DOI: 10.29326/2304-196X-2023-12-4-345-353

Goat-derived cell line (*Capra hircus*) TCh generated by karyological and morphological transformation of YaDK-04 CCL during subcultivation with lanthanide-treated bovine serum

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ABSTRACT

Ability of the continuous cell lines to evolve enables generation of new transformed cell cultures with unlimited life potential and different from the original prototypes in the process of sequential cultivation. There are practically no universal mechanisms and methods for new cell line generation. But it was noted that cell immortalization is associated with chromosomal rearrangements (chromatid morphology) and changes in the number of chromosomes. The paper presents the results of the generation of a new *Testis Capra hircus* (TCh) cell line, suitable for effective replication of dermatotropic and other types of animal viruses, in order to scale up viral material used for the manufacture of the means for animal disease specific prevention and diagnosis. The monolayer TCh cell line was transformed from the continuous YaDK-04 cell line as a result of more than 50 passages in the growth medium supplemented with 10% of lanthanide-treated bovine serum. Use of the bovine serum purified and supplemented with lanthanides during the cultivation of the continuous cell line YaDK-04 led to significant chromosomal rearrangements and contributed to the formation of a stable and productive new TCh cell line, which differed in cytomorphological and karyological characteristics and had unlimited potential for passaging without changing the cell karyotype and morphology. The novel continuous cell line proved to be suitable for effective reproduction of such disease pathogens as lumpy skin disease, sheep pox, peste des petits ruminants agents. These are mainly viruses of dermatotropic origin.

Keywords: continuous cell line, proliferative activity, immortalization, cytopathic effect, infectious activity

For citation: Trofimova E. A., Kononova S. V., Shumilova I. N., Manin B. L. Goat-derived cell line (*Capra hircus*) TCh generated by karyological and morphological transformation of YaDK-04 CCL during subcultivation with lanthanide-treated bovine serum. *Veterinary Science Today.* 2023; 12 (4): 345–353. DOI: 10.29326/2304-196X-2023-12-4-345-353.

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

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УДК 619:616.98:578:57.082.26

Получение клеточной линии козьего происхождения (*Capra hircus*) TCh как результат кариологической и морфологической трансформации ПЛК ЯДК-04 при субкультивировании с применением сыворотки крупного рогатого скота, обработанной лантаноидами

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РЕЗЮМЕ

Способность перевиваемых линий клеток к эволюции дает возможность создавать в процессе последовательного культивирования новые трансформированные клеточные культуры, обладающие неограниченным жизненным потенциалом и отличающиеся от исходных прототипов. Универсальных

механизмов и способов получения новых клеточных линий практически не существует. Но было замечено, что иммортализация клеток связана с хромосомными перестройками (морфология хроматид) и изменением количества хромосом. Представлены результаты получения новой клеточной линии тестикул козленка *Testis Capra hircus* (TCh), пригодной для эффективной репродукции дерматотропных и других видов вирусов животных, с целью наработки вирусного материала, применяемого для изготовления средств специфической профилактики и диагностики заболеваний животных. Монослойная линия клеток TCh трансформировалась из перевиваемой линии клеток ЯДК-04 в результате проведения более 50 пассажей культивирования в ростовой среде с добавлением 10% сыворотки крови крупного рогатого скота, обработанной лантаноидами. Применение сыворотки крови крупного рогатого скота, очищенной и обогащенной лантаноидами, при культивировании постоянной линии клеток ЯДК-04 привело к значительным хромосомным перестройкам и способствовало формированию стабильной и продуктивной новой клеточной линии TCh, которая отличалась по цитоморфологическим и кариологическим признакам и обладала неограниченным потенциалом к пассированию без изменения кариотипа и морфологии клеток. Новая перевиваемая линия клеток оказалась пригодной для эффективной репродукции возбудителей таких болезней, как заразный узелковый дерматит, оспа овец, чума мелких жвачных животных. В основном это вирусы дерматотропного происхождения.

Ключевые слова: перевиваемая (постоянная) линия клеток, пролиферативная активность, иммортализация, цитопатическое действие, инфекционная активность

Для цитирования: Трофимова Е. А., Кононова С. В., Шумилова И. Н., Манин Б. Л. Получение клеточной линии козьего происхождения (*Capra hircus*) TCh как результат кариологической и морфологической трансформации ПЛК ЯДК-04 при субкультивировании с применением сыворотки крупного рогатого скота, обработанной лантаноидами. *Ветеринария сегодня*. 2023; 12 (4): 345–353. DOI: 10.29326/2304-196X-2023-12-4-345-353.

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

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INTRODUCTION

The mechanisms of formation of continuous cell lines (CCL) have not been fully established. But due to quite frequent appearance of infinitely dividing (immortal) CCL, it can be assumed that the mechanism of formation of the continuous cell lines has some patterns, manifested in changes in the cell karyology and morphology during *in vitro* cultivation. One of such patterns involves the increase in proliferative activity – a sign of immortality (immortalization), which occurs by passage 50 after trypsinization of tissues or organs [1, 2, 3]. Changes occurring at the chromosomal and genetic levels of the cells under the effect of various physico-chemical and biological factors are likely to accumulate [4, 5, 6]. The mechanism of stable heterochromatin methylation in metacentric sections of chromosomes is formed and thereby the ability to unlimited division of the main population of cell lines develops under stable cultivation conditions [3, 7, 8, 9].

In veterinary virology, three lines of goat-derived cell lines (*Capra hircus*) obtained from goat gonads are known: CG-91 [10], YaDK-04 [11], TCh [12]. In fact, these lines are derivatives of the single trypsinization, which was carried out in the late 1980s. They were formed under different cultivation conditions and effect of additional chemical factors. Whereas the first two lines have low proliferative activity (split ratio 1:2, 1:4), instability of cultivation and a near-diploid chromosome complement), the third line (TCh), obtained using lanthanide treated bovine serum, has significant chromosomal rearrangements and surpasses its predecessors in performance and stability. This report provides an example of the evolution of the goat gonad cell line and describes the proposed mechanism for the formation of a stable and productive CCL suitable for the effective reproduction of dermatotropic and other animal viruses.

The work was aimed at the study of the karyological and cultural transformation of the TCh cell line obtained by subcultivation of the continuous YaDK-04 cell line, as well as at the assessment of the degree of sensitivity of the new cell subline to the viruses – agents of the animal diseases.

MATERIALS AND METHODS

The cells were cultured according to the generally accepted technique in glass and plastic flasks using classical media MEM, DMEM, DMEM/F-12 supplemented with 10% of the lanthanide treated bovine serum.

Phenotyping of the cell lines was carried out using Olympus CX41 phase contrast microscope (Japan) and ML-2B fluorescent microscope (Russia).

The cell cultures were identified by the karyological method of metaphase spread preparation according to P. S. Moorhead technique [13].

Viral material. Lumpy skin disease virus strain LSD Cattle/Dagestan/2015 with infectivity 5.0 lg $TCID_{50}/cm^3$, production sheep pox virus strain "ARRIAH" with infectivity 5.5 lg $TCID_{50}/cm^3$ and production peste des petits ruminants virus (PPRV) strain "ARRIAH" with infectivity 5.0 lg $TCID_{50}/cm^3$ were used in the study [14, 15, 16, 17].

Cell culture. Cultivation of the viruses was carried out in the cell cultures of various origin, which were obtained from the Cell Cultivation Unit of the Federal Centre for Animal Health.

The infectivity of the obtained viral material was determined by titration using YaDK-04 cell line.

RESULTS AND DISCUSSION

There are no any patterns in the formation of the continuous cell lines of goat origin (*Capra hircus*). Use of a variety of nutrient media and sera, duration of passaging do not allow identification of any significant factor that

would affect the karyological and genetic transformation of the cell lines. The first two variants described below have a unique origin, however they turned out to be similar in characteristics.

It is well known that the effectiveness of cell cultivation is associated with the quality of the nutrient medium, the growth properties of which are largely dependent on the quality of animal serum in its composition. Lanthanide treated bovine serum was used in this study [18]. The use of lanthanides in serum at the stage of its production leads to flocculation of latent microorganisms and endotoxins, which settle and can be removed by separation and ultrafiltration. The lanthanides themselves remain in the medium and participate in the biochemical processes of cultivation. The duration of passaging under homogeneous conditions can lead to certain changes observed in the further study of the cell cytomorphology.

Characteristics of the continuous goat gonad cell line CG-91. The only continuous goat gonad cell line (*Capra hircus*) CG-91, which has no analogues in other countries, was obtained at the Federal Centre for Animal Health in 1990 (RF Patent No. 2061753) [10]. This CCL is quite difficult to cultivate as the split ratio is no more than 1:2–1:4 and the modal class is 59 chromosomes. The CCL subcultivation resulted in almost immediate elimination of the small metacentric Y-chromosome, and the culture was formed as a pseudodiploid.

The CCL is derived (established) from the organs spontaneously, as a result of the long passaging with a variety of nutrient media, as well as with supplementation with animal sera and using some other technological approaches associated with cultivation temperature, subculture methods, duration of cultivation, use of conditioned media, etc. [19, 20, 21]. In this particular case, the CG-91 cell line was stabilized as continuous when cultured in DMEM. As an additive, 10% of different types of bovine sera, including fetal one, were used. Morphological features of CG-91 CCL lie in the fact that spindle-shaped cells predominate in the cell population along with a small number of epithelial and fibroblast-like cells. When the culture is reseeded, the spindle-shaped cells undergo a fibroblast-like transformation under the effect of the dispersing solution or begin taking a spherical shape, while up to 20% of the total number of cells die (Fig. 1). To mitigate the process of trypsinization, 0.5% glucose solution is added to the dispersing solution. The cells survived after the reseeding repair during sedimentation and adhesion to the substrate and form a confluent monolayer in 3–4 days.

CG-91 cell line was found to be sensitive to type A, O, C, Asia-1 foot-and-mouth disease virus, as well as to African horse sickness causative agent.

Characteristics of continuous goat gonad subline YaDK-04. The continuous cell subline YaDK-04 was derived from CG-91 CCL by targeted selection aimed at the increase of the cell biomass and their sensitivity to animal viruses [11]. The selection was carried out using limiting dilution of the cell suspension when reseeded using Eagle's medium supplemented with 0.25% lactalbumin hydrolysate.

Using the limiting dilution method during the cell line reseeding, the subline performance was increased at passage 36 and amounted to 100 million cells from 300 cm^2 culture vial. The morphology of the cells and monolayer remained the same and spindle-shaped cells

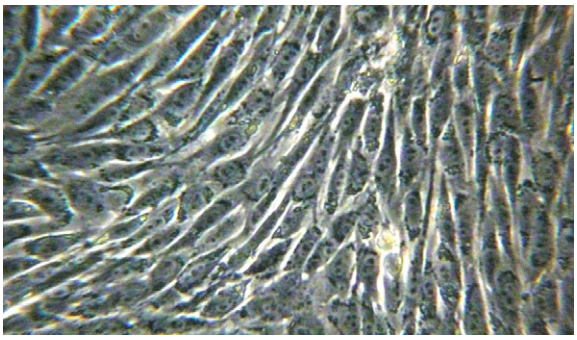

Fig. 1. YaDK-04 cell line morphology

prevailed (Fig. 1). The karyotype became more variable and amounted to 57–60 chromosomes (Fig. 2, 3). The predominant population is the modal class of 59 chromosomes (58%).

As a result of the selection, the list of the viruses efficiently reproduced on this subline was extended. Thus, Aujeszky's disease virus titer reached 8.00–8.75 lg $TCID_{50}/cm^3$, sheep pox virus titer – 5.5–6.0 lg $TCID_{50}/cm^3$, pneumovirus titer – 5.0 lg $TCID_{50}/cm^3$.

Both described variants of goat gonad cells are practically diploid cultures with minimal transformations in karyotype and morphology, the split ratio of which does not exceed 1:4. These CCLs periodically demonstrated suppressed growth activity, therefore, change of growth

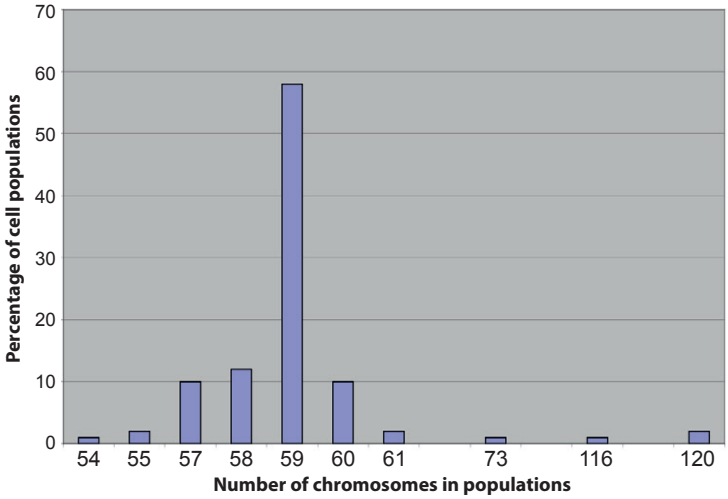

Fig. 2. YaDK-04 cell line karyogram

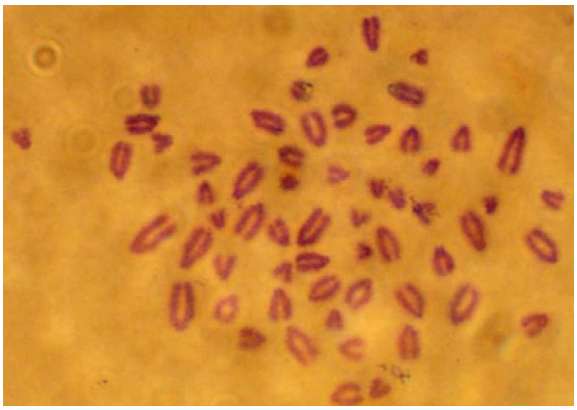

Fig. 3. Metaphase spread of YaDK-04 cell line – 59 chromosomes

ingredients and recovery from the cryobank were required. Propagation of these variants for the production of the specific vaccine products required large material and labor costs.

Production of new continuous TCh cell line. When analyzing YaDK-04 karyogram, we noted sufficient chromosome variability in cell populations – from 54 to 120. This fact suggests that culture selection is possible to isolate cells different from the previous clones. A monolayer goat gonad CCL (YaDK-04) was used as a starting material for this purpose, which was characterized by variable split ratio stability ranging from 1:2 to 1:4.

Use of lanthanide treated bovine serum in the culture technology resulted in the signs of stabilization of the continuous cell line proliferation. Long-term serial cell culture passaging was performed with 72–96 hours cycle and without cryo stage. The activities aimed at the adjustment of the lanthanide treated bovine serum properties was carried out in parallel. Using the tested cell culture, information was obtained about the serum toxicity, its adhesive properties and growth activity. This allowed the serum manufacturers to eliminate latent viruses and endotoxins thus significantly improving the quality of the formed YaDK-04 monolayer and increasing the cell performance [18, 21, 22].

The long-term serial passaging of YaDK-04 without cryo stage was carried out under standard conditions with 10% of the lanthanide treated bovine serum. By passage 44, the cell morphology began to change: the epithelial-like cells prevailed and they became dense by the end of the logarithmic growth phase (Fig. 4). During passages 44 and 55, the karyological examination was carried out, whose results demonstrated significant rearrangements in the karyotype (Fig. 5, 6). Populations with a hyperploid set of chromosomes and 2–4 metacentric elements predominated (Fig. 6, 7). The split ratio increased to 1:6 or higher and maintained by passage 70.

Cultivation for over 50 passages without cryo stage, split ratio increase, morphological and karyological changes indicated production of a new stable lamb testicle subline, which was named TCh (*Testis Capra hircus*). This CCL consisted of predominant epithelial-like cells; spindle-shaped and fibroblast-like cells in smaller numbers concentrated on the substrate amongst the main population.

After the obtained subline was subjected to over 70 serial passages (within 18 months), no signs of degeneration were observed and the CCL was characterized by stable cultural parameters.

Long-term cultivation with lanthanide treated blood serum resulted in a significant transformation of the karyotype. For karyological analysis of the TCh cell subline, chromosomal preparations were prepared using P. S. Moorhead method [13], 100 metaphase spreads

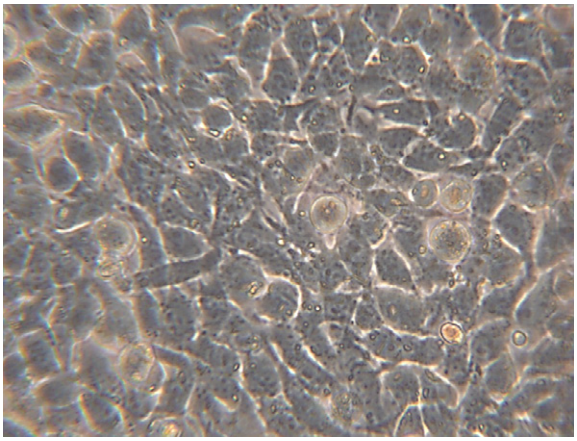

Fig. 4. Morphology of TCh CCL (passage 70) 48 hours

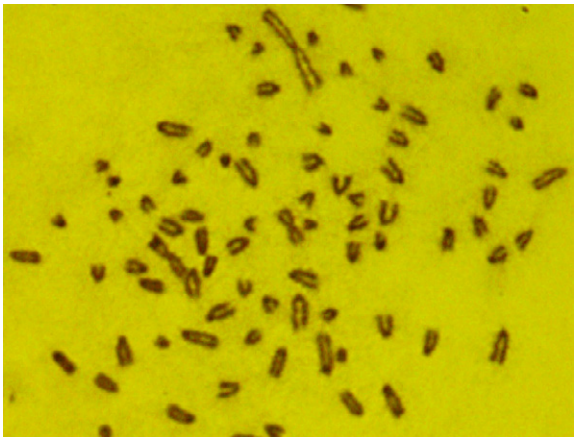

Fig. 6. TCh-2 CCL metaphase spread (passage 70)

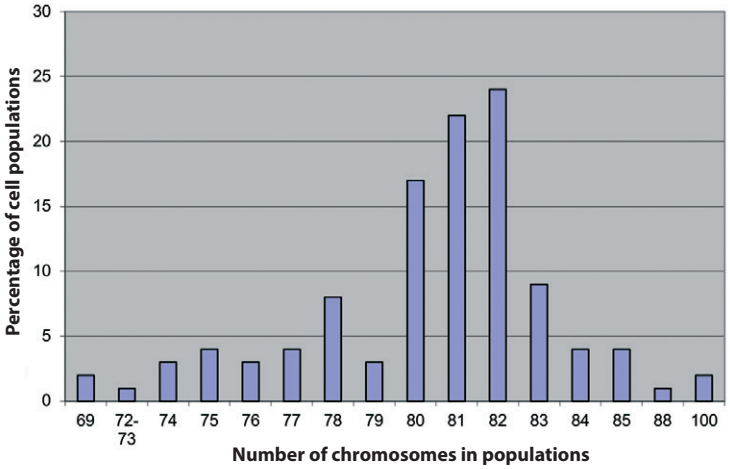

Fig. 5. TCh CCL karyogram (passage 70)

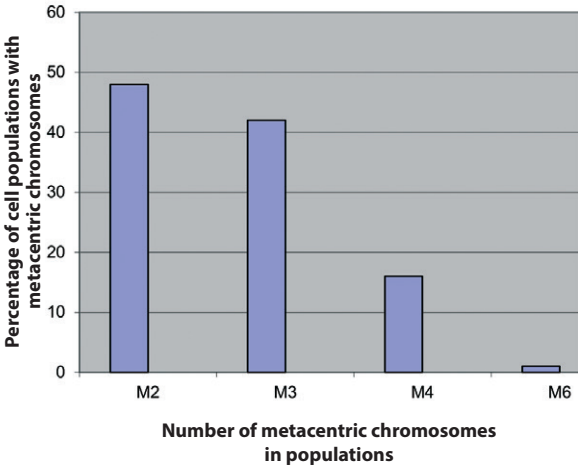

Fig. 7. Percentage of cell populations with different number of metacentric chromosomes in TCh CCL

were photographed, the chromosomes were counted and a karyogram was compiled. According to the analysis results, it was found that the TCh cell subline formed a karyologically heterogeneous population of cells, mainly hyperploid ones. The modal class of the cells in 50 serial passages was 117 chromosomes – 14%, near-tetraploid population – 70%, near-diploid population – 29%. Karyotype variability was 57–122 chromosomes. During passage 66, a new population with predominant cells of the modal class of 82 chromosomes appeared – 24%. The subline stabilized at this karyological level. This population had unlimited potential for passaging without a significant karyotype changes.

By means of karyological analysis, modal class indication and appearance of marker chromosomes, the culture was identified as lamb testicle cells (*Capra hircus* L.).

Characteristics of continuous TCh cell line. The proliferative activity of the resulting cell population was high, which indicates the suitability of the subline for cultivation on an industrial scale. Biotechnological properties of the CCL differed significantly from similar properties of the original cell lines. Possibility of cultivation in roller bottles made it possible to significantly increase the efficiency of the manufacture of the specific antiviral drugs. The potential of the culture turned out to be such that the population recovered from the "foam" to the complete monolayer in case any cells remained after the primary cell subcultivation.

In addition to hyperploidy (modal class – 82 chromosomes), a reliable indicator of the new cell population included the emergence of 2 to 4 metacentric chromosomes (Fig. 6, 7).

The selection with lanthanide treated bovine serum resulted in CCL transformation, and the morphological status of TCh subline cells differed significantly from YaDK-04 and CG-91. The permanent morphology of the TCh CCL was observed during 40 passages of continuous cultivation without cryo stage.

When the TCh CCL was used as a control during the studies of the cytopathic effect (CPE) of various viruses without changing the medium, morphological changes along with the signs of cell and monolayer aging were reported: cytoplasm granulation increased, the intercellular space thickened, vacuoles appeared, small amounts of cellular detritus were localized on the monolayer. All these trophic changes differed from the specific cell degeneration caused by exposure to viruses.

Methods and conditions of TCh cell subline cultivation. TCh monolayer is cultivated in the nutrient medium in two ways: on stationary horizontal surfaces (in culture flasks) and in roller bottles.

Monolayer cultivation in stationary conditions at (37 ± 0.5) °C is the most popular method for the manufacture of culture vaccines and virological studies. TCh cell line is grown in culture flasks with a growth area of 300, 175, 75, 25 cm^2. If necessary, the cell line is used in a microneutralization tests in the plates of various size.

Serial passaging starts with defrosting of the 5 cm^3 ampoule containing cells at the concentration of 5–7 million/mL. In case of the produced TCh subline, there is no need to change the medium every 24 hours. After 72 hours, the cell culture forms a complete monolayer, which can be subcultivated at 1:6. For serial passaging, the semisynthetic nutrient medium + 199 (or DMEM/F-12) is used at 1:3 and 10% of the lanthanide treated bovine serum.

Table 1
Biotechnological specifications of TCh CCL

Growth area of the culture flask, cm^2	Surface type	Split ratio	Monolayer formation, hours	Monolayer characteristics
300	Glass, plastic	1:4	48	Dense, with cell layering
300	Glass, plastic	1:6	72	Dense, with cell layering
300	Glass, plastic	1:8	72–96	Dense, with cell layering
850	Glass, plastic	1:6.6	72	Dense
850	Glass, plastic	1:8.5	72	Dense
1,700	Corrugated plastic	1:12	72–96	Dense

During the serial passaging with the cycle of 72–96 hours, a dense monolayer is formed with partial layering with epithelial-like and spherical cells.

Active proliferation of TCh CCL occurred at pH ranging from 7.3 to 6.8. Acidification of the growth nutrient medium below 6.8 is a sign of the depletion of the stock of ingredients and the need for reseeding or changing the medium.

Roller cultivation of the TCh subline cells is carried out in stationary conditions. Using the same conditions and components, the cells are transferred from the stationary flasks with the growth area of 300 cm^2 into roller bottles with the growth surface of 850 and 1,700 cm^2. The split ratio in this case can reach 1:6.6–1:12 (Table 1). The optimal split ratio for the complete and stable monolayer is 1:6. The culture grown by the roller method is especially in demand for PPRV cultivation.

Reproduction of animal viruses on TCh CCL. The main advantage of the new TCh cell line production involved stability of its cultivation. The TCh cell line began to be actively used for large-scale cultivation of the substrate for the manufacture of vaccine products against such diseases as LSD, sheep and goat pox and PPR. Each of the agents of the above-mentioned infectious animal diseases made its own specific CPE in the TCh cell culture.

During lumpy skin disease virus (LSDV) reproduction in the TCh cell culture, detachment and degeneration of a part of the cell monolayer occurred in the terminal stage. The degenerated cells assembled into aggregates, but part of the monolayer remained on the substrate. There were also spherical, not completely destroyed cells in the suspension (Fig. 8).

The cytopathic effect of the sheep pox virus differed from the CPE of the LSDV and it was manifested by the detachment of the major part of the spherical cells that assembled into aggregates. Degenerative changes were observed in the cells remaining on the substrate: they became spindle-shaped and vacuolated (Fig. 9).

The PPRV totally affected the TCh CCL that was manifested by the degeneration of the individual cells and the whole monolayer to an amorphous state (Fig. 10). In most cases, the monolayer destruction reached 100%.

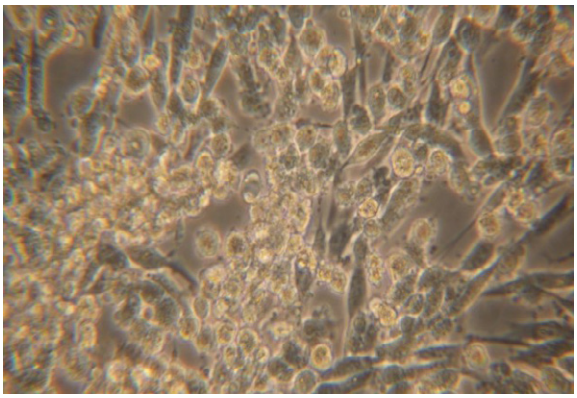

Fig. 8. TCh CCL monolayer cells post infection with LSDV

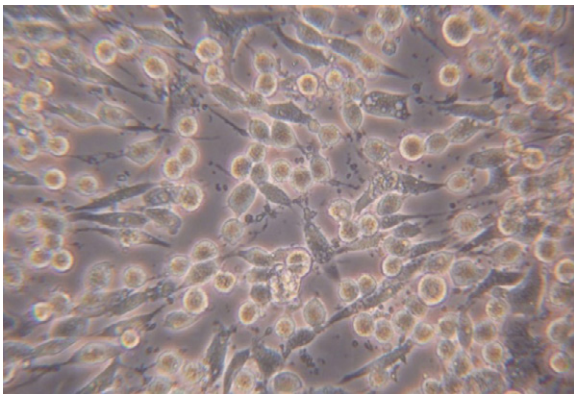

Fig. 9. TCh CCL monolayer cells post infection with sheep pox virus

Comparative analysis of LSDV reproduction in various cell cultures. The following cell lines were used in the comparative analysis: TCh, FBN (continuous fetal bovine nasal septum cell culture), MDBK (continuous bovine kidney cell culture), Taurus-2 (continuous calf kidney cell culture), SIRC (continuous rabbit corneal cell line), PO (continuous sheep kidney cell culture), PT (calf kidney cell subculture) and TYa (lamb testicle cell subculture).

The infectivity of the obtained viral material was determined by titration in 96-well culture plates using YaDK-04 cell suspension. Titration results were recorded according to the virus cytopathic effect during 96–120 hours. The virus titer was calculated by Reed & Muench method and expressed in lg $TCID_{50}/cm^3$.

The data in Table 2 demonstrate that the maximum accumulation of the LSDV was recorded at the level of passage 7 in the homologous continuous TCh cell culture (5.48 ± 0.16 lg $TCID_{50}/cm^3$) and TYa cell subculture (5.17 ± 0.15 lg $TCID_{50}/cm^3$). In FBN and SIRC cell cultures, the infectivity titer amounted to 4.00 ± 0.16 and 4.00 ± 0.12 lg $TCID_{50}/cm^3$, respectively. MDBK, Taurus-2 cell cultures, PO and PT turned out to be insensitive to the LSDV [23, 24].

In order to optimize the parameters of the LSDV cultivation in TCh and TYa cell cultures, the effect of cultivation period on the virus reproduction was studied.

The data in Table 3 demonstrate that the level of LSDV accumulation in TYa and TCh cell cultures after 72 and 96 hours of cultivation did not differ significantly and ranged from 5.00 ± 0.00 to 5.08 ± 0.18 and from 5.25 ± 0.17 to 5.33 ± 0.14 lg $TCID_{50}/cm^3$, respectively, but significantly exceeded the values recorded after 24,

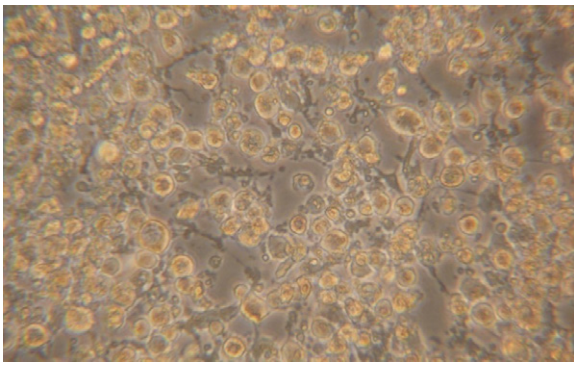

Fig. 10. TCh CCL monolayer cells post infection with PPRV

Table 2
LSDV replication in various cell cultures ($n = 3$)

Cell culture	Virus infectivity, lg $TCID_{50}/cm^3$						
	Number of passages						
	1	2	3	4	5	6	7
TCh	3.33 ± 0.00	4.16 ± 0.16	4.25 ± 0.18	5.25 ± 0.17	5.30 ± 0.14	5.47 ± 0.16	5.48 ± 0.16
TYa	3.02 ± 0.14	4.20 ± 0.16	4.17 ± 0.25	4.50 ± 0.15	5.17 ± 0.14	5.14 ± 0.15	5.17 ± 0.15
FBN	3.01 ± 0.12	3.35 ± 0.15	3.30 ± 0.16	4.17 ± 0.15	4.25 ± 0.16	4.12 ± 0.18	4.00 ± 0.16
SIRC	3.01 ± 0.14	3.15 ± 0.17	3.25 ± 0.12	4.01 ± 0.12	4.15 ± 0.13	4.00 ± 0.15	4.00 ± 0.12
PT	3.02 ± 0.12	2.20 ± 0.25	2.15 ± 0.17	1.35 ± 0.17	n/d	n/d	n/d
MDBK	1.25 ± 0.16	1.15 ± 0.13	n/d	n/d	n/d	n/d	n/d
Taurus-2	1.27 ± 0.13	1.12 ± 0.14	n/d	n/d	n/d	n/d	n/d
PO	3.05 ± 0.15	2.17 ± 0.15	1.35 ± 0.17	n/d	n/d	n/d	n/d

n/d – not detected.

48 and 120 hours of cultivation. The data obtained show that the optimal period of the virus cultivation should be taken as 72–96 hours.

Thus, it was experimentally proved that, despite the fact that the degree of the LSDV accumulation in the TCh cell culture does not statistically differ from that in the TYa subculture, the TCh is a more promising cultivation system for industrial purposes, since this cell line is the most stable and combines such properties as high proliferative activity, sensitivity to LSDV and homology of its origin.

Comparative analysis of sheep pox virus reproduction in various cell cultures. The following cell lines were used in the comparative analysis: TCh, PO, PS (continuous saiga kidney cell culture), PB (ram kidney subculture) and TB (ram testicle subculture) [22, 24, 25, 26]. Three serial passages were performed.

The infectivity of the obtained viral material was determined by titration in 96-well culture plates using YaDK-04 cell suspension. Titration results were recorded according to the cytopathic effect of the virus during 72–120 hours. The virus titer was calculated by Reed & Muench method and expressed in lg $TCID_{50}/cm^3$.

The data in Table 4 demonstrate that the maximum accumulation of the sheep pox virus was observed in TCh, PB and TB cell cultures. By passage 3 the virus titer amounted to 5.50 ± 0.18, 5.50 ± 0.25 and 5.50 ± 0.25 lg $TCID_{50}/cm^3$, respectively. In PO and PS cell cultures, the level of the virus infectivity was low and by passage 3 it amounted to 3.25 ± 0.12 and 3.25 ± 0.25 lg $TCID_{50}/cm^3$, respectively.

Thus, the resulted data indicate that the combination of high proliferative activity, sensitivity to the virus and homology of the origin makes TCh CCL indispensable for the manufacture of the products for sheep pox specific prevention.

Comparative analysis of the PPRV reproduction in various cell cultures The following cell lines were used in the screening process: TCh, PO, PS, TK (goat testicle subculture) and SPEV (continuous pig embryo kidney cell culture). Five serial passages were performed for each cell culture.

The virus was harvested at 80–90% destruction of the cell monolayer. The virus-containing material of each passage was titrated in penicillin vials in YaDK-04 cell culture by the serial 10-fold dilutions. The virus titer was calculated by Ashmarin modified Kärber method and expressed in lg $TCID_{50}/cm^3$.

Already at the first passages, the virus CPE was reported for all cell cultures, and it was most clearly expressed in the TCh cell line (Fig. 10). Cytopathic manifestations of the PPRV in the TCh cell culture consisted in the fact that almost all cells were deadhered on day 3 of cultivation, their membranes and cytoplasm lost their native structure, and partial aggregation of cells occurred.

It was found that at all passage levels the highest virus accumulation was observed in the TCh cell line (virus activity ranged from 5.25 ± 0.00 to 5.33 ± 0.18 lg $TCID_{50}/cm^3$). The virus titer in TK and SPEV cell cultures was significantly lower and amounted to 3.50 ± 0.00 and 4.33 ± 0.18 lg $TCID_{50}/cm^3$ by passage 5, respectively. At passage 1, the virus infectivity was quite high in PO and PS cell cultures (4.58 ± 0.14 and 5.00 ± 0.18 lg $TCID_{50}/cm^3$, respectively), but later a stable and consistent decrease in the level of the virus accumulation was observed. So,

Table 3
Effect of LSDV cultivation period in various lamb testis cell cultures and TCh ($n = 3$)

Cultivation period, hours	Virus titer, lg $TCID_{50}/cm^3$	
	TYa cell culture	TCh cell culture
24	2.66 ± 0.14	2.83 ± 0.22
48	3.78 ± 0.00	3.75 ± 0.17
72	5.08 ± 0.18	5.25 ± 0.17
96	5.00 ± 0.00	5.33 ± 0.14
120	4.75 ± 0.08	5.00 ± 0.00

Table 4
Sheep pox virus accumulation in various cell cultures ($n = 3$)

Cell culture	Virus infectivity, lg $TCID_{50}/cm^3$		
	Number of passages		
	1	2	3
TCh	5.00 ± 0.25	5.48 ± 0.16	5.50 ± 0.18
PO	4.20 ± 0.16	4.50 ± 0.25	3.25 ± 0.12
PS	2.50 ± 0.25	3.08 ± 0.18	3.25 ± 0.25
PB	5.00 ± 0.25	5.14 ± 0.15	5.50 ± 0.25
TB	5.00 ± 0.25	5.17 ± 0.14	5.50 ± 0.25

Table 5
Dynamics of PPRV accumulation in various cell cultures ($n = 3$)

Cell culture	Virus titer, lg $TCID_{50}/cm^3$	
	Passage 1	Passage 5
TCh	5.25 ± 0.00	5.33 ± 0.18
TK	4.20 ± 0.16	3.50 ± 0.00
SPEV	4.15 ± 0.13	4.33 ± 0.18
PO	4.58 ± 0.14	2.08 ± 0.14
PS	5.00 ± 0.18	3.33 ± 0.18

by passage 5, the virus titer in PO cell culture amounted to 2.08 ± 0.14 lg $TCID_{50}/cm^3$, in PS cell culture – to 3.33 ± 0.18 lg $TCID_{50}/cm^3$ (Table 5).

Thus, the study results indicate that the optimal cell line for the PPRV reproduction is the TCh cell culture [12]. The virus titer obtained in this culture was consistently high during five serial passages and ranged from 5.25 ± 0.00 to 5.33 ± 0.18 lg $TCID_{50}/cm^3$, thus indicating the possibility of using this cell culture for the production of the viral raw material for large-scale manufacture of the vaccines based on the tested virus.

CONCLUSION

Long-term use of lanthanide treated bovine blood serum during cultivation of YaDK-04 CCL resulted in the formation of a new TCh cell line, which significantly differed in cytomorphological and karyological features from the original one. We assume that lanthanides, bearing high electrical charge cations, affect

the formation of chromosomal variability and instability of the nucleosomes, especially at the distal ends of acrocentric chromosomes. Two processes occur: emergence and stable reduplication of the hyperploid cell population, as well as accumulation of acrocentrics along with the formation of stable metacentric chromosomes. These rearrangements are correlated with the increase in the proliferative activity of the line and stability of cultivation during long-term passaging.

An important result of the production of the new cell line is the fact that sensitivity to dermatotropic and other viruses did not change. And high productivity of the cell populations enabled cost-effective production of culture vaccines.

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Received 19.07.2023
Revised 11.08.2023
Accepted 05.09.2023

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