DOI: https://doi.org/10.17816/gc630218

Methods of karyoplast cell cycle synchronization for increasing the efficiency of somatic cloning of farm animals

Anastasia S. Zhukova

Federal Science Center for Animal Husbandry named after Academy Member L.K. Ernst, Podolsk, Russia

ABSTRACT

Somatic cloning is a method of obtaining genetically identical offspring, which for some reason have extremely low efficiency. Methods to increase the effectiveness of this procedure aimed at optimizing each of its stages, one of which is karyoplast preparation. In most experiments, to obtain cloned offspring, oocytes are used as recipient cells at the metaphase of the second meiotic division without prior activation, which determines the choice of somatic cells as karyoplasts at the G0/G1 stage, the most optimal for subsequent nuclear reprogramming by oocyte cytoplasmic factors. Serum starvation and/or contact inhibition are the most commonly used methods for arresting cells in this phase, which allows the arrest of up to 90% of cells at the G0/G1 stage. Despite the effectiveness of these methods, they have some significant limitations; therefore, the addition of components to the culture medium of somatic cells that prevent the progression of cells through the cell cycle stages is becoming widespread. Some chemical inhibitors have a protective effect on somatic cells, preventing the induction of apoptotic changes. Although the efficacy of butyrolactone I, mimosine, and aphidicolin application is controversial, several studies have attested the possibility of using these drugs to synchronize the karyoplasts. Thus, several methods can be employed for effective synchronization of the cell cycle of karyoplasts. When choosing the optimal method, the type of cells, species of animals from which they were obtained, and permissible duration of cultivation under given conditions must be considered to minimize the negative effect of conditions on karyoplast viability.

Keywords: SCNT; somatic cloning; cell cycle synchronization; roscovitine; rapamycin; serum starvation; contact inhibition.

To cite this article:
Zhukova AS. Methods of karyoplast cell cycle synchronization for increasing the efficiency of somatic cloning of farm animals. *Genes & cells*. 2024;19(3):319–333. DOI: https://doi.org/10.17816/gc630218

Received: 10.04.2024 **Accepted:** 05.06.2024 **Published online:** 06.08.2024

DOI: https://doi.org/10.17816/gc630218

Способы синхронизации клеточного цикла кариопластов для повышения результативности соматического клонирования сельскохозяйственных животных

А.С. Жукова

Федеральный исследовательский центр животноводства — ВИЖ имени академика Л.К. Эрнста, Подольск, Россия

АННОТАЦИЯ

Соматическое клонирование — способ получения генетически идентичного потомства, обладающий по ряду причин крайне низкой результативностью. Способы повышения эффективности данной процедуры направлены на оптимизацию каждого из её этапов, одним из которых является подготовка кариопластов. В большинстве экспериментов по получению клонированного потомства в качестве клеток-реципиентов применяют ооциты на стадии метафазы II мейотического деления без предварительной активации, что обусловливает выбор в качестве кариопластов соматических клеток на стадии G0/G1, наиболее оптимальной для последующего репрограммирования их ядер факторами цитоплазмы ооцитов. Для остановки клеток в данной фазе наиболее часто используют методы сывороточного голодания и/или контактного ингибирования, позволяющие остановить до 90% клеток на стадии G0/G1. Однако, несмотря на эффективность данных способов, они обладают рядом существенных ограничений, в связи с чем приобретает широкое распространение добавление в среду культивирования соматических клеток компонентов, препятствующих продвижению клеток по стадиям клеточного цикла. Преимуществом применения химических ингибиторов является способность некоторых из них оказывать протекторное воздействие на соматические клетки и в результате предотвращать индукцию апоптотических изменений. Таким образом, в настоящее время существует широкий спектр методов эффективной синхронизации клеточного цикла кариопластов, и при выборе оптимального способа следует обращать внимание на тип клеток; видовую принадлежность животных, от которых они получены; допустимую продолжительность культивирования в заданных условиях с целью минимизации негативного воздействия этих условий на жизнеспособность кариопластов.

Ключевые слова: SCNT; соматическое клонирование; синхронизация клеточного цикла; росковитин; рапамицин; сывороточное голодание; контактное ингибирование.

Как цитировать:
Жукова А.С. Способы синхронизации клеточного цикла кариопластов для повышения результативности соматического клонирования сельскохозяйственных животных // Гены и клетки. 2024. Т. 19, № 3. С. 319–333. DOI: https://doi.org/10.17816/gc630218

Рукопись получена: 10.04.2024 **Рукопись одобрена:** 05.06.2024 **Опубликована online:** 06.08.2024

ЭКО•ВЕКТОР

INTRODUCTION

Somatic cell nuclear transfer (SCNT) involves the production of multicellular organisms using oocytes whose genetic material is replaced by the contents of donor cell nuclei (karyoplasts). In 1952, the first cloned animals were produced from blastocyst stage cells in amphibians [1]. This was followed by studies using mammals of different species and the use of early embryonic blastomeres as karyoplasts [2]. Then, in 1996, the first cloned offspring using somatic cells from an adult animal was obtained [3]. Currently, several mammalian species, including monkeys, have been cloned by SCNT [2]. This method has potential applications in various fields of agriculture and medicine with proven therapeutic potential: production of genetically modified animals [4], reproduction of valuable and conservation of endangered species, and derivation of embryonic stem cells [5]. Despite the demand for this procedure, its efficiency remains low [6–8]. This is largely explained by the fact that SCNT includes a set of consecutive stages and suboptimal conditions, at any of which can have a significant negative impact on the development of cloned embryos and offspring. Moreover, in a number of cases, recipient animals show disorders of placenta formation, and cloned offspring often die from lethal malformations [9]. The main stages of cloning include the preparation of oocytes and somatic cells, enucleation of mature oocytes (cytoplasts) followed by obtaining oocyte–somatic cell complexes, fusion of cytoplasts with karyoplasts, activation of the resulting cytohybrids, and culturing and transplantation of the embryos [10, 11]. After numerous studies conducted to obtain cloned animals, considerable experience has been accumulated to improve SCNT technology. The modern ways to improve the efficiency of cloning include optimization of each of its stages [11–23]. Most published studies reflect advancements in oocyte research, including the modernization of maturation conditions to decelerate aging processes, mitigate apoptotic alterations, and generate reactive oxygen species [13, 21]. Additionally, advancements have been made in enucleation techniques, with the introduction of robotic approaches [19, 23]. A study on methods to restore cytoplasts after enucleation, including the injection of cytoplasm from donor oocytes, is underway [14]. Furthermore, research is being conducted to regulate mitochondrial DNA inheritance [17]. Of particular significance are studies aimed at enhancing the implantation of cloned embryos at the embryological stage [15] and stage of work with recipient animals. Notably, the conditions optimal for SCNT in one animal species may not be suitable for another species.

The critical event that determines the efficacy of SCNT is the reprogramming of the nuclear material of the karyoplast by the oocyte cytoplasm. This process results in totipotency of the cells [24]. During somatic cell differentiation from stem cells, methylation of specific genome regions occurs, which are responsible for binding to transcription factors that maintain the pluripotent state [25]. Despite the stability of the epigenetic status of somatic cells, including terminally differentiated cells, methylation is not irreversibly fixed; rather, it can be reprogrammed to the embryonic state [26]. The reprogramming process entails epigenetic and metabolic remodeling; however, the molecular regulation underlying this phenomenon remains unclear [27]. The developmental potential of cloned embryos is determined by the capacity of the somatic cell nucleus to be reprogrammed by factors present in the oocyte cytoplasm. Following the fusion of an enucleated oocyte and a donor cell, changes take place. These include the destruction of the nuclear envelope of the karyoplast and premature condensation of chromosomes. This process is mediated by a high concentration of maturation-promoting factor (MPF), a $p34^{cdc2}$-cyclin B complex, which is found in the oocyte cytoplasm [28–31]. Moreover, subsequent DNA replication occurs. The replacement of several chromatin structural components with the corresponding components of oocyte origin, which occurs after fusion of the karyoplast and cytoplast membranes, is crucial in the removal of epigenetic marks from DNA, which in turn allows for the reprogramming of donor nuclei into a totipotent state [32–34]. These rearrangements are critical for chromatin decondensation, which is essential for the enhanced binding of transcription regulators to their recognition sites and more efficient reprogramming [35]. Consequently, the intensity of expression and the level of phosphorylation of pivotal histones and chaperones, the degree of acetylation and methylation of oocyte histones, and other factors influence the efficiency of reprogramming [36]. To enhance this index, researchers employ a range of strategies, including the use of inhibitors of DNA deacetylases [37, 38] and methyltransferases [39], induction of demethylase overexpression [40], and correction of aberrant DNA remethylation [41]. However, the cell cycle of the donor somatic cell should be coordinated with that of the recipient oocyte. If not, it may result in chromosome damage due to premature condensation and premature destruction of the nuclear envelope in the donor cells, which are in an inappropriate phase [42, 43].

Studies conducted to identify the optimal stage of the karyoplast cell cycle indicate that embryo development is observed when somatic cells in G0, G1, G2, and M phases are used [44, 45]. Considering that the majority of experiments on the production of cloned animals use oocytes in the MII phase of meiosis (owing to the elevated MPF activity) as recipient cells [46] without prior activation, it is recommended that cells with a diploid nucleus in the G0/G1 phase, awaiting replication, be employed for transfer [31, 47]. Various studies demonstrated that SCNT using somatic cells in this phase as a karyoplast is more efficient [48]. The transfer of cells in the G2 phase results in impaired embryo development because of the replication of the genome, which is directed by the oocyte cytoplasm. The use of karyoplast in the S phase causes chromosome fragmentation [42]. If the cell cycle of the recipient cytoplasm enters the G2/M phase when the

karyoplast is in the G1/S phase, premature chromosome condensation and nuclear envelope disintegration may be initiated while DNA synthesis is not yet complete, resulting in chromosome loss and aneuploidy [49].

The arrest of cell proliferation in the G0/G1 phase can be achieved through several methods, the most prevalent of which are serum starvation (culturing somatic cells in a medium containing an extremely low concentration of fetal bovine serum — FBS) [50–53], contact inhibition (culturing somatic cells to a monolayer with a confluency approaching 100%) [52–55], and a combination of these techniques [43, 56]. Many researchers employ chemical inhibitors of the cell cycle [57, 58].

SERUM STARVATION

Serum starvation is an effective method for the reversible arrest of somatic cells in the G0/G1 phase. Numerous studies have shown that the cultivation of somatic cells in a medium with an extremely low FBS content (0.2–0.5%) results in the arrest of 70–90% of these cells in the aforementioned phase of the cycle [43, 58–64]. This rate increases with the duration of cell cultivation in conditions of reduced FBS content [43, 59, 61, 65]. Furthermore, in a study by Sadeghian-Nodoushan et al., the proportion of sheep granulosa cells in the G0/G1 phase increased significantly when cultured for 48 and 72 h compared to 24 h under serum starvation conditions [61]. Restoration of serum content in the medium to values sufficient for active proliferation (15–20%) resulted in a notable increase in the number of cells in the S and G2/M phases [59, 66].

Despite the widespread use of this methodology to synchronize the cell cycle of karyoplasts during animal cloning, it should be considered that the cultivation of somatic cells in a medium with reduced serum content for 48 h results in damage to the endoplasmic reticulum [67]. Furthermore, extended cultivation periods ⩾72 h result in increased production of reactive oxygen species [57], alterations in mitochondrial morphology [68], increased DNA fragmentation [59], and increased proportion of apoptotic cells [60, 62, 69]. A consequence of apoptosis induction is a change in the membrane potential of cells [70], which can lead to decreased efficiency of electrospraying in cytohybrid production [71]. However, the results of [72] indicate that the use of annexin-positive and annexin-negative cells as a karyoplast does not significantly affect the number of cytohybrids obtained.

The negative effect of using karyoplasts at early apoptosis on the efficiency of SCNT is demonstrated by the results obtained by Miranda et al. According to their study, the use of annexin-positive fibroblasts as donor cells in SCNT reduces the probability of resulting embryos to develop, leads to a reduction in the number of cells in blastocysts, and increases the number of apoptotic cells [72]. In this context, a statistically significant increase in apoptosis during cycle synchronization by serum starvation is observed when somatic cells are cultured in medium containing reduced FBS for 48 and 72 h. Conversely, serum starvation for 24 h does not cause a significant increase in the number of apoptotic cells in culture when compared to cells cultured in medium containing 10% FBS [61].

The low developmental potential of SCNT-derived embryos may be attributed to somatic cell damage caused by culturing under serum starvation conditions. A study conducted by Park et al. demonstrated that the number of porcine embryos developing to the blastocyst stage was lower when karyoplasts were synchronized by serum starvation for 72 h compared to other cell cycle arrest methods [69]. In another study, the number of bovine blastocysts obtained by the SCNT method using karyoplasts cultured for 24 h in a medium containing 0.5% FBS was higher than the corresponding index when fibroblasts were synchronized by the contact inhibition method [65]. These results confirm that an extended duration of karyoplast cultivation under serum starvation conditions negatively affects their condition, which in turn reduces the efficiency of SCNT.

CONTACT INHIBITION

Currently, contact inhibition is the second most prevalent method of inhibiting proliferative activity [54, 73–76]. The formation of a dense monolayer results in an increase in the surface contact area of neighboring cells, which then causes cell cycle inhibition despite sufficient concentration of nutrients and growth factors in the culture medium [77]. Cell arrest in the G0/G1 phase occurs as a result of increased expression of p27Kip1, an inhibitor of cyclin-dependent kinase (CDK) [78] that are involved in cell division, and through the dissociation of cyclin D from the complex with CDK4 [79], which subsequently becomes inactivated. Importantly, contact inhibition reduces the level of intracellular production of reactive oxygen species compared to cells growing at lower densities [80], and activation of PGC1α, a key regulator of energy metabolism involved in reducing the concentration of reactive oxygen species and protecting cells from oxidative stress, is observed [81]. The use of this method allows 65–84% of cells to be arrested in the G0/G1 phase of the cycle [61–63]. Notably, the level of apoptosis in somatic cells synchronized by the contact inhibition method is comparable to that in actively dividing cells [61, 63]. A limitation in the application of this method is that, in some cases, it is not possible to achieve monolayer confluence sufficient for contact inhibition to occur, because individual cultures of cells used as karyoplasts in the SCNT procedure (e.g., after genome editing) may have low proliferative activity.

COMBINED METHOD

Several researchers have shown that somatic cell culturing to a monolayer, followed by the replacement of the

culture medium with one containing a reduced FBS content, cab be used to increase the number of cells present in the G0/G1 phase of the cell cycle. Accordingly, the findings of Ma et al. indicate that the proportion of sheep fibroblasts in this phase was significantly higher in the combined method of synchronization than in contact inhibition and serum starvation: 80.07% vs. 66.82% and 71.24%, respectively [82]. However, a study by Gómez et al. comparing methods of cell cycle synchronization of bovine fibroblasts (serum starvation, contact inhibition, and a combined method) did not reveal significant differences in the number of fibroblasts in the G0/G1 phase [65]. The discrepancies may be attributed to the varying durations of cell cultivation under the specified conditions and potential influence of the species of animals from which the cells were obtained. There is evidence indicating that somatic cells from different animal species may exhibit varying susceptibility to the same methods of cycle synchronization [83, 84].

Moreover, the efficiency of somatic cell synchronization is determined by the type of cells used as karyoplast: the results of the study conducted by Yagcioglu et al. in sheep show that fetal and adult fibroblasts respond differently to cell cycle synchronization [63].

A CHEMICAL WAY TO SYNCHRONIZE THE CELL CYCLE OF KARYOPLASTS

The cell cycle is a unidirectional process in which a cell passes sequentially through its various morphologically and biochemically distinct phases, without skipping or returning to previous phases. The cycle is generally divided into the interphase, including the synthetic (S) period when chromosome doubling occurs, and the intervals separating it from division. These are the G1 and G2 phases. In the G1 phase, proteins are synthesized, organelles are amplified, and cell size increases. In the G2 phase, the correctness of DNA replication is analyzed, and mitosis is prepared. Cells in the G1 phase can exit the cell cycle and enter the G0 phase, which is a reversible quiescent state [85]. Cell progression through the phases of the cell cycle is regulated by the ability of activated CDKs to phosphorylate specific substrates, leading to their activation, inactivation, and localization in the cell. CDK activation occurs when they bind to cyclins, the concentration and activity of which is determined by the phase of the cell cycle [85, 86].

Based on underlying cell progression through the phases of the cell cycle, CDK inhibitors are used to stop them at a specific phase (roscovitine*, butyrolactone I*, statins: lovastatin*, mevastatin*), inhibitors of DNA replication (aphidicolin*, mimosine*, statins), microtubule polymerization (nocodazole*, colchicine) [87], and synthesis of essential proteins (cycloheximide*) [88]. The main principles of somatic cell cycle synchronization for SCNT are its reversibility and absence of toxic effects. Therefore, it should be considered that the effect of a number of inhibitors, in addition to stopping cells in the necessary phase of the cycle, often results in apoptosis. Table 1 [57–61, 63, 69, 89–93] presents information on chemical inhibitors of the cell cycle used for synchronization of donor cells in SCNT of farm animals.

According to the literature, among the drugs for somatic cell synchronization used as karyoplast in SCNT, roscovitine is most often used. This drug promotes reversible cell arrest in the G0/G1 phase by inhibiting CDK1, CDK2, CDK5, CDK7, CDK9, and MPF [65, 94–96]. Various studies have shown that the number of fibroblasts in the G0/G1 phase when roscovitine is added to the culture medium is equivalent to the corresponding indices when cells are synchronized by contact inhibition [60, 63, 69]. The study by Selokar et al. shows a higher concentration of fetal fibroblasts in the G0/G1 stage when cultured for 24 h in medium containing 30 μM roscovitine compared to the corresponding indicators in the groups where cycle synchronization was performed by contact inhibition or serum starvation methods [92]. This index was comparable to that of the combined application of contact inhibition and serum starvation methods.

Data regarding the protective effect of roscovitine on cells are inconsistent: its addition to the culture medium of sheep, fetal pig, and fetal fibroblasts led to a decrease in the number of cells in the apoptotic state [60, 63, 69]. However, incubation of puma fibroblasts in the medium containing 15 μM roscovitine did not have a significant effect on this index [62]. Additionally, the use of fibroblasts cultured in the presence of roscovitine as karyoplasts for SCNT resulted in an increase in the relative proportion of embryos that reached the blastocyst stage [69, 92]. In a study by Hwang et al., the number of recipient gestation pigs was higher in the group where fibroblasts cultured in the presence of roscovitine were used as donor cells for SCNT [91]. Accordingly, most studies indicate that the use of this inhibitor to inhibit the proliferation of somatic cells in the G0/G1 phase of the cell cycle is not superior to the mechanisms of contact inhibition and serum starvation. Nevertheless, the extant data on the protective impact of roscovitine on somatic cells and enhanced production of blastocysts when karyoplasts are cultured in its presence substantiate the employment of this inhibitor as a method of somatic cell synchronization in SCNT.

Rapamycin is a macrolide of bacterial origin that has been found to regulate cell growth, control protein translation, influence energy metabolism, and regulate cytoskeleton formation by inhibiting the mTOR target (mammalian target of rapamycin) [97]. Rapamycin plays a role in maintaining cellular physiology by inducing autophagy, which is crucial for the timely removal of aged proteins, damaged organelles, toxic metabolic products, and peroxisomes from the cell [98, 99].

* hereinafter, the drug is not registered in the Russian State Register of Medicines.

Table 1. Inhibitors used for synchronizing the karyoplasts cell cycle during somatic cloning of farm animals

Inhibitors	Animals	Cell types	Number of cells in G0/G1 phase, %	Reference
Roscovitine	Sheep	Fetal fibroblasts	54.85	[63]
	Sheep	Adult fibroblasts	63.51	
	Cows	Fetal fibroblasts	82.80	[60]
	Sheep	No data	66.64	[89]
	Pigs	Fetal fibroblasts	76.30	[69]
	Cows	Fibroblasts	91.10	[90]
	Pigs	Fibroblasts	84.60	[91]
	Cows	Fetal fibroblasts	96.43	[92]
Rapamycin	Pigs	Fibroblasts	93.60	[58]
	Cows	Fibroblasts	90.0	[57]
Butyrolactone I	Pigs	Fetal fibroblasts	81.0	[59]
Trichostatin A	Sheep	Fetal fibroblasts	74.28	[63]
	Sheep	Adult fibroblasts	68.93	
Mimosine	Sheep	Granulosa	85.20	[61]
	Pigs	Granulosa	85.70	[93]
Aphidicolin	Pigs	Fetal fibroblasts	82.0	[59]
Nocodazole	Sheep	Granulosa	74.60	[61]

It has been demonstrated to reduce the expression of cyclin D1 [100–102] and genes responsible for the synthesis of glucose and amino acid transporters [58], inhibiting cell proliferation and the cell cycle in the G0/G1 phase. The findings of a study conducted by Hyun et al. indicate that culturing pig fibroblasts in a medium containing 1 μM rapamycin for a period of 3 days resulted in a notable increase in the proportion of cells in the G0/G1 phase, reaching 93.6% compared to 61.0% in the control group. The authors observed that when these cells were used as a karyoplast, the number of fractured embryos and embryos that developed to the blastocyst stage was significantly higher than that in the control group. Conversely, DNA fragmentation was less pronounced, and the expression of development-related genes (*CDX2, CDH1*) was higher in the rapamycin group than in the control group [58]. A study conducted on buffaloes yielded comparable outcomes. The content of fibroblasts in the G0/G1 phase was 90% when cultured in a medium containing rapamycin. The number of fibroblasts with a normal karyotype was higher, whereas the number of apoptotic cells and the level of reactive oxygen species production were lower in comparison to the group of cells exposed to serum starvation [57].

A study on fetal piglet fibroblasts [59] demonstrated that butyrolactone I can stop cells in the G0/G1 phase of the cycle by inhibiting CDK1, CDK2, CDK5, and CDC2 [103, 104]. This was achieved by inhibiting the activity of these enzymes, which regulate the cell cycle. The effect of this drug on cell viability is inconclusive. For example, the study of butyrolactone I on tumor cells demonstrated its ability to induce apoptosis [105, 106], whereas the study on granular neurons did not reveal a decrease in their viability [107].

The plant amino acid mimosine has an inhibitory effect on the initiation of DNA replication and is able to arrest cells in the G1 or S phase of the cell cycle in a dose-dependent manner. Application of mimosine at a concentration of 0.5 mM arrested somatic cells in G1 phase, whereas lower concentrations (0.1–0.2 mM) did not prevent entry into S phase [108, 109]. According to the results of these studies, the addition of mimosine to the culture medium allows the arrest of 85.2% of sheep granulosa cells [61] and 85.7% of porcine granulosa cells [93] in the G0/G1 phase, confirming its efficacy in synchronizing karyoplasts during SCNT.

Cell cycle inhibitors with conflicting data include aphidicolin and nocodazole. Aphidicolin is a reversible inhibitor of DNA polymerases. It arrests cells in the early S phase [110]. However, according to the results of the study by Kues et al., the cultivation of porcine fetal fibroblasts in medium containing aphidicolin resulted in a cell concentration of 82% in the G0/G1 phase, whereas only 3.9% of the fibroblasts stopped in the S phase [59]. Additionally, the inconsistency of the available data on the inhibitory activity of nocodazole is highlighted. The findings of Sadeghian-Nodoushan et al. indicate that 74.6% of sheep granulosa cells were in the G0/G1 stage when cultured in the presence of nocodazole [61]. In contrast, another study demonstrated that the sequential application of aphidicolin and nocodazole could effectively synchronize the cell cycle of porcine cells [111]. The use of cine-induced pluripotent stem cells permitted the cessation of 77.6% of cells on day 2 of

culturing and 72.2% on day 3, which was observed to be in the G2/M phase. Conversely, the number of cells in the G0/G1 phase was found to be only 9.4 and 11.3% on days 2 and 3, respectively. In this context, nocodazole induces its effect by disrupting microtubule polymerization, which contributes to cell arrest in the G2/M phase [112]. Therefore, this drug is not recommended for the synchronization of somatic cells for their use as karyoplast in SCNT.

CONCLUSION

The success of cloning, in conjunction with the physiological state and viability of the recipient oocyte, largely determines the potential for somatic cell nuclear material reprogramming. It has been demonstrated that karyoplasts at the G0/G1 stage of the cell cycle are most capable of reprogramming. Therefore, selecting optimal conditions for somatic cell cultivation that ensure a high level of synchronization of their cell cycle enhances the efficiency of somatic cloning. Despite various existing methods for cell arrest in the G0/G1 phase of the cell cycle, there is currently no consensus on the most optimal method. In selecting a method of cell cycle synchronization, it is crucial to consider the species of animal from which the somatic cells are derived and the permitted duration of cell cultivation under conditions that induce cell cycle arrest. The impact of the selected method on cell viability and the induction of apoptotic changes in cells is of significant interest. The investigation of the influence of chemical inhibitors on the cell cycle of karyoplasts is constrained to the evaluation of cell content within a specific phase of the cycle. However, the majority of research is directed toward data pertaining to the number of embryos obtained, their engraftment, and the subsequent birth of viable offspring.

ADDITIONAL INFORMATION

Funding source. This work was supported by the Ministry of Science and Higher Education of the Russian Federation (State assignment FGGN-2024-0014).

Competing interests. The author declares that there are no competing interests.

REFERENCES

1. Briggs R, King TJ. Transplantation of living nuclei from blastula cells into enucleated frogs' eggs. *Proc Natl Acad Sci U S A*. 1952;38(5):455–463. doi: 10.1073/pnas.38.5.455

2. Klinger B, Schnieke A. 25th anniversary of cloning by somatic cell nuclear transfer. Twenty-five years after Dolly: how far have we come? *Reproduction*. 2021;162(1):F1–F10. doi: 10.1530/REP-20-0652

3. Wilmut I, Schnieke A, McWhir J, et al. Viable offspring derived from fetal and adult mammalian cells. *Nature*. 1997;385(6619):810–813. Corrected and republished from: *Nature* 1997;386(6621):200. doi: 10.1038/385810a0

4. Shakweer WME, Krivoruchko AY, Dessouki SM, Khattab AA. A review of transgenic animal techniques and their applications. *J Genet Eng Biotechnol*. 2023;21(1):55. doi: 10.1186/s43141-023-00502-z

5. Lee JE, Chung YG, Eum JH, et al. An efficient SCNT technology for the establishment of personalized and public human pluripotent stem cell banks. *BMB Rep*. 2016;49(4):197–198. doi: 10.5483/bmbrep.2016.49.4.055

6. Keefer CL. Artificial cloning of domestic animals. *Proc Natl Acad Sci USA*. 2015;12(29):8874–8878. doi: 10.1073/pnas.1501718112

7. Ogura A, Inoue K, Wakayama T. Recent advancements in cloning by somatic cell nuclear transfer. *Philos Trans R Soc Lond B Biol Sci*. 2013;368(1609):20110329. doi: 10.1098/rstb.2011.0329

8. Zhang X, Gao S, Liu X. Advance in the role of epigenetic reprogramming in somatic cell nuclear transfer-mediated embryonic development. *Stem Cells Int*. 2021;2021b:6681337. doi: 10.1155/2021/6681337

9. Malin K, Witkowska-Piłaszewicz O, Papis K. The many problems of somatic cell nuclear transfer in reproductive cloning of mammals. *Theriogenology*. 2022;189:246–254. doi: 10.1016/j.theriogenology.2022.06.030

10. Lopukhov AV, Singina GN, Zinovieva NA. Biotechnological bases of the development of cloned pig embryos. *Vavilov Journal of Genetics and Breeding*. 2019;23(5):527–533. EDN: LCBRWK doi: 10.18699/VJ19.521

11. Srirattana K, Kaneda M, Parnpai R. Strategies to improve the efficiency of somatic cell nuclear transfer. *Int J Mol Sci*. 2022;23:1969. doi: 10.3390/ijms23041969

12. Gouveia C, Huyser C, Egli D, et al. Lessons learned from somatic cell nuclear transfer. *Int J Mol Sci*. 2020;21(7):2314. doi: 10.3390/ijms21072314

13. Zhao Q, Qiu J, Feng Z, et al. Robotic label-free precise oocyte enucleation for improving developmental competence of cloned embryos. *IEEE Trans Biomed Eng*. 2021;68(8):2348–2359. doi: 10.1109/TBME.2020.3036494

14. Vazquez-Avendanõ JR, Ambríz-García DA, Cortez-Romero C. Current state of the efficiency of sheep embryo production through somatic cell nuclear transfer. *Small Rumin Res*. 2022;212:106702. doi: 10.1016/j.smallrumres.2022.106702

15. Samiec M. Molecular mechanism and application of somatic cell cloning in mammals — past, present and future. *Int J Mol Sci*. 2022;23(22):13786. doi: 10.3390/ijms232213786

16. Yao Y, Yang A, Li G, et al. Melatonin promotes the development of sheep transgenic cloned embryos by protecting donor and recipient cells. *Cell Cycle*. 2022;21(13):1360–1375. doi: 10.1080/15384101.2022.2051122

17. Wang Y, Qi JJ, Yin YJ, et al. Ferulic acid enhances oocyte maturation and the subsequent development of bovine oocytes. *Int J Mol Sci*. 2023;24(19):14804. doi: 10.3390/ijms241914804

18. Himaki T, Hano K. Effects of alpha lipoic acid treatment during in vitro maturation on the development of porcine somatic cell nuclear transfer embryos. *Anim Sci J*. 2023;94(1):e13889. doi: 10.1111/asj.13889

19. Vajta G, Lewis IM, Trounson AO, et al. Handmade somatic cell cloning in cattle: Analysis of factors contributing to

high efficiency in vitro. *Biol Reprod*. 2003;68(2):571–578. doi: 10.1095/biolreprod.102.008771

20. Kang JS, Joo MD, Lee SH, et al. Effect of additional cytoplasm injection on the cloned bovine embryo organelle distribution and stress mitigation. *Theriogenology*. 2024;216:12–19. doi: 10.1016/j.theriogenology.2023.11.031

21. Srirattana K, St John JC. Manipulating the mitochondrial genome to enhance cattle embryo development. *G3 (Bethesda)*. 2017;7(7):2065–2080. doi: 10.1534/g3.117.042655

22. Srirattana K, St. John JC. Additional mitochondrial DNA influences the interactions between the nuclear and mitochondrial genomes in a bovine embryo model of nuclear transfer. *Sci Rep*. 2018;8(1):7246. doi: 10.1038/s41598-018-25516-3

23. Liao Z, Zhang J, Sun S. et al. Reprogramming mechanism dissection and trophoblast replacement application in monkey somatic cell nuclear transfer. *Nat Commun*. 2024;15(1):5. doi: 10.1038/s41467-023-43985-7

24. Lu F, Zhang Y. Cell totipotency: molecular features, induction, and maintenance. *Natl Sci Rev*. 2015;2(2):217–225. doi: 10.1093/nsr/nwv009

25. Weidgang CE, Seufferlein T, Kleger A, Mueller M. Pluripotency factors on their lineage move. *Stem Cells Int*. 2016;2016:6838253. doi: 10.1155/2016/6838253

26. Jaenisch R, Young R. Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming. *Cell*. 2008;132(4):567–582. doi: 10.1016/j.cell.2008.01.015

27. Hanna JH, Saha K, Jaenisch R. Pluripotency and cellular reprogramming: facts, hypotheses, unresolved issues. *Cell*. 2010;143(4):508–525. doi: 10.1016/j.cell.2010.10.008

28. Czołowska R, Modliński JA, Tarkowski AK. Behaviour of thymocyte nuclei in non-activated and activated mouse oocytes. *J Cell Sci*. 1984;69:19–34. doi: 10.1242/jcs.69.1.19

29. Barnes FL, Collas P, Powell R, et al. Influence of recipient oocyte cell cycle stage on DNA synthesis, nuclear envelope breakdown, chromosome constitution, and development in nuclear transplant bovine embryos. *Mol Reprod Dev*. 1993;36(1):33–41. doi: 10.1002/mrd.1080360106

30. Campbell KH, Ritchie WA, Wilmut I. Nuclear-cytoplasmic interactions during the first cell cycle of nuclear transfer reconstructed bovine embryos: implications for deoxyribonucleic acid replication and development. *Biol Reprod*. 1993;49:933–942. doi: 10.1095/biolreprod49.5.933

31. Campbell KHS, Choi I, Zhu J, et al. Cell cycle regulation in cloning. In: Cibelli J, Wilmut I, Jaenisch R, et al, editors. *Principles of cloning (second edition)*. San Diego: Academic Press; 2014. P. 149–160. doi: 10.1016/B978-0-12-386541-0.00012-6

32. Jullien J, Pasque V, Halley-Stott RP, et al. Mechanisms of nuclear reprogramming by eggs and oocytes: A deterministic process? *Nat Rev Mol Cell Biol*. 2011;12(7):453–459. doi: 10.1038/nrm3140

33. Xu R, Zhang S, Lei A. Chromatin changes in reprogramming of mammalian somatic cells. *Rejuvenation Res*. 2014;17(1):3–10. doi: 10.1089/rej.2013.1455

34. Glanzner WG, de Macedo MP, Gutierrez K, Bordignon V. Enhancement of chromatin and epigenetic reprogramming in porcine SCNT embryos-progresses and perspectives. *Front Cell Dev Biol*. 2022;10:940197. doi: 10.3389/fcell.2022.940197

35. Gaspar-Maia A, Alajem A, Meshorer E, Ramalho-Santos M. Open chromatin in pluripotency and reprogramming. *Nat Rev Mol Cell Biol*. 2011;12(1):36–47. Corrected and republished from: *Nat Rev Mol Cell Biol*. 2011;12(4):273. doi: 10.1038/nrm3036

36. Gonzales-Munoz E, Cibelli JB. Somatic cell reprogramming informed by the oocyte. *Stem Cells Dev*. 2018:27(13):871–887. doi: 10.1089/scd.2018.0066

37. Zhao J, Hao Y, Ross JW, et al. Histone deacetylase inhibitors improve in vitro and in vivo developmental competence of somatic cell nuclear transfer porcine embryos. *Cell Reprogram*. 2010;12(1):75–83. doi: 10.1089/cell.2009.0038

38. Taweechaipaisankul A, Jin JX, Lee S, et al. Improved early development of porcine cloned embryos by treatment with quisinostat, a potent histone deacetylase inhibitor. *J Reprod Dev*. 2019;65(2):103–112. doi: 10.1262/jrd.2018-098

39. Zhai Y, Zhang Z, Yu H, et al. Dynamic methylation changes of DNA and H3K4 by RG108 improve epigenetic reprogramming of somatic cell nuclear transfer embryos in pigs. *Cell Physiol Biochem*. 2018;50(4):1376–1397. doi: 10.1159/000494598

40. Matoba S, Liu Y, Lu F, et al. Embryonic development following somatic cell nuclear transfer impeded by persisting histone methylation. *Cell*. 2014;159:884–895. doi: 10.1016/j.cell.2014.09.055

41. Gao R, Wang C, Gao Y, et al. Inhibition of aberrant DNA re-methylation improves post-implantation development of somatic cell nuclear transfer embryos. *Cell Stem Cell*. 2018;23:426–435.e5. doi: 10.1016/j.stem.2018.07.017

42. Wang Z, Meissner A, Jaenisch R. Nuclear cloning and epigenetic reprogramming. In: Lanza R, Gearhart J, Hogan B, et al, editors. *Handbook of stem cells*. London: Elsevier Academic press; 2004. P. 119–127. doi: 10.1016/B978-012436643-5/50019-5

43. Nguyen VK, Somfai T, Salamone D, et al. Optimization of donor cell cycle synchrony, maturation media and embryo culture system for somatic cell nuclear transfer in the critically endangered Vietnamese — pig. *Theriogenology*. 2021;166:21–28. doi: 10.1016/j.theriogenology.2021.02.008

44. Lai L, Tao T, Macháty Z, et al. Feasibility of producing porcine nuclear transfer embryos by using G2/M-stage fetal fibroblasts as donors. *Biol Reprod*. 2001;65(5):1558–1564. doi: 10.1095/biolreprod65.5.1558

45. Tani T, Kato Y, Tsunoda Y. Direct exposure of chromosomes to nonactivated ovum cytoplasm is effective for bovine somatic cell nucleus reprogramming. *Biol Reprod*. 2001;64(4):324–330. doi: 10.1095/biolreprod64.1.324

46. Wu B, Ignotz G, Currie WB, Yang X. Dynamics of maturation-promoting factor and its constituent proteins during in vitro maturation of bovine oocytes. *Biol Reprod*. 1997;56(1):253–259. doi: 10.1095/biolreprod56.1.253

47. Polejaeva IA. 25th anniversary of cloning by somatic cell nuclear transfer: generation of genetically engineered livestock using somatic cell nuclear transfer. *Reproduction*. 2021;162(1):F11. doi: 10.1530/REP-21-0072

48. Kato Y, Tsunoda Y. Role of the donor nuclei in cloning efficiency: can the ooplasm reprogram any nucleus? *Int J Dev Biol*. 2010;54:1623–1629. doi: 10.1387/ijdb.103203yk

49. Kikyo N, Wolffe AP. Reprogramming nuclei: Insights from cloning, nuclear transfer and heterokaryons. *J Cell Sci*. 2000;113(Pt 1):11–20. doi: 10.1242/jcs.113.1.11

50. Hosseini SM, Hajian M, Moulavi F, et al. Cloned sheep blastocysts derived from oocytes enucleated manually using

a pulled Pasteur pipette. *Cell Reprogram*. 2013;15(1):15–23. doi: 10.1089/cell.2012.0033

51. Wang Y, Liu Q, Kang J, et al. Overexpression of PGC7 in donor cells maintains the DNA methylation status of imprinted genes in goat embryos derived from somatic cell nuclear transfer technology. *Theriogenology*. 2020;151:86–94. doi: 10.1016/j.theriogenology.2020.04.013

52. Huang Y, Zhang J, Li X, et al. Chromatin accessibility memory of donor cells disrupts bovine somatic cell nuclear transfer blastocysts development. *FASEB J*. 2023;37(9):e23111. doi: 10.1096/fj.202300131RRR

53. Sangalli JR, Sampaio RV, De Bem THC, et al. Cattle cloning by somatic cell nuclear transfer. *Methods Mol Biol*. 2023;2647:225–244. doi: 10.1007/978-1-0716-3064-8_12

54. Rim CS, Kim YS, Rim CH, et al. Effect of roscovitine pretreatment for increased utilization of small follicle-derived oocytes on developmental competence of somatic cell nuclear transfer embryos in pigs. *Anim Reprod Sci*. 2022;241:106987. doi: 10.1016/j.anireprosci.2022.106987

55. Cai L, Hyun SH, Kim E. Stem cell factor's role in enhancing the quality of fertilized and cloned porcine embryos for improved embryonic stem cell derivation. *Front Vet Sci*. 2023;10:1285530. doi: 10.3389/fvets.2023.1285530

56. Hosseini SM, Moulavi F, Foruzanfar M, et al. Effect of donor cell type and gender on the efficiency of in vitro sheep somatic cell cloning. *Small Ruminant Research*. 2008;78(1–3):162–168. doi: 10.1016/j.smallrumres.2008.06.004

57. Xu J, Shi P, Zhao X, et al. Cell synchronization by rapamycin improves the developmental competence of buffalos (Bubalus bubalis) somatic cell nuclear transfer embryos. *Reprod Domest Anim*. 2021;56(2):313–323. doi: 10.1111/rda.13868

58. Hyun H, Lee SE, Son YJ, et al. Cell synchronization by rapamycin improves the developmental competence of porcine SCNT embryos. *Cell Reprogram*. 2016;18(3):195–205. doi: 10.1089/cell.2015.0090

59. Kues WA, Anger M, Carnwath JW, et al. Cell cycle synchronization of porcine fetal fibroblasts: Effects of serum deprivation and reversible cell cycle inhibitors. *Biol Reprod*. 2000;62(2):412–419. doi: 10.1095/biolreprod62.2.412

60. Cho SR, Ock SA, Yoo JG, et al. Effects of confluent, roscovitine treatment and serum starvation on the cell-cycle synchronization of bovine foetal fibroblasts. *Reprod Domest Anim*. 2005;40(2):171–176. doi: 10.1111/j.1439-0531.2005.00577.x

61. Sadeghian-Nodoushan F, Eftekhari-Yazdi P, Dalman A, et al. Mimosine as well as serum starvation can be used for cell cycle synchronization of sheep granulosa cells. *Chinese Journal of Biology*. 2014;2014:851736. doi: 10.1155/2014/851736

62. Rodrigues LLV, Moura YBF, Viana JVDS, et al. Full confluency, serum starvation, and roscovitine for inducing arrest in the G0/G1 phase of the cell cycle in puma skin-derived fibroblast lines. *Anim Reprod*. 2023;20(1):e20230017. doi: 10.1590/1984-3143-AR2023-0017

63. Yagcioglu S, Ersoy N, Demir K, et al. Can roscovitine and trichostatin A be alternatives to standard protocols for cell cycle synchronization of ovine adult and foetal fibroblast cells? *Reprod Domest Anim*. 2023;58(9):1251–1260. doi: 10.1111/rda.14425

64. Zhao B, Li H, Zhang H, et al. The effect of L-carnitine supplementation during in vitro maturation on oocyte maturation and somatic cloned embryo development. *Reprod Biol*. 2024;24(2):100853. doi: 10.1016/j.repbio.2023.100853

65. Gómez NA, Ramírez MM, Ruiz-Cortés ZT. Primary fibroblast cell cycle synchronization and effects on handmade cloned (HMC) bovine embryos. *Ciênc Anim Bras*. 2018;19:e48555. doi: 10.1590/1809-6891v19e-48555

66. Chen M, Huang J, Yang X, et al. Serum starvation induced cell cycle synchronization facilitates human somatic cells reprogramming. *PLoS One*. 2012;7(4):e28203. doi: 10.1371/journal.pone.0028203

67. Zhang Y, Qu P, Ma X, et al. Tauroursodeoxycholic acid (TUDCA) alleviates endoplasmic reticulum stress of nuclear donor cells under serum starvation. *PLoS One*. 2018;13(5):e0196785. doi: 10.1371/journal.pone.0196785

68. Giannasi C, Niada S, Della Morte E, et al. Serum starvation affects mitochondrial metabolism of adipose-derived stem/stromal cells. *Cytotherapy*. 2023;25(7):704–711. doi: 10.1016/j.jcyt.2023.03.004

69. Park HJ, Koo OJ, Kwon DK, et al. Effect of roscovitine-treated donor cells on development of porcine cloned embryos. *Reprod Domest Anim*. 2010;45(6):1082–1088. doi: 10.1111/j.1439-0531.2009.01499.x

70. Franco R, Bortner C, Cidlowski J. Potential roles of electrogenic ion transport and plasma membrane depolarization in apoptosis. *J Membrane Biol*. 2006;209(1):43–58. doi: 10.1007/s00232-005-0837-5

71. Rols MP. Parameters affecting cell viability following electroporation in vitro. In: Miklavčič D, editor. *Handbook of electroporation*. Cham: Springer International Publishing; 2017. P. 1449–1465. doi: 10.1007/978-3-319-32886-7_149

72. Miranda MDS, Bressan FF, Zecchin KG, et al. Serum-starved apoptotic fibroblasts reduce blastocyst production but enable development to term after SCNT in cattle. *Cloning Stem Cells*. 2009;11(4):565–573. doi: 10.1089/clo.2009.0028

73. Carvalho BP, Cunha ATM, Silva BDM, et al. Production of transgenic cattle by somatic cell nuclear transfer (SCNT) with the human granulocyte colony-stimulation factor (hG-CSF). *J Anim Sci Technol*. 2019;61(2):61–68. doi: 10.5187/jast.2019.61.2.61

74. Al-Ghadi MQ, Alhimaidi AR, Iwamoto D, et al. The in vitro development of cloned sheep embryos treated with Scriptaid and Trichostatin (A). *Saudi J Biol Sci*. 2020;27(9):2280–2286. doi: 10.1016/j.sjbs.2020.04.039

75. Cortez JV, Hardwicke K, Cuervo-Arango J, Grupen CG. Cloning horses by somatic cell nuclear transfer: Effects of oocyte source on development to foaling. *Theriogenology*. 2023;203:99–108. doi: 10.1016/j.theriogenology.2023.03.018

76. Yadav PS, Kumar D, Saini M, et al. Evaluation of postnatal growth, hematology, telomere length and semen attributes of multiple clones and re-clone of superior buffalo breeding bulls. *Theriogenology*. 2024;213:24–33. doi: 10.1016/j.theriogenology.2023.09.024

77. Curto M, Cole BK, Lallemand D, et al. Contact-dependent inhibition of EGFR signaling by Nf2/Merlin. *J Cell Biol*. 2007;177(5):893–903. doi: 10.1083/jcb.200703010

78. Fuse T, Tanikawa M, Nakanishi M, et al. p27Kip1 expression by contact inhibition as a prognostic index of human glioma. *J Neurochem*. 2000;74(4):1393–1399. doi: 10.1046/j.1471-4159.2000.0741393.x

79. Wieser RJ, Faust D, Dietrich C, et al. p16INK4 mediates contact-inhibition of growth. *Oncogene*. 1999;18(1):277–281. doi: 10.1038/sj.onc.1202270

80. Pani G, Colavitti R, Bedogni B, et al. A redox signaling mechanism for density-dependent inhibition of cell growth. *J Biol Chem*. 2000;275(49):38891–38899. doi: 10.1074/jbc.M007319200

81. Yang S, Hwang S, Jang J, et al. PGC1α is required for the induction of contact inhibition by suppressing ROS. *Biochem Biophys Res Commun*. 2018;501(3):739–744. doi: 10.1016/j.bbrc.2018.05.059
82. Ma L, Liu X, Wang F, et al. Different donor cell culture methods can influence the developmental ability of cloned sheep embryos. *PLoS One*. 2015;10(8):e0135344. doi: 10.1371/journal.pone.0135344
83. Wittayarat M, Thongphakdee A, Saikhun K, et al. Cell cycle synchronization of skin fibroblast cells in four species of family Felidae. *Reprod Domest Anim*. 2013;4(2):305–310. doi: 10.1111/j.1439-0531.2012.02149.x
84. Młodawska W, Mrowiec P, Bochenek M, et al. Effect of serum starvation and contact inhibition on dermal fibroblast cell cycle synchronization in two species of wild felids and domestic cat. *Annals of Animal Science*. 2022;22(4):1245–1255. doi: 10.2478/aoas-2022-0042
85. Visotskaja LV. Mitotic cycle and its regulation. *Vavilov Journal of Genetics and Breeding*. 2014;18(1):81–92. EDN: SJCEOR
86. Wang Z. Cell cycle progression and synchronization: an overview. *Methods Mol Biol*. 2022;2579:3–23. doi: 10.1007/978-1-0716-2736-5_1
87. Banfalvi G. Overview of cell synchronization. *Methods Mol Biol*. 2011;761:1–23. doi: 10.1007/978-1-61779-182-6_1
88. Martinez Diaz MA, Suzuki M, Kagawa M, et al. Effects of cycloheximide treatment on in-vitro development of porcine parthenotes and somatic cell nuclear transfer embryos. *Jpn J Vet Res*. 2003;50(4):147–155. doi: 10.14943/jjvr.50.4.147
89. Eren A, Arat S, Tuna M, et al. Effects of confluency, roscovitine and serum starvation on the cell-cycle synchronization and viability of sheep and goat adult fibroblasts. *J Biotechnol*. 2014;185:S46. doi: 10.1016/j.jbiotec.2014.07.156
90. Sun X, Wang S, Zhang Y, et al. Cell-cycle synchronization of fibroblasts derived from transgenic cloned cattle ear skin: effects of serum starvation, roscovitine and contact inhibition. *Zygote*. 2008;16(2):111–116. doi: 10.1017/S0967199407004522
91. Hwang S, Oh KB, Kwon DJ, et al. Improvement of cloning efficiency in minipigs using post-thawed donor cells treated with roscovitine. *Mol Biotechnol*. 2013;55(3):212–216. doi: 10.1007/s12033-013-9671-7
92. Selokar NL, Saini M, Muzaffer M, et al. Roscovitine treatment improves synchronization of donor cell cycle in G0/G1 stage and in vitro development of handmade cloned buffalo (Bubalus bubalis) embryos. *Cell Reprogram*. 2012;14(2):146–154. doi: 10.1089/cell.2011.0076
93. Vacková I, Engelová M, Marinov I, Tománek M. Cell cycle synchronization of porcine granulosa cells in G1 stage with mimosine. *Anim Reprod Sci*. 2003;77(3-4):235–245. doi: 10.1016/s0378-4320(03)00034-4
94. Alessi F, Quarta S, Savio M, et al. The cyclin-dependent kinase inhibitors olomoucine and roscovitine arrest human fibroblasts in G1 phase by specific inhibition of CDK2 kinase activity. *Exp Cell Res*. 1998;245(1):8–18. doi: 10.1006/excr.1998.4216
95. Banfalvi G. Erratum. *Methods Mol Biol*. 2017;1524:E1. doi: 10.1007/978-1-4939-6603-5_22
96. Zhang M, Zhang L, Hei R, et al. CDK inhibitors in cancer therapy, an overview of recent development. *Am J Cancer Res*. 2021;11(5):1913–1935.
97. Cuyàs E, Corominas-Faja B, Joven J, Menendez JA. Cell cycle regulation by the nutrient-sensing mammalian target of rapamycin (mTOR) pathway. *Methods Mol Biol*. 2014;1170:113–144. doi: 10.1007/978-1-4939-0888-2_7
98. Rubinsztein DC, Mariño G, Kroemer G. Autophagy and aging. *Cell*. 2011;146(5):682–695. doi: 10.1016/j.cell.2011.07.030
99. Lee J, Park JI, Yun JI, et al. Rapamycin treatment during in vitro maturation of oocytes improves embryonic development after parthenogenesis and somatic cell nuclear transfer in pigs. *J Vet Sci*. 2015;16(3):373–380. doi: 10.4142/jvs.2015.16.3.373
100. Albers MW, Williams RT, Brown EJ, et al. FKBP-rapamycin inhibits a cyclin-dependent kinase activity and a cyclin D1-Cdk association in early G1 of an osteosarcoma cell line. *J Biol Chem*. 1993;268(30):22825–22829. doi: 10.1016/S0021-9258(18)41602-X
101. Han J, Yan H. Inhibitory effect of rapamycin on the proliferation of Rhesus retinal vascular endothelial cells. *Chinese Journal of Experimental Ophthalmology*. 2014;3:216–219.
102. Jiang F, Wang Y, Du S, et al. Rapamycin prevents retinal neovascularization by downregulation of cyclin D1 in a mouse model of oxygen-induced retinopathy. *BMC Ophthalmol*. 2020;20(1):44. doi: 10.1186/s12886-020-1325-5
103. Kitagawa M, Okabe T, Ogino H, et al. Butyrolactone I, a selective inhibitor of CDK2 and CDC2 kinase. *Oncogene*. 1993;8(9):2425–2432.
104. Liu F, Ma XH, Ule J, et al. Regulation of cyclin-dependent kinase 5 and casein kinase 1 by metabotropic glutamate receptors. *Proc Natl Acad Sci USA*. 2001;98(20):11062–11068. doi: 10.1073/pnas.191353898
105. Wada M, Hosotani R, Lee JU, et al. An exogenous cdk inhibitor, butyrolactone-I, induces apoptosis with increased Bax/Bcl-2 ratio in p53-mutated pancreatic cancer cells. *Anticancer Res*. 1998;18(4A):2559–2566.
106. Ghfar AA, El-Metwally MM, Shaaban M, et al. Production of terretonin N and butyrolactone I by Thermophilic Aspergillus terreus TM8 promoted apoptosis and cell death in human prostate and ovarian cancer cells. *Molecules*. 2021;26(9):2816. doi: 10.3390/molecules26092816
107. Monaco EA 3rd, Beaman-Hall CM, Mathur A, Vallano ML. Roscovitine, olomoucine, purvalanol: Inducers of apoptosis in maturing cerebellar granule neurons. *Biochem Pharmacol*. 2004;67(10):1947–1964. doi: 10.1016/j.bcp.2004.02.007
108. Krude T. Mimosine arrests proliferating human cells before onset of DNA replication in a dose-dependent manner. *Exp Cell Res*. 1999;247(1):148–159. doi: 10.1006/excr.1998.4342
109. Galgano PJ, Schildkraut CL. G1/S phase synchronization using mimosine arrest. *CSH Protoc*. 2006;2006(4):pdb.prot4488. doi: 10.1101/pdb.prot4488
110. Jackman J, O'Connor PM. Methods for synchronizing cells at specific stages of the cell cycle. *Curr Protoc Cell Biol*. 1998;8:8.3. doi: 10.1002/0471143030.cb0803s00
111. Kim E, Zheng Z, Jeon Y, et al. An improved system for generation of diploid cloned porcine embryos using induced pluripotent stem cells synchronized to metaphase. *PLoS One*. 2016;11(7):e0160289. doi: 10.1371/journal.pone.0160289
112. Ho YS, Duh JS, Jeng JH, et al. Griseofulvin potentiates antitumorigenesis effects of nocodazole through induction of apoptosis and G2/M cell cycle arrest in human colorectal cancer cells. *Int J Cancer*. 2001;91(3):393–401. doi: 10.1002/1097-0215(200002)9999:9999<::aid-ijc1070>3.0.co;2-#

СПИСОК ЛИТЕРАТУРЫ

1. Briggs R., King T.J. Transplantation of living nuclei from blastula cells into enucleated frogs' eggs // Proc Natl Acad Sci U S A. 1952. Vol. 38, N 5. P. 455–463. doi: 10.1073/pnas.38.5.455

2. Klinger B., Schnieke A. 25th anniversary of cloning by somatic cell nuclear transfer. Twenty-five years after Dolly: how far have we come? // Reproduction. 2021. Vol. 162, N 1. P. F1–F10. doi: 10.1530/REP-20-0652

3. Wilmut I., Schnieke A., McWhir J., et al. Viable offspring derived from fetal and adult mammalian cells // Nature. 1997. Vol. 385, N 6619. P. 810–813. Corrected and republished from: Nature. 1997. Vol. 386, N 6621. P. 200. doi: 10.1038/385810a0

4. Shakweer W.M.E., Krivoruchko A.Y., Dessouki S.M., et al. A review of transgenic animal techniques and their applications // J Genet Eng Biotechnol. 2023. Vol. 21, N 1. P. 55. doi: 10.1186/s43141-023-00502-z

5. Lee J.E., Chung Y.G., Eum J.H., et al. An efficient SCNT technology for the establishment of personalized and public human pluripotent stem cell banks // BMB Rep. 2016. Vol. 49, N 4. P. 197–198. doi: 10.5483/bmbrep.2016.49.4.055

6. Keefer C.L. Artificial cloning of domestic animals // Proc Natl Acad Sci USA. 2015. Vol. 12, N 29. P. 8874–8878. doi: 10.1073/pnas.1501718112

7. Ogura A., Inoue K., Wakayama T. Recent advancements in cloning by somatic cell nuclear transfer // Philos Trans R Soc Lond B Biol Sci. 2013. Vol. 368, N 1609. P. 20110329. doi: 10.1098/rstb.2011.0329

8. Zhang X., Gao S., Liu X. Advance in the role of epigenetic reprogramming in somatic cell nuclear transfer-mediated embryonic development // Stem Cells Int. 2021. Vol. 2021. P. 6681337. doi: 10.1155/2021/6681337

9. Malin K., Witkowska-Piłaszewicz O., Papis K. The many problems of somatic cell nuclear transfer in reproductive cloning of mammals // Theriogenology. 2022. Vol. 189. P. 246–254. doi: 10.1016/j.theriogenology.2022.06.030

10. Лопухов А.В., Сингина Г.Н., Зиновьева Н.А. Биотехнологические основы получения клонированных эмбрионов свиней // Вавиловский журнал генетики и селекции. 2019.Т. 23, № 5. С. 527–533. EDN: LCBRWK doi: 10.18699/VJ19.521

11. Srirattana K., Kaneda M., Parnpai R. Strategies to improve the efficiency of somatic cell nuclear transfer // Int J Mol Sci. 2022. Vol. 23, N 4. P. 1969. doi: 10.3390/ijms23041969

12. Gouveia C., Huyser C., Egli D., Pepper MS. Lessons learned from somatic cell nuclear transfer // Int J Mol Sci. 2020. Vol. 21, N 7. P. 2314. doi: 10.3390/ijms21072314

13. Zhao Q., Qiu J., Feng Z., et al. Robotic label-free precise oocyte enucleation for improving developmental competence of cloned embryos // IEEE Trans Biomed Eng. 2021. Vol. 68, N 8. P. 2348–2359. doi: 10.1109/TBME.2020.3036494

14. Vazquez-Avendanõ J.R., Ambríz-García D.A., Cortez-Romero C. Current state of the efficiency of sheep embryo production through somatic cell nuclear transfer // Small Rumin Res. 2022. Vol. 212. P. 106702. doi: 10.1016/j.smallrumres.2022.106702

15. Samiec M. Molecular mechanism and application of somatic cell cloning in mammals — past, present and future // Int J Mol Sci. 2022. Vol. 23, N 22. P. 13786. doi: 10.3390/ijms232213786

16. Yao Y., Yang A., Li G., et al. Melatonin promotes the development of sheep transgenic cloned embryos by protecting donor and recipient cells // Cell Cycle. 2022. Vol. 21, N 13. P. 1360–1375. doi: 10.1080/15384101.2022.2051122

17. Wang Y., Qi J.J., Yin Y.J., et al. Ferulic acid enhances oocyte maturation and the subsequent development of bovine oocytes // Int J Mol Sci. 2023. Vol. 24, N 19. P. 14804. doi: 10.3390/ijms241914804

18. Himaki T., Hano K. Effects of alpha lipoic acid treatment during in vitro maturation on the development of porcine somatic cell nuclear transfer embryos // Anim Sci J. 2023. Vol. 94, N 1. P. e13889. doi: 10.1111/asj.13889

19. Vajta G., Lewis I.M., Trounson A.O., et al. Handmade somatic cell cloning in cattle: Analysis of factors contributing to high efficiency in vitro // Biol Reprod. 2003. Vol. 68, N 2. P. 571–578. doi: 10.1095/biolreprod.102.008771

20. Kang J.S., Joo M.D., Lee S.H., et al. Effect of additional cytoplasm injection on the cloned bovine embryo organelle distribution and stress mitigation // Theriogenology. 2024. Vol. 216. P. 12–19. doi: 10.1016/j.theriogenology.2023.11.031

21. Srirattana K., St John J.C. Manipulating the mitochondrial genome to enhance cattle embryo development // G3 (Bethesda). 2017. Vol. 7, N 7. P. 2065–2080. doi: 10.1534/g3.117.042655

22. Srirattana K., St John J.C. Additional mitochondrial DNA influences the interactions between the nuclear and mitochondrial genomes in a bovine embryo model of nuclear transfer // Sci Rep. 2018. Vol. 8, N 1. P. 7246. doi: 10.1038/s41598-018-25516-3

23. Liao Z., Zhang J., Sun S., et al. Reprogramming mechanism dissection and trophoblast replacement application in monkey somatic cell nuclear transfer // Nat Commun. 2024. Vol. 15, N 1. P. 5. doi: 10.1038/s41467-023-43985-7

24. Lu F., Zhang Y. Cell totipotency: molecular features, induction, and maintenance // Natl Sci Rev. 2015. Vol. 2, N 2. P. 217–225. doi: 10.1093/nsr/nwv009

25. Weidgang C.E., Seufferlein T., Kleger A., Mueller M. Pluripotency factors on their lineage move // Stem Cells Int. 2016. Vol. 2016. P. 6838253. doi: 10.1155/2016/6838253

26. Jaenisch R., Young R. Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming // Cell. 2008. Vol. 132, N 4. P. 567–582. doi: 10.1016/j.cell.2008.01.015

27. Hanna J.H., Saha K., Jaenisch R. Pluripotency and cellular reprogramming: facts, hypotheses, unresolved issues // Cell. 2010. Vol. 143, N 4. P. 508–525. doi: 10.1016/j.cell.2010.10.008

28. Czołowska R., Modliński J.A., Tarkowski A.K. Behaviour of thymocyte nuclei in non-activated and activated mouse oocytes // J Cell Sci. 1984. Vol. 69. P. 19–34. doi: 10.1242/jcs.69.1.19

29. Barnes F.L., Collas P., Powell R., et al. Influence of recipient oocyte cell cycle stage on DNA synthesis, nuclear envelope breakdown, chromosome constitution, and development in nuclear transplant bovine embryos // Mol Reprod Dev. 1993. Vol. 36, N 1. P. 33–41. doi: 10.1002/mrd.1080360106

30. Campbell K.H., Ritchie W.A., Wilmut I. Nuclear-cytoplasmic interactions during the first cell cycle of nuclear transfer reconstructed bovine embryos: implications for deoxyribonucleic acid replication and development // Biol Reprod. 1993. Vol. 49, N 5. P. 933–942. doi: 10.1095/biolreprod49.5.933

31. Campbell K.H.S., Choi I., Zhu J., et al. Cell cycle regulation in cloning. In: Cibelli J., Wilmut I., Jaenisch R., et al, editors. Principles

of cloning (second edition). San Diego: Academic Press, 2014. P. 149–160. doi: 10.1016/B978-0-12-386541-0.00012-6

32. Jullien J., Pasque V., Halley-Stott R.P., et al. Mechanisms of nuclear reprogramming by eggs and oocytes: a deterministic process? // Nat Rev Mol Cell Biol. 2011. Vol. 12, N 7. P. 453–459. doi: 10.1038/nrm3140

33. Xu R., Zhang S., Lei A. Chromatin changes in reprogramming of mammalian somatic cells // Rejuvenation Res. 2014. Vol. 17, N 1. P. 3–10. doi: 10.1089/rej.2013.1455

34. Glanzner W.G., de Macedo M.P., Gutierrez K., Bordignon V. Enhancement of chromatin and epigenetic reprogramming in porcine SCNT embryos-progresses and perspectives // Front Cell Dev Biol. 2022. Vol. 10. P. 940197. doi: 10.3389/fcell.2022.940197

35. Gaspar-Maia A., Alajem A., Meshorer E., Ramalho-Santos M. Open chromatin in pluripotency and reprogramming // Nat Rev Mol Cell Biol. 2011. Vol. 12, N 1. P. 36–47. Corrected and republished from: Nat Rev Mol Cell Biol. 2011. Vol. 12, N 4. P. 273. doi: 10.1038/nrm3036

36. Gonzales-Munoz E., Cibelli J.B. Somatic cell reprogramming informed by the oocyte // Stem Cells Dev. 2018. Vol. 27, N 13. P. 871–887. doi: 10.1089/scd.2018.0066

37. Zhao J., Hao Y., Ross J.W., et al. Histone deacetylase inhibitors improve in vitro and in vivo developmental competence of somatic cell nuclear transfer porcine embryos // Cell Reprogram. 2010. Vol. 12, N 1. P. 75–83. doi: 10.1089/cell.2009.0038

38. Taweechaipaisankul A., Jin J.X., Lee S., et al. Improved early development of porcine cloned embryos by treatment with quisinostat, a potent histone deacetylase inhibitor // J Reprod Dev. 2019. Vol. 65, N 2. P. 103–112. doi: 10.1262/jrd.2018-098

39. Zhai Y., Zhang Z., Yu H., et al. Dynamic methylation changes of DNA and H3K4 by RG108 improve epigenetic reprogramming of somatic cell nuclear transfer embryos in pigs // Cell Physiol Biochem. 2018. Vol. 50, N 4. P. 1376–1397. doi: 10.1159/000494598

40. Matoba S., Liu Y., Lu F., et al. Embryonic development following somatic cell nuclear transfer impeded by persisting histone methylation // Cell. 2014. Vol. 159, N 4. P. 884–895. doi: 10.1016/j.cell.2014.09.055

41. Gao R., Wang C., Gao Y., et al. Inhibition of aberrant DNA re-methylation improves post-implantation development of somatic cell nuclear transfer embryos // Cell Stem Cell. 2018. Vol. 23, N 3. P. 426–435. doi: 10.1016/j.stem.2018.07.017

42. Wang Z., Meissner A., Jaenisch R. Nuclear cloning and epigenetic reprogramming. In: Lanza R., Gearhart J., Hogan B., et al, editors. Handbook of stem cells. London: Elsevier Academic press, 2004. P. 119–127. doi: 10.1016/B978-012436643-5/50019-5

43. Nguyen V.K., Somfai T., Salamone D., et al. Optimization of donor cell cycle synchrony, maturation media and embryo culture system for somatic cell nuclear transfer in the critically endangered Vietnamese — pig // Theriogenology. 2021. Vol. 166. P. 21–28. doi: 10.1016/j.theriogenology.2021.02.008

44. Lai L., Tao T., Macháty Z., et al. Feasibility of producing porcine nuclear transfer embryos by using G2/M-stage fetal fibroblasts as donors // Biol Reprod. 2001. Vol. 65, N 5. P. 1558–1564. doi: 10.1095/biolreprod65.5.1558

45. Tani T., Kato Y., Tsunoda Y. Direct exposure of chromosomes to nonactivated ovum cytoplasm is effective for bovine somatic cell nucleus reprogramming // Biol Reprod. 2001. Vol. 64, N 1. P. 324–330. doi: 10.1095/biolreprod64.1.324

46. Wu B., Ignotz G., Currie W.B., et al. Dynamics of maturation-promoting factor and its constituent proteins during in vitro maturation of bovine oocytes // Biol Reprod. 1997. Vol. 56, N 1. P. 253–259. doi: 10.1095/biolreprod56.1.253

47. Polejaeva I.A. 25th anniversary of cloning by somatic cell nuclear transfer: generation of genetically engineered livestock using somatic cell nuclear transfer // Reproduction. 2021. Vol. 162, N 1. P. F11. doi: 10.1530/REP-21-0072

48. Kato Y., Tsunoda Y. Role of the donor nuclei in cloning efficiency: can the ooplasm reprogram any nucleus? // Int J Dev Biol. 2010. Vol. 54, N 11-12. P. 1623–1629. doi: 10.1387/ijdb.103203yk

49. Kikyo N., Wolffe A.P. Reprogramming nuclei: Insights from cloning, nuclear transfer and heterokaryons // J Cell Sci. 2000. Vol. 113, Pt 1. P.11–20. doi: 10.1242/jcs.113.1.11

50. Hosseini S.M., Hajian M., Moulavi F., et al. Cloned sheep blastocysts derived from oocytes enucleated manually using a pulled Pasteur pipette // Cell Reprogram. 2013. Vol. 15, N 1. P. 15–23. doi: 10.1089/cell.2012.0033

51. Wang Y., Liu Q., Kang J., et al. Overexpression of PGC7 in donor cells maintains the DNA methylation status of imprinted genes in goat embryos derived from somatic cell nuclear transfer technology // Theriogenology. 2020. Vol. 151. P. 86–94. doi: 10.1016/j.theriogenology.2020.04.013

52. Huang Y., Zhang J., Li X., et al. Chromatin accessibility memory of donor cells disrupts bovine somatic cell nuclear transfer blastocysts development // FASEB J. 2023. Vol. 37, N 9. P. e23111. doi: 10.1096/fj.202300131RRR

53. Sangalli J.R., Sampaio R.V., De Bem T.H.C., et al. Cattle cloning by somatic cell nuclear transfer // Methods Mol Biol. 2023. Vol. 2647. P. 225–244. doi: 10.1007/978-1-0716-3064-8_12

54. Rim C.S., Kim Y.S., Rim C.H., et al. Effect of roscovitine pretreatment for increased utilization of small follicle-derived oocytes on developmental competence of somatic cell nuclear transfer embryos in pigs // Anim Reprod Sci. 2022. Vol. 241. P. 106987. doi: 10.1016/j.anireprosci.2022.106987

55. Cai L., Hyun S.H., Kim E. Stem cell factor's role in enhancing the quality of fertilized and cloned porcine embryos for improved embryonic stem cell derivation // Front Vet Sci. 2023. Vol. 10. P. 1285530. doi: 10.3389/fvets.2023.1285530

56. Hosseini S.M., Moulavi F., Foruzanfar M., et al. Effect of donor cell type and gender on the efficiency of in vitro sheep somatic cell cloning // Small Ruminant Research. 2008. Vol. 78, N 1–3. P. 162–168. doi: 10.1016/j.smallrumres.2008.06.004

57. Xu J., Shi P., Zhao X., et al. Cell synchronization by rapamycin improves the developmental competence of buffalos (Bubalus bubalis) somatic cell nuclear transfer embryos // Reprod Domest Anim. 2021. Vol. 56, N 2. P. 313–323. doi: 10.1111/rda.13868

58. Hyun H., Lee S.E., Son Y.J., et al. Cell synchronization by rapamycin improves the developmental competence of porcine SCNT embryos // Cell Reprogram. 2016. Vol. 18, N 3. P. 195–205. doi: 10.1089/cell.2015.0090

59. Kues W.A., Anger M., Carnwath J.W., et al. Cell cycle synchronization of porcine fetal fibroblasts: effects of serum deprivation and reversible cell cycle inhibitors // Biol Reprod. 2000. Vol. 62, N 2. P. 412–419. doi: 10.1095/biolreprod62.2.412

60. Cho S.R., Ock S.A., Yoo J.G., et al. Effects of confluent, roscovitine treatment and serum starvation on the cell-cycle synchronization

of bovine foetal fibroblasts // Reprod Domest Anim. 2005. Vol. 40, N 2. P. 171–176. doi: 10.1111/j.1439-0531.2005.00577.x

61. Sadeghian-Nodoushan F., Eftekhari-Yazdi P., Dalman A., et al. Mimosine as well as serum starvation can be used for cell cycle synchronization of sheep granulosa cells // Chinese Journal of Biology. 2014. Vol. 2014. P. 851736. doi: 10.1155/2014/851736

62. Rodrigues L.L.V., Moura Y.B.F., Viana J.V.D.S., et al. Full confluency, serum starvation, and roscovitine for inducing arrest in the G0/G1 phase of the cell cycle in puma skin-derived fibroblast lines // Anim Reprod. 2023. Vol. 20, N 1. P. e20230017. doi: 10.1590/1984-3143-AR2023-0017

63. Yagcioglu S., Ersoy N., Demir K., et al. Can roscovitine and trichostatin A be alternatives to standard protocols for cell cycle synchronization of ovine adult and foetal fibroblast cells? // Reprod Domest Anim. 2023. Vol. 58, N 9. P. 1251–1260. doi: 10.1111/rda.14425

64. Zhao B., Li H., Zhang H., et al. The effect of L-carnitine supplementation during in vitro maturation on oocyte maturation and somatic cloned embryo development // Reprod Biol. 2024. Vol. 24, N 2. P. 100853. doi: 10.1016/j.repbio.2023.100853

65. Gómez N.A., Ramírez M.M., Ruiz-Cortés Z.T. Primary fibroblast cell cycle synchronization and effects on handmade cloned (HMC) bovine embryos // Ciênc Anim Bras. 2018. Vol. 19. P. e48555. doi: 10.1590/1809-6891v19e-48555

66. Chen M., Huang J., Yang X., et al. Serum starvation induced cell cycle synchronization facilitates human somatic cells reprogramming // PLoS One. 2012. Vol. 7, N 4. P. e28203. doi: 10.1371/journal.pone.0028203

67. Zhang Y., Qu P., Ma X., et al. Tauroursodeoxycholic acid (TUDCA) alleviates endoplasmic reticulum stress of nuclear donor cells under serum starvation // PLoS One. 2018. Vol. 13, N 5. P. e0196785. doi: 10.1371/journal.pone.0196785

68. Giannasi C., Niada S., Della Morte E., et al. Serum starvation affects mitochondrial metabolism of adipose-derived stem/stromal cells // Cytotherapy. 2023. Vol. 25, N 7. P. 704–711. doi: 10.1016/j.jcyt.2023.03.004

69. Park H.J., Koo O.J., Kwon D.K., et al. Effect of roscovitine-treated donor cells on development of porcine cloned embryos // Reprod Domest Anim. 2010. Vol. 45, N 6. P. 1082–1088. doi: 10.1111/j.1439-0531.2009.01499.x

70. Franco R., Bortner C., Cidlowski J. Potential roles of electrogenic ion transport and plasma membrane depolarization in apoptosis // J Membrane Biol. 2006. Vol. 209, N 1. P. 43–58. doi: 10.1007/s00232-005-0837-5

71. Rols M.P. Parameters affecting cell viability following electroporation in vitro. In: Miklavčič D., editor. Handbook of electroporation. Cham: Springer International Publishing, 2017. P. 1449–1465. doi: 10.1007/978-3-319-32886-7_149

72. Miranda Mdos S., Bressan F.F., Zecchin K.G., et al. Serum-starved apoptotic fibroblasts reduce blastocyst production but enable development to term after SCNT in cattle // Cloning Stem Cells. 2009. Vol. 11, N 4. P. 565–573. doi: 10.1089/clo.2009.0028

73. Carvalho B.P., Cunha A.T.M., Silva B.D.M., et al. Production of transgenic cattle by somatic cell nuclear transfer (SCNT) with the human granulocyte colony-stimulation factor (hG-CSF) // J Anim Sci Technol. 2019. Vol. 61, N 2. P. 61–68. doi: 10.5187/jast.2019.61.2.61

74. Al-Ghadi M.Q., Alhimaidi A.R., Iwamoto D., et al. The in vitro development of cloned sheep embryos treated with Scriptaid and Trichostatin (A) // Saudi J Biol Sci. 2020. Vol. 27, N 9. P. 2280–2286. doi: 10.1016/j.sjbs.2020.04.039

75. Cortez J.V., Hardwicke K., Cuervo-Arango J., Grupen C.G. Cloning horses by somatic cell nuclear transfer: effects of oocyte source on development to foaling // Theriogenology. 2023. Vol. 203. P. 99–108. doi: 10.1016/j.theriogenology.2023.03.018

76. Yadav P.S., Kumar D., Saini M., et al. Evaluation of postnatal growth, hematology, telomere length and semen attributes of multiple clones and re-clone of superior buffalo breeding bulls // Theriogenology. 2024. Vol. 213. P. 24–33. doi: 10.1016/j.theriogenology.2023.09.024

77. Curto M., Cole B.K., Lallemand D., et al. Contact-dependent inhibition of EGFR signaling by Nf2/Merlin // J Cell Biol. 2007. Vol. 177, N 5. P. 893–903. doi: 10.1083/jcb.200703010

78. Fuse T., Tanikawa M., Nakanishi M., et al. p27Kip1 expression by contact inhibition as a prognostic index of human glioma // J Neurochem. 2000. Vol. 74, N 4. P. 1393–1399. doi: 10.1046/j.1471-4159.2000.0741393.x

79. Wieser R.J., Faust D., Dietrich C., Oesch F. p16INK4 mediates contact-inhibition of growth // Oncogene. 1999. Vol. 18, N 1. P. 277–281. doi: 10.1038/sj.onc.1202270

80. Pani G., Colavitti R., Bedogni B., et al. A redox signaling mechanism for density-dependent inhibition of cell growth // J Biol Chem. 2000. Vol. 275, N 49. P. 38891–38899. doi: 10.1074/jbc.M007319200

81. Yang S., Hwang S., Jang J., et al. PGC1α is required for the induction of contact inhibition by suppressing ROS // Biochem Biophys Res Commun. 2018. Vol. 501, N 3. P. 739–744. doi: 10.1016/j.bbrc.2018.05.059

82. Ma L., Liu X., Wang F., et al. Different donor cell culture methods can influence the developmental ability of cloned sheep embryos // PLoS One. 2015. Vol. 10, N 8. P. e0135344. doi: 10.1371/journal.pone.0135344

83. Wittayarat M., Thongphakdee A., Saikhun K., et al. Cell cycle synchronization of skin fibroblast cells in four species of family Felidae // Reprod Domest Anim. 2013. Vol. 4, N 2. P. 305–310. doi: 10.1111/j.1439-0531.2012.02149.x

84. Młodawska W., Mrowiec P., Bochenek M., et al. Effect of serum starvation and contact inhibition on dermal fibroblast cell cycle synchronization in two species of wild felids and domestic cat // Annals of Animal Science. 2022. Vol. 22, N 4. P. 1245–1255. doi: 10.2478/aoas-2022-0042

85. Высоцкая Л.В. Митотический цикл и его регуляция // Вавиловский журнал генетики и селекции. 2014. Т. 18, № 1. С. 81–92. EDN: SJCEOR

86. Wang Z. Cell cycle progression and synchronization: an overview // Methods Mol Biol. 2022. Vol. 2579. P. 3–23. doi: 10.1007/978-1-0716-2736-5_1

87. Banfalvi G. Overview of cell synchronization // Methods Mol Biol. 2011. Vol. 761. P. 1–23. doi: 10.1007/978-1-61779-182-6_1

88. Martinez Diaz M.A., Suzuki M., Kagawa M., et al. Effects of cycloheximide treatment on in-vitro development of porcine parthenotes and somatic cell nuclear transfer embryos // Jpn J Vet Res. 2003. Vol. 50, N 4. P. 147–155. doi: 10.14943/jjvr.50.4.147

89. Eren A., Arat S., Tuna M., et al. Effects of confluency, roscovitine and serum starvation on the cell-cycle synchronization and viability of sheep and goat adult fibroblasts // J Biotechnol. 2014. Vol. 185. P. S46. doi: 10.1016/j.jbiotec.2014.07.156

90. Sun X., Wang S., Zhang Y., et al. Cell-cycle synchronization of fibroblasts derived from transgenic cloned cattle ear skin: Effects of

serum starvation, roscovitine and contact inhibition // Zygote. 2008. Vol. 16, N 2. P. 111–116. doi: 10.1017/S0967199407004522
91. Hwang S., Oh K.B., Kwon D.J., et al. Improvement of cloning efficiency in minipigs using post-thawed donor cells treated with roscovitine // Mol Biotechnol. 2013. Vol. 55, N 3. P. 212–216. doi: 10.1007/s12033-013-9671-7
92. Selokar N.L., Saini M., Muzaffer M., et al. Roscovitine treatment improves synchronization of donor cell cycle in G0/G1 stage and in vitro development of handmade cloned buffalo (Bubalus bubalis) embryos // Cell Reprogram. 2012. Vol. 14, N 2. P. 146–154. doi: 10.1089/cell.2011.0076
93. Vacková I., Engelová M., Marinov I., Tománek M. Cell cycle synchronization of porcine granulosa cells in G1 stage with mimosine // Anim Reprod Sci. 2003. Vol. 77, N 3-4. P. 235–245. doi: 10.1016/s0378-4320(03)00034-4
94. Alessi F., Quarta S., Savio M., et al. The cyclin-dependent kinase inhibitors olomoucine and roscovitine arrest human fibroblasts in G1 phase by specific inhibition of CDK2 kinase activity // Exp Cell Res. 1998. Vol. 245, N 1. P. 8–18. doi: 10.1006/excr.1998.4216
95. Banfalvi G. Erratum // Methods Mol Biol. 2017. Vol. 1524. P. E1. doi: 10.1007/978-1-4939-6603-5_22
96. Zhang M., Zhang L., Hei R., et al. CDK inhibitors in cancer therapy, an overview of recent development // Am J Cancer Res. 2021. Vol. 11, N 5. P. 1913–1935.
97. Cuyàs E., Corominas-Faja B., Joven J., et al. Cell cycle regulation by the nutrient-sensing mammalian target of rapamycin (mTOR) pathway // Methods Mol Biol. 2014. Vol. 1170. P. 113–144. doi: 10.1007/978-1-4939-0888-2_7
98. Rubinsztein D.C., Mariño G., Kroemer G. Autophagy and aging // Cell. 2011. Vol. 146, N 5. P. 682–695. doi: 10.1016/j.cell.2011.07.030
99. Lee J., Park J.I., Yun J.I., et al. Rapamycin treatment during in vitro maturation of oocytes improves embryonic development after parthenogenesis and somatic cell nuclear transfer in pigs // J Vet Sci. 2015. Vol. 16, N 3. P. 373–380. doi: 10.4142/jvs.2015.16.3.373
100. Albers M.W., Williams R.T., Brown E.J., et al. FKBP-rapamycin inhibits a cyclin-dependent kinase activity and a cyclin D1-Cdk association in early G1 of an osteosarcoma cell line // J Biol Chem. 1993. Vol. 268, N 30. P. 22825–22829. doi: 10.1016/S0021-9258(18)41602-X
101. Han J., Yan H. Inhibitory effect of rapamycin on the proliferation of Rhesus retinal vascular endothelial cells // Chinese Journal of Experimental Ophthalmology. 2014. N 3. P. 216–219.
102. Jiang F., Wang Y., Du S., et al. Rapamycin prevents retinal neovascularization by downregulation of cyclin D1 in a mouse model of oxygen-induced retinopathy // BMC Ophthalmol. 2020. Vol. 20, N 1. P. 44. doi: 10.1186/s12886-020-1325-5
103. Kitagawa M., Okabe T., Ogino H., et al. Butyrolactone I, a selective inhibitor of CDK2 and CDC2 kinase // Oncogene. 1993. Vol. 8, N 9. P. 2425–2432.
104. Liu F., Ma X.H., Ule J., et al. Regulation of cyclin-dependent kinase 5 and casein kinase 1 by metabotropic glutamate receptors // Proc Natl Acad Sci USA. 2001. Vol. 98, N 20. P. 11062–11068. doi: 10.1073/pnas.191353898
105. Wada M., Hosotani R., Lee J.U., et al. An exogenous CDK inhibitor, butyrolactone-I, induces apoptosis with increased Bax/Bcl-2 ratio in p53-mutated pancreatic cancer cells // Anticancer Res. 1998. Vol. 18, N 4A. P. 2559–2566.
106. Ghfar A.A., El-Metwally M.M., Shaaban M., et al. Production of terretonin N and butyrolactone I by Thermophilic Aspergillus terreus TM8 promoted apoptosis and cell death in human prostate and ovarian cancer cells // Molecules. 2021. Vol. 26, N 9. P. 2816. doi: 10.3390/molecules26092816
107. Monaco E.A. 3rd, Beaman-Hall C.M., Mathur A., Vallano M.L. Roscovitine, olomoucine, purvalanol: Inducers of apoptosis in maturing cerebellar granule neurons // Biochem Pharmacol. 2004. Vol. 67, N 10. P. 1947–1964. doi: 10.1016/j.bcp.2004.02.007
108. Krude T. Mimosine arrests proliferating human cells before onset of DNA replication in a dose-dependent manner // Exp Cell Res. 1999. Vol. 247, N 1. P. 148–159. doi: 10.1006/excr.1998.4342
109. Galgano P.J., Schildkraut C.L. G1/S phase synchronization using mimosine arrest // CSH Protoc. 2006. Vol. 2006, N 4. P. pdb.prot4488. doi: 10.1101/pdb.prot4488
110. Jackman J., O'Connor P.M. Methods for synchronizing cells at specific stages of the cell cycle // Curr Protoc Cell Biol. 1998. Chapter 8, Unit 8.3. doi: 10.1002/0471143030.cb0803s00
111. Kim E., Zheng Z., Jeon Y., et al. An improved system for generation of diploid cloned porcine embryos using induced pluripotent stem cells synchronized to metaphase // PLoS One. 2016. Vol. 11, N 7. P. e0160289. doi: 10.1371/journal.pone.0160289
112. Ho Y.S., Duh J.S., Jeng J.H., et al. Griseofulvin potentiates antitumorigenesis effects of nocodazole through induction of apoptosis and G2/M cell cycle arrest in human colorectal cancer cells // Int J Cancer. 2001. Vol. 91, N 3. P. 393–401. doi: 10.1002/1097-0215(200002)9999:9999<::aid-ijc1070>3.0.co;2-#

AUTHOR'S INFO

Anastasia S. Zhukova, Cand. Sci. (Biology);
address: 60 Dubrovitsy, 142132 Podolsk, Russia;
ORCID: 0000-0003-1155-014X;
eLibrary SPIN: 4590-3971;
e-mail: anastasia.s.belyaeva@gmail.com

ОБ АВТОРЕ

Жукова Анастасия Сергеевна, канд. биол. наук;
адрес: Россия, 142132, Подольск, пос. Дубровицы, д. 60;
ORCID: 0000-0003-1155-014X;
eLibrary SPIN: 4590-3971;
e-mail: anastasia.s.belyaeva@gmail.com