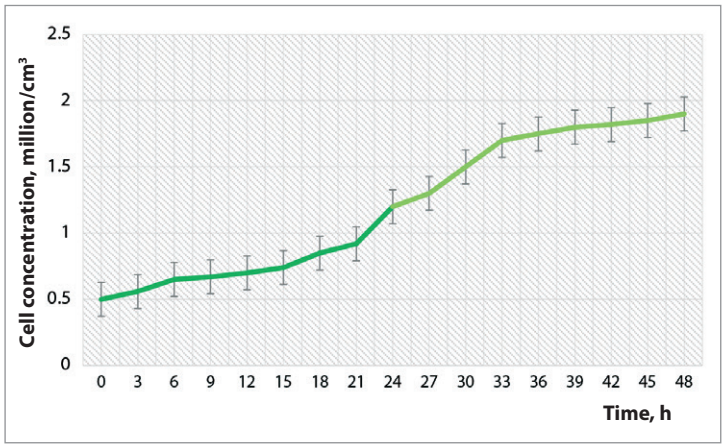


*Fig. 2. BHK-21/SUSP/ARRIAH cell concentration dynamics during rabies virus reproduction (n = 3, p < 0.05)*

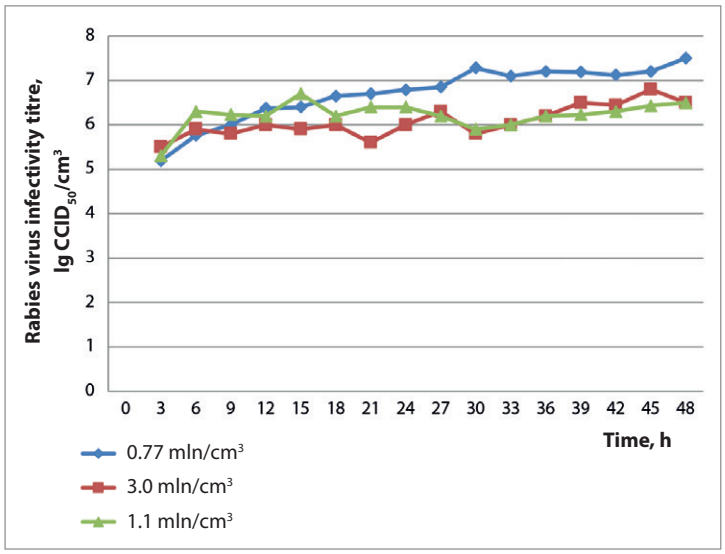


*Fig. 3. Rabies virus accumulation dynamics during its reproduction in cells with different initial concentrations (n = 3, p < 0.05)*

Subsequently, the virus accumulated 1.1–1.2 times more intensively in the culture with initial cell concentration of (0.77 ± 0.10) million/cm³. This is probably due to the limited amount of nutrients for the reproduction and growth of cells with a higher concentration.

Tests were carried out to study changes in cell cycle phases during rabies virus cultivation in suspension BHK-21/SUSP/ARRIAH cells. Under standard optimal conditions, intact populations are distributed according to cell cycle phases as follows: G1 – 30–75%, G2 + M – 2–18%, S – 2–33%, polyploids – up to 4% and debris – 1–20% [23].

It was found that the dynamics of changes in the apoptotic cell population in the control and test samples was similar within 36 hours of cultivation (a small time shift of 3 hours between the test and control samples was observed after 9 and 12 hours of reproduction). The proportion of apoptotic infected cells was 28–42% higher than that of apoptotic control cells 36 hours after the start of cultivation (Fig. 4).

After 9 hours, the proportion of cells undergoing G1 phase increased by 11.7% in the test samples, whereas it decreased by 16.6% in the control samples. Subsequently, the number of G1 phase cells in the control and test samples changed in the same way: a 40% decrease was observed after 15–18 hours, it was followed by a 45–46% growth jump, then again a 39–40% decrease and an increase were observed. After 33 hours of reproduction and till the end of cultivation, the proportion of infected cells undergoing G1 phase was significantly higher (by 12–21%) as compared with the control cells.

The comparison of the number of cells undergoing apoptosis + debris and G1 phase revealed a certain synchronicity between these phases: after 9 hours of the virus cultivation, G1 phase increased by 10–12%, at the same time apoptosis decreased by 10–11%, then a decrease in the percentage of G1 phase cells and an increase in the proportion of cells in apoptosis and debris were observed. A more pronounced "mirroring" of these cell cycle phases was observed after 24 hours of cultivation. A similar pattern was seen in the study of the cell cycle during foot-and-mouth disease virus reproduction [22].

The diploid phase of the cell cycle (G1), during which the synthesis of mRNA, structural proteins and other cell components began, prevailed in the cell cycle of intact BHK-21/SUSP/ARRIAH population. This phase accounted for 30 to 75% of cells, depending on the cultivation conditions, which is reflected in earlier studies [23].

It is known that during G1 phase, cells start to grow in size, mRNA and enzymes required for subsequent DNA replication are activated [21].

G1 phase cells in the suspension culture were maintained at 37–70% throughout the entire virus reproduction time, i.e. cells infected with the virus served as a source of energy and material for the synthesis of viral components, as well as for the assembly of complete virions.

The transition from diploid (G1) phase to synthetic (S) phase is one of the check points of the cell cycle. Depending on the amount of nutrients and energy, as well as on external factors of cultivation, the cell "decides" whether to enter the cell cycle or to go into a non-dividing quiescent state known as G0 phase that leads to apoptosis. The main event of S phase is DNA replication, which has its specific features [21].

In our tests, the percentage of S phase cells in the test and control samples was the same during the first day of the virus reproduction, with sharp jump-like

3.4- and 2.4-fold increases having been observed after 15 and 24 hours, respectively (Fig. 5). After 24 hours, the infected and control cells began to demonstrate differences, which gradually increased from 8 to 137% by the end of reproduction. It can be assumed that the cells undergoing the said phase also participated in the virus reproduction.

In G2 phase, the last of three successive phases of the cell cycle interphase, and M phase, a tetraploid population was formed. In the intact and rabies virus infected cells of BHK-21/SUSP/ARRIAH line, the phase of preparation for mitosis and mitosis itself accounted for 2 to 20% of the entire population. After 30 hours of reproduction, the proportion of test sample cells undergoing these phases began to decrease by 17–28% as compared with the control cells (Fig. 6). This was probably due to the fact that the overall biosynthesis and functions of the host cell were inhibited as a result of the biosynthesis of rabies virus components; therefore, the percentage of G2 + M phase cells decreased.

The number of polyploid cells in the test samples was approximately equal during 45 hours, with a sharp 2.0–2.5-fold increase having been observed only within the last hours of the virus reproduction. In the control cells, the population ploidy was abrupt: 2.06-, 2.7-, 1.56- and 3.18-fold increases were observed after 9, 21, 42 and 48 hours, respectively. It can be assumed that polyploid cells were partially resistant to the virus. This fact has already been noted earlier in the study of foot-and-mouth disease virus reproduction in BHK-21 cells [22].

The DNA histograms of BHK-21/SUSP/ARRIAH cells during rabies virus reproduction 24 hours after the start of infection and after 42 hours of cultivation (Fig. 7) summarize the graphs shown in Figures 4–6.

## CONCLUSION

The phases of the cell cycle during rabies virus reproduction in the host cell were examined, the results obtained allowed for the estimation of duration and predominance of the corresponding cell cycle G1, S, G2 + M phases at different stages of cultivation.

When studying the dynamics of changes in the DNA of BHK-21/SUSP/ARRIAH cells infected with rabies virus, it was found that after 24 hours of the virus cultivation, the proportion of cells in G1 phase increased as compared with the control cells (by 12–21%). Since mRNA, structural proteins, other cell components that are also necessary for rabies virus virion assembly are synthesized during G1 phase, the growth of cells in this phase of the cycle can be considered expectable.

It was found that since the main event of S phase is DNA replication, a linear decrease of S phase during rabies virus cultivation after 24 hours of reproduction may also be indicative of the cell switch-over from cell DNA replication to viral RNA synthesis.

It was revealed that G2 phase and M phase (postsynthetic, or premitotic phase, and mitosis itself) of the test sample cell life cycle were relatively decreased (by 17–28%) as compared with the control cells, i.e. the infected cells spent less time in the division stage than the uninfected ones. This was probably due to the fact that the overall biosynthesis and functions of the host cell were inhibited as a result of the biosynthesis of rabies virus components.

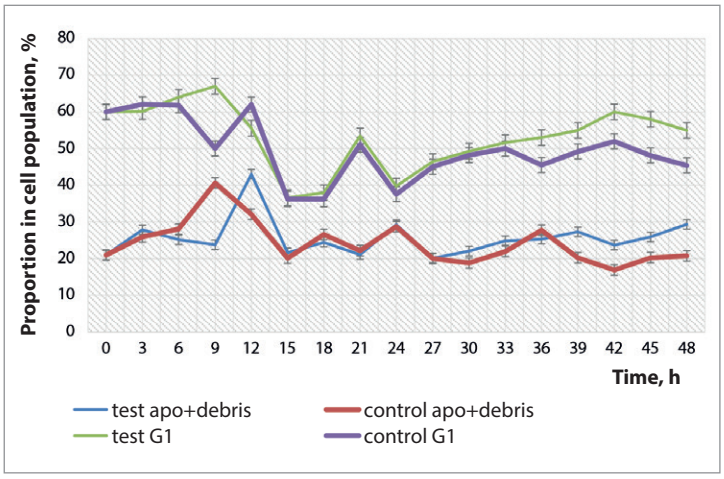


*Fig. 4. Changes in percentages of cells undergoing apoptosis + debris and G1 phase in BHK-21/SUSP/ARRIAH culture during rabies virus reproduction (n = 3, p < 0.05)*

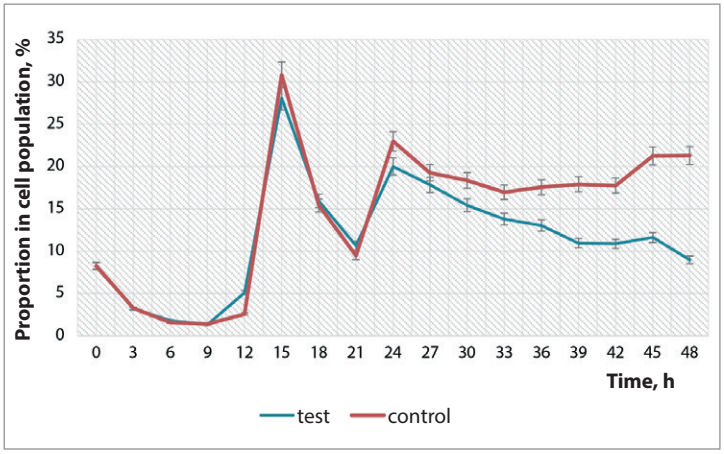


*Fig. 5. Changes in percentages of cells undergoing S phase in BHK-21/SUSP/ARRIAH culture during rabies virus reproduction (n = 3, p < 0.05)*

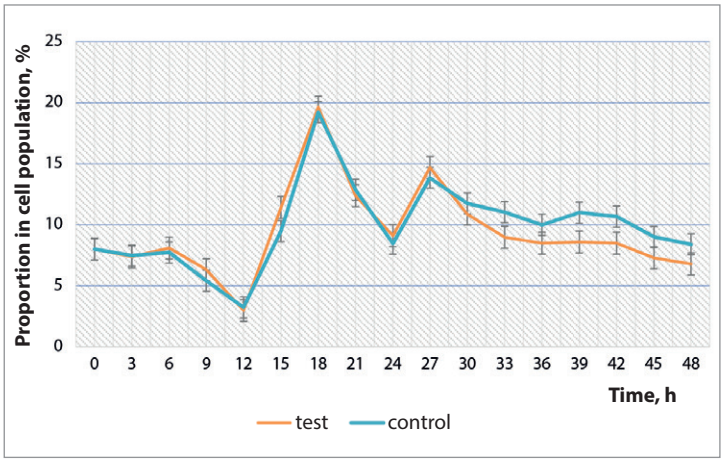


*Fig. 6. Changes in percentages of G2 + M phase cells in BHK-21/SUSP/ARRIAH culture during rabies virus reproduction (n = 3, p < 0.05)*

The polyploid cells formed during the cell and virus cultivation were found to be partially resistant to rabies virus and probably had the least sensitivity.

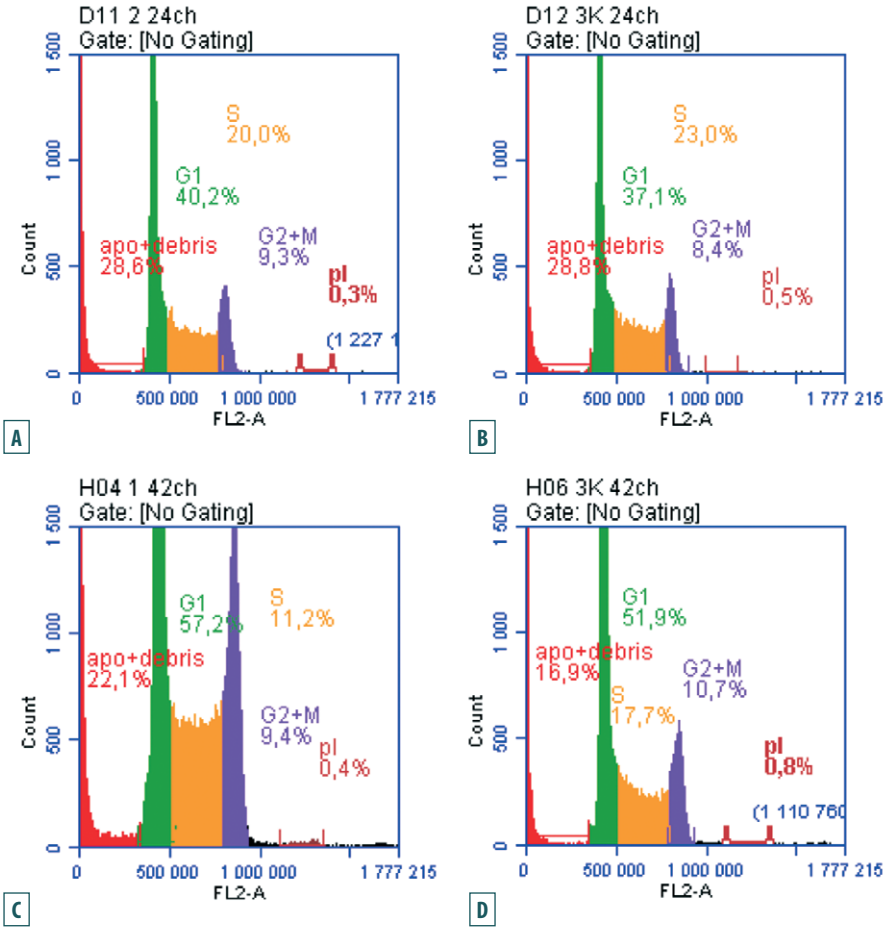


*Fig. 7. Comparison of DNA histograms of BHK-21/SUSP/ARRIAH cells during rabies virus reproduction and those of control samples after 24 hours (A – test, B – control) and after 42 hours (C – test, D – control)*

It was established that BHK-21/SUSP/ARRIAH cell switch-over to the synthesis of complete rabies virus particles occurred after 24 hours of reproduction, as indicated by changes in the host cell cycle phases, as well as by the slowing down of the cell population growth.

## REFERENCES

1. Syurin V. N., Samuilenko A. Ya., Solovyev B. V., Fomina N. V. Viral Diseases of Animals. Moscow: VNITIBP; 2001. 928 p. (in Russ.)

2. Gruzdev K. N., Metlin A. E. Animal Rabies. Vladimir: FGBI "ARRIAH"; 2019. 394 p. EDN: FCSNOH (in Russ.)

3. Makarov V. V., Gulyukin A. M., Gulyukin M. I. Rabies: Natural History at Centuries Boundary. Moscow: ZooVetKniga; 2015. 121 p. EDN: TXECJR (in Russ.)

4. Makarov V. V., Lozovoy D. A., Briko N. I. Cheiropter rabies and man. *Epidemiology and Infectious Diseases. Current Items*. 2015; 6: 46–53. EDN: VEAETL (in Russ.)

5. Metlin A. Ye. Modern aspects of lyssavirus classification. *Veterinary Science Today.* 2017; (3): 52–57. EDN: ZIGAFP (in Russ.)

6. Guidance on virology. Human and animal viruses and viral infections. Ed. by D. K. Lvov. Moscow: Meditsinskoe informatsionnoe agentstvo; 2013. 1200 p. (in Russ.)

7. Bourhy H., Cowley J. A., Larrous F., Holmes E. C., Walker P. J. Phylogenetic relationships among rhabdoviruses inferred using the L polymerase gene. *Journal of General Virology.* 2005; 86 (10): 2849–2858. https://doi.org/10.1099/vir.0.81128-0

8. Albertini A. A. V., Schoehn G., Weissenhorn W., Ruigrok R. W. H. Structural aspects of rabies virus replication. *Cellular and Molecular Life Sciences*. 2008; 65 (2): 282–294. https://doi.org/10.1007/s00018-007-7298-1

9. Barabash E. Yu., Kalinina E. P., Gvozdenko T. A., Denisenko Yu. K., Novgorodtseva T. P., Antonyuk M. V., Khodosova K. K. Regulation of immune response of patients with partially controlled vs controlled bronchial asthma. *Medical Immunology (Russia).* 2017; 19 (1): 65–72. https://doi.org/10.15789/1563-0625-2017-1-65-72 (in Russ.)

10. Voitkova V. V. Study of apoptosis with use of flow cytometry (review of literature). *Bulletin of Eastern-Siberian Scientific Center.* 2010; 6-1 (76): 220–225. EDN: OHWEVV (in Russ.)

11. Ishchenko I. O., Oppedisano M. D. L. Improvement of antituminal immunity assessment using the method of flow cytometry. *Forcipe*. 2019; 2 (4): 24–31. EDN: STCTRI (in Russ.)

12. Kozyreva V. S., Shilova A. N., Shkoda O. S. Flow cytometry for measuring residual leukocytes in blood plasma. *Russian Journal of Hematology and Transfusiology.* 2019; 64 (1): 66–72. https://doi.org/10.35754/0234-5730-2019-64-1-66-72 (in Russ.)

13. Yagunov A. S., Kartashov A. V., Tokalov S. V., Kiseleva L. N. Main milestones of development and application of ductal cytometry at the Russian center for research in radiological and surgical techniques, St. Petersburg. *Voprosy Onkologii = Problems in Oncology.* 2008; 54 (4): 494–497. EDN: JVSUZB (in Russ.)

14. Stakhneva E. M., Ragino Yu. I. Modern methods for studying atherosclerosis and coronary artery disease: flow cytometry. *Bulletin of Siberian Medicine*. 2021; 20 (2): 184–190. https://doi.org/10.20538/1682-0363-2021-2-184-190

15. Barnum K. J., O'Connell M. J. Cell cycle regulation by checkpoints. *In: Cell Cycle Control. Methods in Molecular Biology*. *Eds. E. Noguchi, M. Gadaleta*. 2014; 1170: 29–40. https://doi.org/10.1007/978-1-4939-0888-2_2

16. Manin B. L., Nochevny V. T., Khaidukov S. V., Laskavy V. N. Efficiency of flow cytometric analysis used to study of mechanisms of reparation of BHK-21 cells in the process of cultivation and cryoconservation. *Veterinary Medicine*. 2011; 95: 66–70. EDN: SMUJVJ (in Russ.)

17. Sherman J., Wang R. Rapid profiling of G2 phase to mitosis progression by flow cytometry in asynchronous cells. *Cell Cycle*. 2020; 19 (21): 2897–2905. https://doi.org/10.1080/15384101.2020.1827510

18. Rieger A. M. Flow cytometry and cell cycle analysis: An overview. *In: Cell-Cycle Synchronization. Methods in Molecular Biology. Ed. Z. Wang*. 2022; 2579: 47–57. https://doi.org/10.1007/978-1-0716-2736-5_4

19. Kim K. H., Sederstrom J. M. Assaying cell cycle status using flow cytometry. *Current Protocols in Molecular Biology*. 2015; 111: 28.6.1–28.6.11. https://doi.org/10.1002/0471142727.mb2806s111

20. Nair A., Manohar S. M. A flow cytometric journey into cell cycle analysis. *Bioanalysis*. 2021; 13 (21): 1627–1644. https://doi.org/10.4155/bio-2021-0071

21. Cells. Eds. B. Lewin, L. Cassimeris, V. R. Lingappa, G. Plopper. Sudbury (Massachusetts): Jones and Bartlett Publishers; 2007. 863 p.

22. Guseva M. N., Shevchenko M. A., Mikhalishin D. V., Mikhalishin V. V., Manin B. L. Study of DNA transformation dynamics in BHK-21/2-17 cell culture using flow cytometry during FMD virus reproduction. *Veterinary Science Today*. 2018; (4): 39–43. https://doi.org/10.29326/2304-196X-2018-4-27-39-43

23. Shevchenko M. A., Guseva M. N., Mikhalishin D. V., Manin B. L., Shishkova A. A. Studies of the BHK-21/2-17 lineage using flow cytometry. *Veterinary Science Today*. 2017; (4): 58–62. EDN: YKWAND (in Russ.)

24. Lozovoj D. A., Guseva M. N., Mikhalishin D. V., Doronin M. I., Manin B. L., Shishkova A. A., et al. BHK-21/SUSP/ARRIAH – continuous suspension subline of newborn Syrian hamster kidney cells, intended for reproduction of foot-and-mouth disease viruses, rabies, parainfluenza-3, Aujeszky's disease in producing antiviral vaccines, as well as for making diagnostic and preventive veterinary biopreparations. Patent No. 2722671 C1 Russian Federation. Int. Cl. C12N 5/10 (2006.01). FGBI "ARRIAH". No. 2019131190. Date of filing: 01.10.2019. Date of publication: 02.06.2020. Bull. No. 16.

*Received 22.08.2023*
*Revised 09.10.2023*
*Accepted 08.11.2023*

## INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

**Marina N. Guseva,** Cand. Sci. (Biology), Senior Researcher, Laboratory for FMD Prevention, Federal Centre for Animal Health, Vladimir, Russia; *https://orcid.org/0000-0002-3997-3390, e-mail: guseva_mn@arriah.ru*

**Гусева Марина Николаевна,** канд. биол. наук, старший научный сотрудник лаборатории профилактики ящура ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0002-3997-3390, e-mail: guseva_mn@arriah.ru*

**Maksim I. Doronin,** Dr. Sci. (Biology), Leading Researcher, Laboratory for FMD Prevention, Federal Centre for Animal Health, Vladimir, Russia; *https://orcid.org/0000-0002-4682-6559, e-mail: doronin@arriah.ru*

**Доронин Максим Игоревич,** д-р биол. наук, ведущий научный сотрудник лаборатории профилактики ящура ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0002-4682-6559, e-mail: doronin@arriah.ru*

**Maxim A. Shevchenko,** Leading Veterinarian, Laboratory for FMD Prevention, Federal Centre for Animal Health, Vladimir, Russia; *https://orcid.org/0009-0006-5874-8363, e-mail: shevchenko_ma@arriah.ru*

**Шевченко Максим Александрович,** ведущий ветеринарный врач лаборатории профилактики ящура ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0009-0006-5874-8363, e-mail: shevchenko_ma@arriah.ru*

**Dmitry V. Mikhalishin,** Dr. Sci. (Veterinary Medicine), Head of Laboratory for FMD Prevention, Federal Centre for Animal Health, Vladimir, Russia; *https://orcid.org/0000-0003-1718-1955, e-mail: mihalishindv@arriah.ru*

**Михалишин Дмитрий Валерьевич,** д-р вет. наук, заведующий лабораторией профилактики ящура ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0003-1718-1955, e-mail: mihalishindv@arriah.ru*

**Alexey V. Borisov,** Cand. Sci. (Veterinary Medicine), Head of Department, Laboratory for FMD Prevention, Federal Centre for Animal Health, Vladimir, Russia; *https://orcid.org/0000-0001-9880-9657, e-mail: borisov_av@arriah.ru*

**Борисов Алексей Валерьевич,** канд. вет. наук, начальник отдела лаборатории профилактики ящура ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0001-9880-9657, e-mail: borisov_av@arriah.ru*

**Yulia S. El'kina,** Cand. Sci. (Veterinary Medicine), Junior Researcher, Laboratory for FMD Prevention, Federal Centre for Animal Health, Vladimir, Russia; *https://orcid.org/0000-0002-2986-8992, e-mail: elkina_ys@arriah.ru*

**Елькина Юлия Сергеевна,** канд. вет. наук, младший научный сотрудник лаборатории профилактики ящура ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0000-0002-2986-8992, e-mail: elkina_ys@arriah.ru*

**Tatyana V. Okovytaya,** Cand. Sci. (Biology), Senior Researcher, Laboratory for FMD Prevention, Federal Centre for Animal Health, Vladimir, Russia; *https://orcid.org/0009-0002-8403-8665, e-mail: okovitaya@arriah.ru*

**Оковытая Татьяна Владимировна,** канд. биол. наук, старший научный сотрудник лаборатории профилактики ящура ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *https://orcid.org/0009-0002-8403-8665, e-mail: okovitaya@arriah.ru*

**Valery M. Zakharov,** Dr. Sci. (Veterinary Medicine), Professor, Federal Centre for Animal Health, Vladimir, Russia; *Author ID: 7402991265, e-mail: zaharov@arriah.ru*

**Захаров Валерий Михайлович,** д-р вет. наук, профессор, ФГБУ «ВНИИЗЖ», г. Владимир, Россия; *Author ID: 7402991265, e-mail: zaharov@arriah.ru*

**Valery V. Mikhalishin,** Dr. Sci. (Veterinary Medicine), Professor, Chief Researcher, Information and Analysis Centre, Federal Centre for Animal Health, Vladimir, Russia;
*https://orcid.org/0009-0001-9538-5657, e-mail: mihalishin@arriah.ru*

**Михалишин Валерий Васильевич,** д-р вет. наук, профессор, главный научный сотрудник информационно-аналитического центра ФГБУ «ВНИИЗЖ» г. Владимир, Россия;
*https://orcid.org/0009-0001-9538-5657, e-mail: mihalishin@arriah.ru*

**Contribution:** Guseva M. N. – conceptualization, data interpretation, text preparation, literature searches, analysis and synthesis of study findings; Doronin M. I. – conceptualization, bioinformatic analysis, statistical analysis; Shevchenko M. A. – experimentation, data collection; Mikhalishin D. V. – conceptualization, administration, funding acquisition, resource provision; Borisov A. V. – data interpretation, text preparation, literature searches; El'kina Yu. S. – experimentation, data collection; Okovytaya T. V. – experimentation, data collection; Zakharov V. M. – administration, text editing; Mikhalishin V. V. – conceptualization, analysis and synthesis of study findings, final version approval.

**Вклад авторов:** Гусева М. Н. – концепция исследования, интерпретация данных, подготовка текста, работа с литературой, анализ и обобщение результатов исследования; Доронин М. И. – концепция исследования, биоинформатический анализ, проведение статистического анализа; Шевченко М. А. – проведение экспериментов, сбор материала; Михалишин Д. В. – концепция исследования, администрирование, получение финансирования, ресурсное обеспечение исследования; Борисов А. В. – интерпретация данных, подготовка текста, работа с литературой; Елькина Ю. С. – проведение экспериментов, сбор материала; Оковытая Т. В. – проведение экспериментов, сбор материала; Захаров В. М. – администрирование, редактирование текста; Михалишин В. В. – концепция исследования, анализ и обобщение результатов исследования, утверждение окончательного варианта.