ISSN 1062-3604, Russian Journal of Developmental Biology, 2018, Vol. 49, No. 6, pp. 356–361. © Pleiades Publishing, Inc., 2018.
Original Russian Text © Yu.Yu. Silaeva, Yu.K. Kirikovich, L.N. Skuratovskaya, A.V. Deikin, 2018, published in Ontogenez, 2018, Vol. 49, No. 6, pp. 352–358.

DEVELOPMENTAL BIOLOGY OF MAMMALS

Optimal Number of Embryos for Transplantation in Obtaining Genetic-Modified Mice and Goats

Yu. Yu. Silaeva[a], Yu. K. Kirikovich[c], L. N. Skuratovskaya[b], and A. V. Deikin[a, b, *]

[a] *Gene Biology Institute, Russian Academy of Sciences, Moscow, Russia*
[b] *Research Institute of General Pathology and Pathophysiology, Moscow, Russia*
[c] *Scientific and Practical Center for Animal Breeding, Zhodino, Republic of Belarus*
**e-mail: deikin@igb.ac.ru*

Received December 11, 2017; in final form, June 27, 2018

Abstract—The technology of creating genetically modified animals (placental mammals) by microinjection into the pronucleus of a fertilized egg suggests, as one of the key stages, the transplantation of early embryos into female recipients. However, there is a wide range of opinions among researchers about the optimal number of embryos to be transferred to the female recipient. Thus, data on transplantation of 20–60 mouse embryos and from 2 to 6 goat embryos to one recipient are given in the methodological literature and experimental articles devoted to the method of creating genetically modified animals. Thus, the standard recommendation is the transfer of a much larger number of embryos than that which develops in animals of both species in physiological pregnancy. At the same time, technology of transplantation of bovine embryos (cattle) involves the transfer of one embryo, which is the physiological norm for this species of animals. Clinical protocols of assisted reproductive technologies for the transplantation of human embryos also recommend the transfer of one embryo, because transferring the number of embryos greater than in physiological pregnancy leads to increased risks. In our work, we analyze the results of experiments on obtaining genetically modified mice and goats and provide data indicating the need to revise the standard recommendations on the number of transferred embryos downward. We believe that the number of transferred embryos should not exceed the number of embryos characteristic for physiological pregnancy. Excess of the number of transplanted embryos leads to a pathological course of pregnancy and a significant decrease in overall performance.

Keywords: genetically modified animals, mice, goats, embryo transplantation, pregnancy pathology
DOI: 10.1134/S106236041806005X

INTRODUCTION

Embryo transplantation is one of the key steps in the production of transgenic mice and goats using various methods of genome modification. However, the development of this technology is mainly focused on reproductive programs in humans or large farm animals. Accordingly, in medicine and veterinary medicine, standards have been adopted that allow one to minimize the risks of the procedure and ensure the most effective results. The general principle of technology for human (Zander-Fox et al., 2011; Pandian et al., 2013) and large farm animals (Hasler, 2014; Scherzer et al., 2008) is a strict ban on transplantation of embryos in an amount greater than the physiological norm. Thus, the number of transplanted embryos for cattle is strictly limited to 1–2. In recent years, more and more people tend to transplant a single embryo in human reproductive programs. In the work on obtaining transgenic mice and goats today, there are no clear methodological instructions on the number of transplanted embryos, and, accordingly, there is no generally accepted understanding of the most effective practice. A different number of transplanted embryos is recommended in different methodological guidelines: 40 (20 per each uterine horn) (Voncken, 2011), 30–60 (15–30 per each uterine horn) (Ittner and Götz, 2007), 30–40 (15–20 per each uterine horn) (Cho et al., 2009), 20–30 (10–15 per each uterine horn) (Damert and Kusserow, 2003), 30 (15 per each uterine horn) (Kadulin et al., 2006).

In the articles on the creation of transgenic animals, the transplantation of 20 (Rodriguez et al., 1995), 28 (Niavarani et al., 2005), 28 (Lisauskas et al., 2008), 25 (Hansson et al., 1994) mice embryos and 5–6 (Amiri Yekta et al., 2013), 3.8 (Zhang et al., 2008), 6.2 (Baldassarre et al., 2003), 6.6 (Freitas et al., 2012; Batista et al., 2014), 2.4 (Yu et al., 2012, 2013) of goat embryos is reported. Thus, it is recommended in all studies to transplant the number of embryos that significantly exceed the number of embryos developing in physiological pregnancy in animals of both species. Thus, the physiological norm for a house mouse (*Mus musculus*) is 2–10 cubs in a litter, usually 5–6

(Sokolov, 1989), and that for goats (*Capra hircus*) is 1–2, rarely three baby goats in a litter (Sokolov, 1989).

The purpose of this work was to systematize and analyze the results obtained in the work on transplantation of goat and mouse embryos when implementing projects for obtaining transgenic animals and determining the optimal number of embryos to obtain the most effective result as a whole.

MATERIALS AND METHODS

Accommodation conditions of animals. Mice of hybrid line F1 (CBAXC57BL/6) (Stolbovaya nursery) were used in the work. In the process of the experimental work, the mice were kept in the vivarium of the Center for Shared Use of the Gene Biology Institute (Russian Academy of Sciences). The vivarium was equipped with equipment that allows one to monitor the air temperature, adjust the light cycle (12/12), and perform supply and exhaust air ventilation. Mice were kept in conditions of constant access to water and fodder (water from a centralized source of water supply, mixed feed was fully extruded (Laboratorkorm, Russia)). Litter in cages, dry fine wood sawdust (Laboratorkorm, Russia), was changed once a week. Cells and water bottles were treated once a week with sterilizing solutions.

Work with goats was carried out on the basis of the Laboratory of Reproduction, Transplantation of Embryos and Transgenesis of Animals in the Biotechnological Research and Experimental Production for Transgenesis of Animals (Budagovo village), Minsk region, Scientific and Practical Center of the National Academy of Sciences of Belarus for Animal Breeding.

Feeding and maintenance of goats was carried out in accordance with the norms of the USSR All-Union Academy of Agricultural Sciences (1985).

Solutions and media. M2 medium (Sigma-Aldrich, United States) and BWWH-5 or Hepes-KSOM media or DPBS medium with the addition of 0.1 mg/mL bovine serum albumin were used for washing out murine and goat oocytes respectively. M16 (Sigma-Aldrich, United States) and BWW-5 or KSOM or Bovihold (Minitube, Germany) media were used for the cultivation of mouse and goat oocytes respectively. The cultivation was carried out with mineral oil (Sigma, United States).

Anesthesia. To immobilize and anesthetize mice, avertine, which is a 2.5% aqueous solution of 1 g of 2,2,2-tribromoethanol (Aldrich, United States) in 1 mL of 2-methyl-2-butanol (Sigma-Aldrich, United States) was used. Avertin was administered intraperitoneally at a rate of 15 μL per gram of animal weight. In a number of experiments, a mixture of Zolilet 100 (VIRBAC, France) and Romethar 20 mg/mL (Bioveta, a. s., Czech Republic) were used to immobilize and anesthetize animals. To prepare the mixture, 0.5 mL of Zoletil 100 was mixed with 0.25 mL of Romethar 20 mg/mL and adjusted to a total volume of 10 mL with physiological solution. The Zoletil-Romethar mixture was also administered intraperitoneally at a rate of 6 μL per gram of animal weight.

Narcotic drugs using a solution of xylazine (Xyla) at a rate of 0.15 mL/kg of live weight were injected to anesthetize goats 1–1.5 h before the operation. Before the administration of xylazine, the premedication of the animal was performed by one of the following preparations: 1% solution of atropine sulfate (subcutaneously) or 2.5% solution of aminazine at a dose of 1 mL per 25 kg of animal weight. Beginning of sensitivity loss (with pricking in various parts of the body of the animal) and relaxation of the muscles (the animal lies down) was noted.

The protocols for the induction of polyovulation, the production of fertilized eggs in the pronuclei stage in mice and goats, and the procedures for the preparation of vasectomized males of recipient animals were published by us earlier (Zvezdova et al., 2010; Goldman et al., 2012).

Microinjection. Microinjections were performed in HEPES-KSOM medium under a Zeiss Axiovert 200M microscope at a magnification of 400×–600× using Narishige micromanipulators. A Sutter Instrument Co-P-97 (United States) was used to make microinjection needles, Narishige PC-10 puller and Narishige MF-900 microforge were used for the production of retention pipette. The concentration of genetic constructs was 1 ng/μL.

Transplantation of oocytes into the oviduct of the pseudo-pregnant recipient. We earlier described the transplantation of microinjected zygotes to pseudo-pregnant recipients (Zvezdova et al., 2010; Goldman et al., 2012). The operation was performed on the right and left oviducts.

Transplantation of embryos to goats was performed surgically 24–48 h after microinjection of recombinant DNA or for intact embryos on the seventh day after detection of goat recipients in the hunt.

All procedures with animals performed in studies corresponded to the ethical standards of the institution where the studies were conducted and to the approved legal acts of the Russian Federation and international organizations.

RESULTS AND DISCUSSION

We performed an analysis of the efficiency of embryo transplantation in the works on obtaining transgenic animals using 1106 recipient mice and 113 goat recipients as an example (Gursky et al., 2009; Deykin et al., 2009; Zvezdova et al., 2010; Goldman et al., 2012; Shelkovnikova et al., 2013; Silaeva et al., 2013, 2014; Deĭkin et al., 2014; Robinson et al., 2015; Gurskiy et al., 2016). In total, 12479 mouse embryos and 281 goat embryos were transplanted. Pregnancy occurred in 28% of the recipients used. In the course

Table 1. Results of the experiment on transplantation of microinjected embryos

Cells transplanted	Recipients used	Pregnant recipients	Implanted embryos by the end of 2/3 of pregnancy	Received cubs
		Mice		
12479	1106	307 (28%)	No data	713 (no data)
		Goats		
281	113	32 (28%)	37 (46%**)	33 (89%***)

** % of the oocyte number transplanted to pregnant recipients.
*** % of implanted embryos.

of the work, 713 mice and 33 baby goats were born. General data on the scope of work are presented in Table 1. We suppose that the amount of data obtained during the experiments is sufficient for carrying out a statistical analysis of the efficiency of transplantation in goats and mice.

Determination of the optimal number of embryos for transplantation. To determine the optimal number of embryos for transplantation, an efficiency coefficient calculated as the number of born calves/number of transplanted embryos was introduced. The number of recipients used is not taken into account in the formula for calculating the efficiency coefficient, since the most laborious and critical stages of the work are the production of microinjected eggs and their transplantation to pseudo-pregnant recipients. At the same time, the preparation of pseudo-pregnant recipients is much less complex, and their number can easily be increased by increasing the population of experimental animals. When calculating the effectiveness of transplantation, animals in which pregnancy did not occur were excluded from the sample, since the lack of pregnancy can be due to the condition of the recipient in this case.

Figures 1 and 2 show the graphs of the change in the coefficient of transplantation efficiency with an increase in the number of transplanted embryos. As can be seen from Fig. 1, the maximum efficiency of transplantation in mice is achieved with the transfer of a small number of oocytes—no more than 11 to one recipient (which corresponds to the physiological norm of the number of developing embryos for the *Mus musculus* species (Sokolov, 1989). Further increase in the number of transferred eggs does not lead to an increase in the efficiency coefficient; moreover, with a significant increase in the number of transferred embryos (more than 20 eggs per recipient), the coefficient of transplantation efficiency decreases. Thus, when the amount of embryos used in the transplantation is close to the physiological one, the efficiency ratio exceeds 30% and is significantly different from that in excess of physiological norm by two or more times.

Apparently, this means that the number of born mice after transplantation of microinjected eggs is determined not only by the viability of the eggs themselves but by the ability of the mother's organism to tolerate a certain number of fetuses. The results obtained by us testify that the transplantation of the

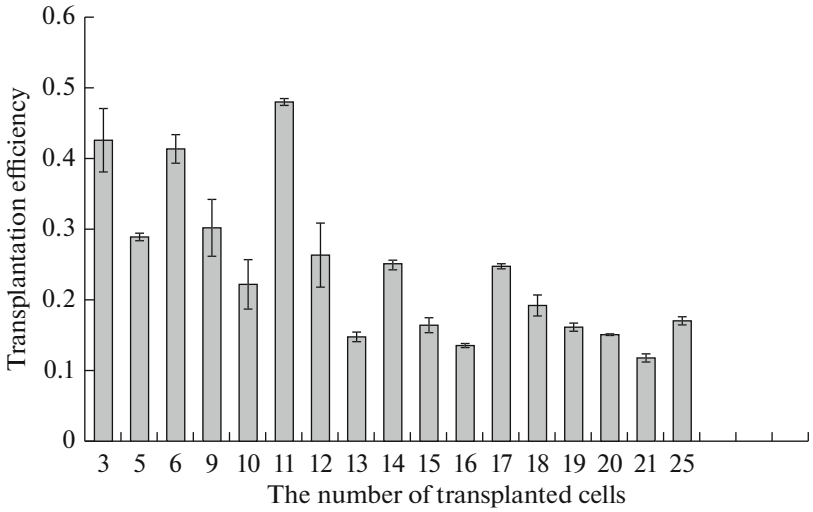

Fig. 1. Dependence of transplantation efficiency on the number of transplanted embryos in mice.

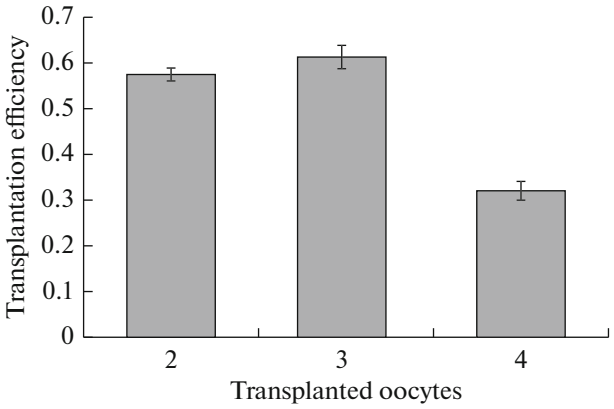

Fig. 2. Dependence of transplantation efficiency on the number of transplanted embryos in goats. The mean coefficient of transplant efficiency for transplantation of 2–3 embryos is significantly higher than that for 4 embryos ($p = 0.05$).

number of microinjected eggs significantly exceeding the physiological norm for mice leads to unjustified losses of embryos at early stages of development.

Similar results were obtained in experiments on the transplantation of microinjected zygotes to goats-recipients (Fig. 2): transplantation efficiency does not differ in the case of transplantation of two and three microinjected eggs and significantly decreases with a double increase in the number of transferred eggs compared to the physiological norm for current species (Sokolov, 1989).

Study of the effect of an increase in the number of transferred embryos on the course of pregnancy. In view of the possible toxicity of genetic constructs, there is a question concerning the need to increase the number of embryos due to their possible death as a result of unsuccessful modification of the genome. In order to investigate the effect of increasing the number of embryos during the course of pregnancy, a control experiment on transplantation was conducted. In this experiment, ten intact fertilized eggs were transferred to pseudo-pregnant recipients into each horn of the uterus (i.e., an amount increasing the physiological norm for *Mus musculus* by two times). Mice were dissected on day 14 after transfer and the relative number of implanted embryos was determined. The results of the control experiment are shown in Table 2. It can be seen from Table 2 that, when the double amount of embryos compared with physiological norm are transplanted into one horn of the uterus, by the 14th day of pregnancy, a high embryo implantation rate is recorded in the mouse, on average 76% of the embryo transplanted, and a high level of embryonic death, so that only 63% of implanted embryos are alive by day 14, which corresponds to the physiological norm and represents 4.8 embryos per horn of the uterus on average. In this case, pregnancy occurs against a background of a large number of decaying fetuses, which leads to abortions of a significant part of pregnancies.

The data from a similar experiment conducted on goats are represented in Table 2. It turned out that, with a double increase (compared to the physiological norm) in the number of embryos transferred in a given species of animals, the loss of a part of the embryos and the birth of baby goats in accordance with the physiological norm of 1.25 baby goats per goat is observed.

Thus, the results obtained in the control experiment disprove the theory of the need to increase the number of transferred embryos due to the possible toxicity of the genetic construct (Hansson et al., 1994; Rodriguez et al., 1995; Baldassarre et al., 2003; Niavarani et al., 2005; Lisauskas et al., 2008; Zhang et al., 2008; Freitas et al., 2012; Yu et al., 2012, 2013; Amiri Yekta et al., 2013; Batista et al., 2014), because "extra" embryos die after the middle part of pregnancy, and an increase in the number of transplanted embryos does not lead to an increase in the number of animals born. In addition, the results obtained during the control experiment are completely consistent with the results obtained during the experiments on the transplantation of microinjected eggs: an increase in the number of eggs transferred does not lead to an increase in the efficiency of transplantation and the birth of a larger number of calves but, on the contrary, decreases the efficiency of work. Based on the results obtained, we believe that, when working with toxic genetic constructs, it is necessary to reduce the number of transferred eggs in comparison with the physiological norm

Table 2. Results of the control experiment on the transplantation of intact embryos

Cells transplanted	Recipients used *	Pregnant recipients by the end of 2/3 of pregnancy	Implanted embryos by the end of 2/3 of pregnancy	Live embryos by the end of 2/3 of pregnancy
		Mice		
110	11	7 (63%)	53 (76%**)	34 (63%***)
		Goats		
84	42	24 (57.1%)	34 (70.8%**)	30 (88.2%***)

* Transplantation of ten zygotes in mice and two zygotes in goats in one horn of the uterus.
** % from the oocyte number transplanted to pregnant recipients.
*** % of implanted embryos.

in order to minimize the ratio between dead and living embryos and thereby increase the chances of surviving embryos for full development.

Thus, regardless of the specific characteristics of the recipient in the work on transplantation of embryos in the production of transgenic animals, it is necessary to focus on the physiological norm for the number of calves/implanting embryos. It should also be taken into account that 1.3 babies per goat is a norm for goats, and there are 4–6 calves at the first birth in C57BL6xCBA hybrid line mice. The average number of mice of approximately ten heads can be obtained from the females of the CD1 line at repeated births and further.

The transplantation of more embryos than the physiological norm results in abortions against the background of a large number of implanted embryos. Expecting the effectiveness of works on obtaining genetically modified animals, it is necessary to take into account that the stage of microinjection is the most traumatic for the future organism. Obtaining eggs, their genetic modification, and cultivation are the most costly stages of the work, requiring the involvement of high-tech equipment and specialists and occupying the greatest working time (Maksimenko et al., 2013). Transplantation, which is a more utilitarian process, well developed in solving medical and veterinary problems of reproduction, sharply reduces the overall performance in this case due to unreasonable overestimation of the number of transplanted embryos. We assume that the use of physiological quantities of transplanted embryos (1 per horn of the uterus in goats and 3–5 per horn of the uterus in mice) will allow to avoid pregnancy pathologies and increase the yield of calves from the number of transplanted zygotes and, hence, the number of genetically modified primary transgenic animals obtained in the framework of one series of experiments. As with the implementation of reproductive programs in humans and farm animals, special attention should be given to the quality of transplanted embryos and an individual approach to work with them. Transplantation of deliberately non-viable embryos will only distort statistics, and the artificial creation of competition between viable embryos will increase the risk of pregnancy and abortion pathologies.

ACKNOWLEDGMENTS

This work was performed with support from the Russian Science Foundation, project no. 16-14-00150 (2470 mouse embryos were transplanted into 269 recipients, 277 calves were obtained. The results of transplantation and statistical processing were systematized).

The work was conducted using equipment of the Center for Shared Use of the Gene Biology Institute of the Russian Academy of Sciences.

We would like to thank A.I. Budevich and I.L. Goldman for invaluable assistance in mastering the technology of creating genetically modified animals.

REFERENCES

Amiri Yekta, A., Dalman, A. Eftekhari-Yazdi, P., et al., Production of transgenic goats expressing human coagulation factor IX in the mammary glands after nuclear transfer using transfected fetal fibroblast cells, *Transgenic Res.*, 2013, vol. 22, no. 1, pp. 131–142.

Baldassarre, H., Wang, B., Kafidi, N., et al., Production of transgenic goats by pronuclear microinjection of in vitro produced zygotes derived from oocytes recovered by laparoscopy, *Theriogenology*, 2003, vol. 59, nos. 3–4, pp. 831–839.

Batista, R., Melo, C., Souza-Fabjan, J., Teixeira, D., et al., Phenotypic features of first-generation transgenic goats for human granulocyte-colony stimulation factor production in milk, *Biotechnol. Lett.*, 2014, vol. 36, no. 11, pp. 2155–2162.

Cho, A., Haruyama, N., and Kulkarni, A., *Generation of Transgenic Mice*, Current Protocols in Cell Biology [Internet], Hoboken, NJ, USA: John Wiley and Sons, Inc., 2009.

Damert, A. and Kusserow, H., Generation of transgenic mice by pronuclear injection, in *Blood–Brain Barrier*, New Jersey: Humana Press, 2003, pp. 513–528.

Deĭkin, A.V., Kovrazhkina, E.A., Ovchinnikov, R.K., et al., A mice model of amyotrophic lateral sclerosis expressing mutant human FUS protein, *Zh. Nevrol. Psikhiatr. im. S.S. Korsakova*, 2014, vol. 114, no. 8, pp. 62–69.

Deykin, A.V., Ermolkevich, T.G., Gursky, Y.G., et al., The state of health and the reproductive potential of transgenic mice secreting recombinant human lactoferrin in milk, *Dokl. Biochem. Biophys.*, 2009, vol. 427, pp. 195–198.

Freitas, V., Serova, I., Moura, R., et al., The establishment of two transgenic goat lines for mammary gland hG-CSF expression, *Small Rumin. Res.*, 2012, vol. 105, nos. 1–3, pp. 105–113.

Goldman, I., Georgieva, S., Gurskiy, Y., et al., New opportunities of using transgenic milk animals for pharmaceutical human protein production, *Transgenic Res.*, 2012a, vol. 21, no. 4, p. 923.

Goldman, I., Georgieva, S., Gurskiy, Y., et al., Production of human lactoferrin in animal milk, *Biochem. Cell Biol.*, 2012b, vol. 90, no. 3, pp. 513–519.

Gurskiy, Y., Garbuz, D., Soshnikova, N., et al., The development of modified human Hsp70 (HSPA1A) and its production in the milk of transgenic mice, *Cell Stress Chaperones*, 2016, vol. 21, no. 6, pp. 1055–1064.

Gursky, Y., Bibilashvili, R., Minashkin, M., et al., Expression of full-length human pro-urokinase in mammary glands of transgenic mice, *Transgenic Res.*, 2009, vol. 18, no. 5, pp. 747–756.

Hansson, L., Edlund, M., Edlund, A., et al., Expression and characterization of biologically active human extracellular superoxide dismutase in milk of transgenic mice, *J. Biol. Chem.*, 1994, vol. 269, no. 7, pp. 5358–5363.

Hasler, J., Forty years of embryo transfer in cattle: a review focusing on the journal theriogenology, the growth of

the industry in North America, and personal reminisces, *Theriogenology*, 2014, vol. 81, no. 1, pp. 152–169.

Ittner, L. and Götz, J., Pronuclear injection for the production of transgenic mice, *Nat. Protoc.*, 2007, vol. 2, no. 5, pp. 1206–1215.

Kadulin, S., Ermolkevich, T., and Andreeva, L., Analysis of transfer of microinjected zygotes in production of transgenic mice, *Russ. J. Dev. Biol.*, 2006, vol. 37, no. 2, pp. 85–89.

Lisauskas, S., Cunha, N., Vianna, G., et al., Expression of functional recombinant human factor ix in milk of mice, *Biotechnol. Lett.*, 2008, vol. 30, no. 12, pp. 2063–2069.

Maksimenko, O.G., Deykin, A.V., Khodarovich, Y.M., et al., Use of transgenic animals in biotechnology: prospects and problems, *Acta Naturae*, 2013, vol. 5, no. 1, pp. 33–46.

Niavarani, A., Dehghanizadeh, S., Zeinali, S., et al., Development of transgenic mice expressing calcitonin as a beta-lactoglobulin fusion protein in mammary gland, *Transgenic Res.*, 2005, vol. 14, no. 5, pp. 719–727.

Pandian, Z., Marjoribanks, J., Ozturk, O., et al., *Number of Embryos for Transfer Following in vitro Fertilisation or Intra-Cytoplasmic Sperm Injection*, Cochrane Database of Systematic Reviews, Chichester: UK: John Wiley and Sons, Ltd., 2013.

Robinson, H.K., Deykin, A.V., Bronovitsky, E.V., et al., Early lethality and neuronal proteinopathy in mice expressing cytoplasm-targeted FUS that lacks the RNA recognition motif, *Amyotroph. Lateral. Scler. Front. Degener.*, 2015, vol. 16, nos. 5–6, pp. 402–409.

Rodriguez, A., Castro, F.O., Aguilar, A., et al., Expression of active human erythropoietin in the mammary gland of lactating transgenic mice and rabbits, *Biol. Res.*, 1995, vol. 28, no. 2, pp. 141–153.

Scherzer, J., Fayrer-Hosken, R., Ray, L., et al., Advancements in large animal embryo transfer and related biotechnologies, *Reprod. Domest. Anim.*, 2008, vol. 43, no. 3, pp. 371–376.

Shelkovnikova, T.A., Peters, O.M., Deykin, A.V., et al., Fused in sarcoma (FUS) protein lacking nuclear localization signal (NLS) and major RNA binding motifs triggers proteinopathy and severe motor phenotype in transgenic mice, *J. Biol. Chem.*, 2013, vol. 288, no. 35, pp. 25266–25274.

Silaeva, Y.Y., Kalinina, A.A., Vagida, M.S., et al., Decrease in pool of T lymphocytes with surface phenotypes of effector and central memory cells under influence of TCR transgenic β-chain expression, *Biochemistry* (Moscow), 2013, vol. 78, no. 5, pp. 549–559.

Silaeva, Y.Y., Grinenko, T.S., Vagida, M.S., et al., Immune selection of tumor cells in TCR β-chain transgenic mice, *J. Immunotoxicol.*, 2014, vol. 11, no. 4, pp. 393–399.

Sokolov, V.E., *Zhizn zhivotnykh* (Life of Animals), Moscow: Prosveshchenie, 1989, vol. 7.

Voncken, J.W., Genetic modification of the mouse: general technology—pronuclear and blastocyst injection, in *Transgenic Mouse Methods and Protocols*, Totowa, NJ: Humana Press, 2011, pp. 11–36.

Yu, H., Chen, J., Sun, W., et al., The dominant expression of functional human lactoferrin in transgenic cloned goats using a hybrid lactoferrin expression construct, *J. Biotechnol.*, 2012, vol. 161, no. 3, pp. 198–205.

Yu, H., Chen, J., Liu, S., et al., Large-scale production of functional human lysozyme in transgenic cloned goats, *J. Biotechnol.*, 2013, vol. 168, no. 4, pp. 676–683.

Zander-Fox, D.L., Tremellen, K., and Lane, M., Single blastocyst embryo transfer maintains comparable pregnancy rates to double cleavage-stage embryo transfer but results in healthier pregnancy outcomes: the benefits of single blastocyst transfer, *Aust. N. Z. J. Obstet. Gynaecol.*, 2011, vol. 51, no. 5, pp. 406–410.

Zhang, J., Li, L., Cai, Y., et al., Expression of active recombinant human lactoferrin in the milk of transgenic goats, *Protein Expr. Purif.*, 2008, vol. 57, no. 2, pp. 127–135.

Zvezdova, E.S., Silaeva, Y.Y., Vagida, M.S., et al., Generation of transgenic animals expressing the α and β chains of the autoreactive t-cell receptor, *Mol. Biol.*, 2010, vol. 44, no. 2, p. 277.

Translated by P. Kuchina