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Efficacy of blastodermal cells and CRISPR/CAS9 method in the creation of transgenic duck *(Anas Platyrhynchos)*

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The aim of the work was to develop a methodology for the creation of transgenic chimeras of ducks by using donor blastodermal cells after transfection with DNA vector and Lipofectamine 2000® (Invitrogen, USA). The CRISPR/Cas9 system with homology directed repair (HDR) was used to edit the target site of the duck genome. Materials and research methods. Transgenic duck chimeras were created using donor blastodermal cells after transfection with plasmid DNA and Lipofectamine 2000. To edit the target region of the duck genome, we used the CRISPR / cas9 system with HDR. The EGFP reporter gene was used as the transgene. Conclusions. *Среди выжившиx фертильных животных было 13/20 животных G0 (65 %): 10/12 (83,3 %)* Of the 200 eggs, in which the transfected blastodermal cells were introduced, 20 offspring were obtained, including 8 males and 12 females. Thus, the survival of embryos was 10 %. Among the surviving fertile animals, 13/20 were animals G0 (65 %): 10/12 (83.3 %) females and 3/8 (37.5 %) males. The procedure of obtaining chimeras has a stronger effect on the survival and fertility of male chimeras. From 13 of 20 birds G0, we received a total of 197 offspring (including 117 (59.4 %) daughters and 80 (40.6 %) sons), 59 of which were EGFP-

positive (30.3 %), including 10 males (16.9 %) and 49 females (83.1 %). The technique used by us can be successfully applied in further researches and for creation of a transgenic duck.

Keywords: CRISPR/Cas9,EGFP,Transgenic Duck, Blastodermal Cells, chimera

Introduction

Transgenesis has now become a routine procedure that allows obtaining uniquely valuable pharmaceutical producing animals [1–3], models of evolution[4, 5] and hereditary diseases [6], and a valuable animal protein for human nutrition [7].

A transgenic animal is defined as an animal that has a transgene stably incorporated into its germline and is able to transmit the transgene to its offspring [8]. To obtain a transgenic animal, it is necessary to edit the zygote genome. However, the strategy used for mammals [9] was not successful when editing the avian genome because of the differences in the reproductive system and embryo development *in ovo* [10].

The bird has unique economically valuable features consisting of reduced resource costs and short time from the start of the experiment up to obtaining transgenic birds. Due to the compact size of birds, control and zootechnical manipulations (artificial insemination, feeding, egg collection, *etc.*) do not require highly skilled labor and most of the technological processes of growing and keeping the birds are automated. Short interval between generations (6–7 months), speed of reproduction (up to 200 ducklings from one duck per year) and high feed conversion rate are considerably superior to other farm animals [11]. The 54 % of egg white in its chemical composition is represented by ovalbumin [1, 12], which allows more efficient purification of recombinant proteins after transgenesis, while the glycosylation profile close to that of humans, makes it possible to obtain the proteins toxic for mammals, so the bird egg is considered to be the best model for the production of recombinant proteins [13–15]. Transgenic EGFP construct had no effect on duck egg productivity [16].

Three methods are mostly used to obtain transgenic birds: 1) transfection of the DNA vector with sperm [17, 18]; 2) DNA injections into the embryonic cavity of a newly laid egg [19–21]; 3) introduction of donor cells [22–24]. Certain complications have been established in the implementation of the transgene with the introduction of DNA vector using the method of transfection with sperm [17].

In a freshly incubated egg — stage X according to Eyal-Giladi and Kochav, 1976 (EGK-X), the embryo has developed up to 50000-60000 cells [25], therefore, methods of obtaining transgenic bird chimeras have become more widespread in poultry [11], using blastodermal cells produced from non incubated eggs (EGK-X) and PGCs — primordial germ cells — primary reproductive cells produced from blood of 2.5-3-day-old embryos (stages 13–17 according to Hamburger and Hamilton 1951); and from the gonads of 5–7-day-old embryos (stages 26–31) [26]. It has been shown that the blastodermal cells freshly isolated from the EGK-X stage chicken embryos can contribute to all somatic tissues as well as to the germline after injection

of the EGK-X recipient embryos into the subgerminal cavity [27–31].

The use of primordial germ cells (PGC) in chickens and quail is considered to be the most effective method for transferring genetic information to the next generation [32, 33]. Moreover, the methods mediated by PGC require a lot of time, expensive equipment for PGC selection and cultivation, as well as highly qualified laboratory staff.

After the first transgenic chicken was created using a viral vector, various approaches to obtaining genetically modified chicken were tested [11].

Currently, the CRISPR/Cas9 genome editing system is considered to be an advanced technology for avian transgenesis [10, 34], since this system is more specific and versatile compared to other site-specific nucleases such as ZFNs (Zinc-finger nucleases) and TALENs (Transcription activator-like effector nuclease) [35, 36]. To create double-strand DNA breaks (DSB) in the target genome site using the CRISPR/Cas9 system, only the sequence of 20-nucleotide guiding sgRNA should be changed, whereas the construction of ZFNs and TALENs is labor intensive and their specificity is lower, ZFN: from 5 to 7 bp, and TALEN: from 12 to 20 bp [37, 38]. Double-strand DNA breaks (DSB) stimulate the mechanisms of cellular DNA repair, including non-homologous end joining (NHEJ) and homology directed repair (HDR) [39] when the donor DNA is injected together with CRISPR/Cas9 [40].

The main part of research on the creation of transgenic birds was done on chickens and quails [41–43, 22, 13]. However, the technology of creating transgenic ducks using blastodermal cells is hampered by the peculiarities of the waterfowl's eggs shell, which contains wide pores [44] and provides ways for contamination of the embryo by bacterial infections.

Therefore, the aim of the work was to develop a methodology for the creation of transgenic chimeras of ducks by using donor blastodermal cells after transfection with DNA vector with the help of Lipofectamine 2000. The CRISPR/Cas9 system with homology directed repair (HDR) was used to edit the target site of the duck genome. The EGFP reporter gene was used as a transgene [45, 46].

Materials and Research Methods

Research bird. The study was conducted on Shanma and Shaoxing poultry. The poultry were kept in breeding facilities of Zhejiang Guowei Technology Co. LTD (Zhuji, China), which is a research platform of the Institute of Animal Husbandry and Veterinary Medicine of the Zhejiang Academy of Agricultural Sciences (Hangzhou, China).

All experiments with animals were carried out in accordance with the provisions of the European Convention on the Protection of Spine Animals used for research and other scientific purposes. The experiment was conducted in three stages during 2016–2018s. The birds were kept in individual cages in the vivarium. The first stage began in February 2016 and included transgene injection, egg incubation, and identification of offspring (G0) with wing markers (Fig. 1). The second stage began in March 2017, the samples (blood, feathers, sperm, biopsy sample) were taken from the birds for DNA isolation and identification of the presence of the transgene by PCR method,

as well as a mating campaign was conducted to determine the transfer of the transgene to offspring, the G1 descendants were received. The third stage began in March 2018 and included blood sampling from the offspring (G1), transgene determination.

Transgenic construction design. Integration of the EGFP reporter gene into the duck genome was performed using homology directed repair after double-strand breaks (DSBs) by the Cas9 system. The plasmid containing the Cas9 gene was obtained from Addgene (http://www.addgene.org/42230/). The plasmids encoding RNA guides (pBR322-sgRNA1, pBR322-sgRNA2) and the plasmid with the EGFP reporter gene and homologous regions of the genomic locus (pBR322-HDR-EGFP) were modeled on the basis of NCBI «*Anas platyrhynchos* Spindlin 1 (SPIN1)» (Gene ID: 101791720). The plasmids are described in

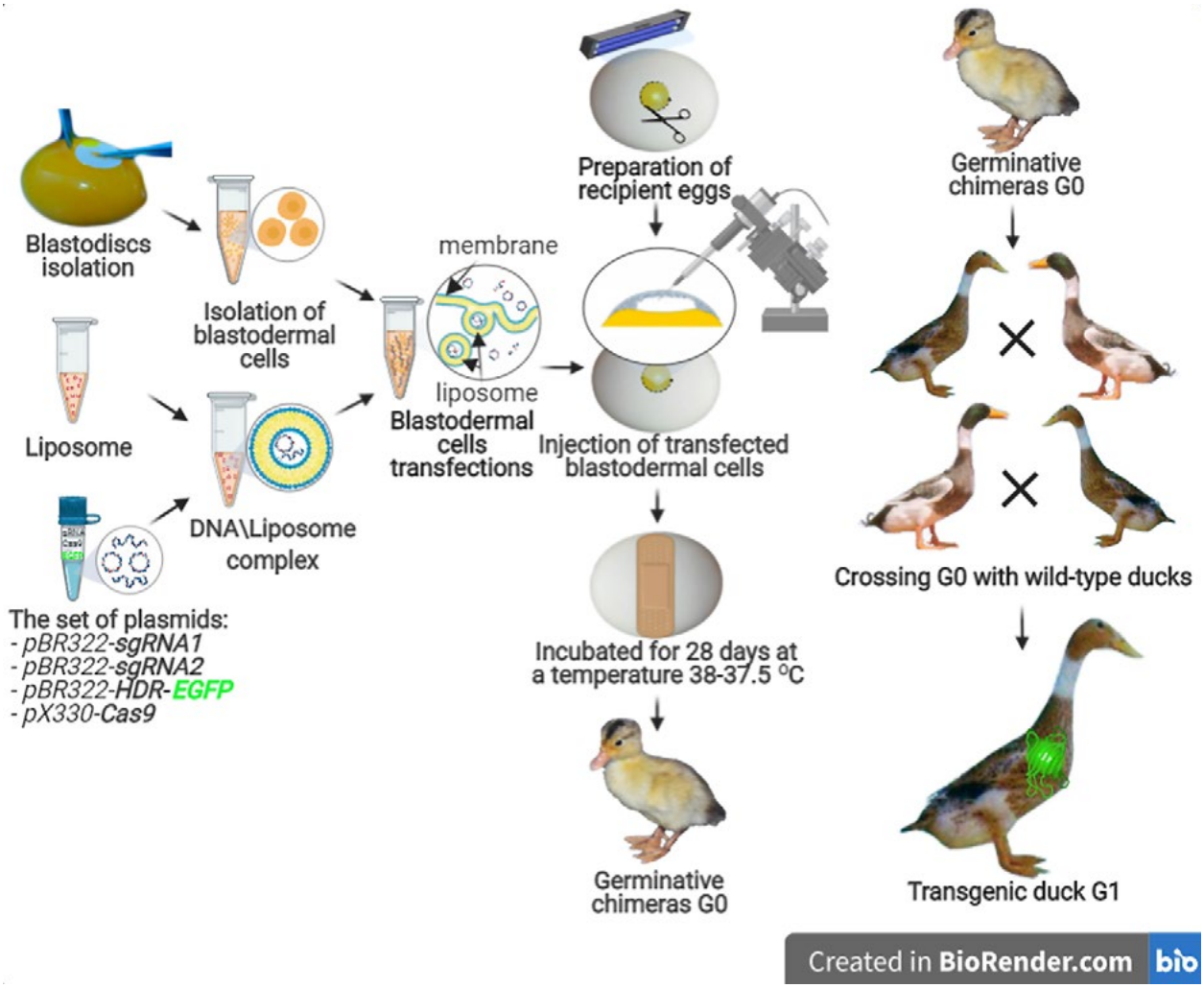

Fig. 1. Scheme of the experiment on the creation of transgenic chimeras

more detail in our previous work, where we used sperm to create transgenic ducks [17,47].

Isolation of blastodermal cells. Blastodermal cells were isolated by the method developed by M. T. Tagirov on chickens [48]. Blastodermal cells were isolated from embryos of the Shaoxing breed, at stage EGK-X by means of a filtration papery ring [49]. Each blastodisk was washed twice from the yolk in a solution of phosphate-buffered saline (PBS). Then 10–12 blastodiscs were transferred using Pasteur pipette into 1 ml of PBS containing 0.25 % trypsin, 0.04 % ethylenediaminetriacetic acid (EDTA), incubated for 10 min at 37 ◦C and then centrifuged (10 s at 1500 rpm). Newly precipitated cells were resuspended in 1 ml of RPMI 1640 culture medium containing 10 % fetal bovine serum. The cell suspension was concentrated by centrifugation (10 s at 1500 rpm) with further removal of 0.7 ml of supernatant. Then the cells were resuspended in the remained medium.

Preparation of the DNA\Lipofectamine 2000 complex, transfection of blastodermal cells. For transfection of blastodermal cells, two solutions were prepared simultaneously: 1) solution 1. 30 µl of OPTI-MEM + 6 µl of Lipofectamine 2000 — incubated at room temperature for 5 min; 2) solution 2. 30 µl of OPTI-MEM + 4 µl of plasmid DNA (25 ng/ml of each vector: sgRNA1, sgRNA2, HDR-EGFP, Cas9) — incubated for 5 min. Further, solution 1 and solution 2 were mixed and incubated for another 20 min.

DNA/Lipofectamine 2000 complex was added to the precipitated blastodermal cells and incubated at 37.8 °C for 4 hours.

Preparation of recipient eggs. The recipients were Shanma embryos at stage EGK-X. For a more viable introduction of transfected blastodermal cells (TBCs) into the recipient's gonads, it was necessary to decrease the total level of primordial germ cells in the recipient's gonads. For this purpose, the recipient eggs were exposed to ultraviolet irradiation for one hour before the injection [50].

To gain access to the embryo, a 0.7-cm opening was cut in the shell. The prepared TBCs were injected into the subgerminal cavity using a micro-needle injector (up to 70 µm in diameter). Each embryo was injected with 2–3 µL of the suspension containing approximately 600 TBCs.

After TBCs injection into the recipients' subgerminal cavity, RPMI 1640 culture medium (with a mixture of ampicillin and streptomycin) was added with a pasteurized pipette to complete the filling so that no air was left in the eggs, then the donor eggs protein was applied around the cut openings as glue, and covered with a piece of UV-sterilized food film, 3x3 cm in size, which was additionally fastened over the openings in the eggs with 2x5 cm patch. Then the eggs were incubated for 28 days at a temperature of 38–37.5 °C.

Selection of samples for DNA isolation. Samples of feathers, blood, and sperm were taken for DNA extraction. In each animal, two or three feathers were plucked from the chest and placed in individual tubes. From the brachial vein (*Vena cutanea ulnaris)* 1–2 ml of blood was collected in a vacuum tube with anticoagulant (EDTA). Sperm samples from males were taken by the transverse body massage [51].

All samples, after selection, were frozen and stored at –20 °C until DNA was isolated.

EGFP identification. Identification of transgenic DNA was performed by PCR. We used two primers located within the EGFP to amplify a 903 bp fraction: the anterior one (5′ GTGTACGGTGGGGAGGTC 3′) and the posterior one (5′ AAATGTGGTGGTGGCTGAT TATG 3′).

The program of the polymerase chain reaction included

– Initial stage at 94 °C, 3 min, 35 cycles
– denaturation at 94 °C, 15 seconds
– evaporation at 55 °C, 15 seconds
– elongation at 72 °C, 30 seconds
– final stage at 72 °C, 3 min.

The obtained PCR product was sequenced, the resulting sequence was compared with the sequence of the EGFP gene in the NCBI base, the result of the comparison is 100 % identity. PCR and sequencing experiments were performed by Genery Biotechnology Company (Shanghai, China)

(http://www.generay.com.cn/english)

Identification of chimerism by microsatellite loci. Analysis of the chimerism of G0 offspring was performed by assessing the phenotype and genotype of three generations of ducks P, G0, G1 at microsatellite loci. A total of 19 microsatellite loci were used for the

Table 1. **Description of microsatellite loci used for analysis of three generations of ducks**

№	Locus	Sequence of primers		Fluorescent dyes	Annealing temperature, °C
1	APL2	APL2-F	CGCTCTTGGCAAATGTCC	FAM	60
		APL2-R	GATTCAACCTTAGCTATCAGTCTCC		
2	APL11	APL11-F	TTGCATCAGGGTCTGTATTTTC	HEX	60
		APL11-R	AACTACAGGGCACCTTATTTCC		
3	APL12	APL12-F	AAGAGACACTGAGAAGTGCTATTG	FAM	60
		APL12-R	AGTTGACCCTAATGTCAGCATC		
4	APL23	APL23-F	GCTGAGATGCTCCCAGGAC	HEX	60
		APL23-R	GAAGAGGCAGTGGCAACG		
5	APL36	APL36-F	TCCACTGGGTGCAAACAAG	HEX	60
		APL36-R	ATGCTTTGCTGTTGGAGAGC		
6	APL80	APL80-F	TTGCCTTGTTTATGAGCCATTA	HEX	58
		APL80-R	GGATGTTGCCCCACATATTT		
7	APL79	APL79-F	CATCCACTAGAACACAGACATT	FAM	58
		APL79-R	ACATCTTTGGCATTTTGAA		
8	APL77	APL77-F	GTATGACAGCAGACACGGTAA	FAM	55
		APL77-R	TCACTTGCTCTTCACTTTCTTT		
9	SMO10	SMO10-F	CATTGTTCATTGTTTCTTCTTCA	HEX	55
		SMO10-R	TCCTAGCGACAGCAATTCTAATG		
0	SMO13	SMO13-F	GGGCTTGAGGCATACACTCCCTA	FAM	58
		SMO13-R	ACCATCTTCCTTTCCTCCCAACC		

analysis [52, 53], of which only 10 were polymorphic (Table 1).

Research results

After preparation and transfection of blastodermal cells, injections were performed in 200 recipient eggs that were incubated before hatching. To control embryonic development, three ovoscopies were performed during the incubation period (at day[s] 8, 15 and 25). During the first incubation period 95 (47.5 %) of the eggs perished, 15 (7.5 %) of the eggs perished during the second incubation period, and 70 (35.0 %) of the eggs perished during the third incubation period.

Of the 200 eggs, into which the transfected blastodermal cells were injected, 20 siblings were obtained, including 8 males and 12 females. Thus, the survival rate of embryos was 10 %. Among the surviving fertile were 13/20 animals: 10/12 (83.3 %) females and 3/8 (37.5 %) males (Table 2). This can indicate that the procedure of chimera acquisition has a stronger effect on the survival and fertility

Table 2. **Transmission of EGFP to the next generation by chimeric transgenic ducks**

Founder number (G0)	Sex	Number of descendants (G1)	Number of transgenic descendants						Number of descendants analyzed for MS	Found alleles which were not in parents
			Totally		Females		Males			
29	Female	33	13	39.4 %	12	92.3 %	1	7.7 %	13	4
45	Female	9	2	22.2 %	1	50.0 %	1	50.0 %	9	1
46	Female	12	4	33.3 %	3	75.0 %	1	25.0 %	11	4
47	Female	12	1	8.3 %	1	100.0 %	0	0.0 %	7	2
48	Female	13	3	23.1 %	3	100.0 %	0	0.0 %	11	3
49	Female	13	2	15.4 %	2	100.0 %	0	0.0 %	8	2
50	Female	20	7	35.0 %	6	85.7 %	1	14.3 %	11	3
51	Female	15	6	40.0 %	5	83.3 %	1	16.7 %	13	5
52	Female	19	4	21.1 %	4	100.0 %	0	0.0 %	10	5
53	Female	6	4	66.7 %	4	100.0 %	0	0.0 %	6	1
27	Male	8	3	37.5 %	2	66.7 %	1	33.3 %	8	5
28	Male	21	7	33.3 %	6	85.7 %	1	14.3 %	19	7
30	Male	16	3	18.8 %	0	0.0 %	3	100.0 %	16	11
Totally from the females		152	46	30.3 %	41	89.1 %	5	10.9 %	99	30 (30.3 %)
Totally from the males		45	13	28.9 %	8	61.5 %	5	38.5 %	43	23 (53.5 %)
Totally		197	59	29.9 %	49	83.1 %	10	16.9 %	142	53 (37.3 %)

of males. This refers both to the number of those who survived 8/20 (40 %) — males, 12/20 (60 %) females and to the fertility of survived animals of different status.

In order to identify G0 chimerism, we analyzed G0 and G1 microsatellites. The animals that had alleles in their fathers were identified (Table 2).

From 13 out of 20 (G0) birds, a total of 197 offspring were obtained (incl. 117 (59.4 %) daughters and 80 (40.6 %) sons), of which 59 were EGFP-positive (30.3 %), including 10 males (16.9 %) and 49 females (83.1 %) (Fig. 2).

Out of 12 chimeric ducks (G0), ten were pregnant and 152 ducks (G1) were obtained from them (including 94 (61.8 %) daughters and 58 (38.2 %) sons). 46 (30.3 %) of all G1 from females (G0) were transgenic. G0 females gave 5 transgenic sons (10.9 %) and 41 transgenic daughters (89.1 %). Nine out of ten G0 females transmitted the transgene only to their daughters, which may indicate that the construct may have been incorporated into the W chromosome and passed from mother to daughter. Only one female equally (50 % females, 50 % males) passed on to transgenic offspring, which may indicate the location of the embedded structure in the autosome and gender-independent inheritance.

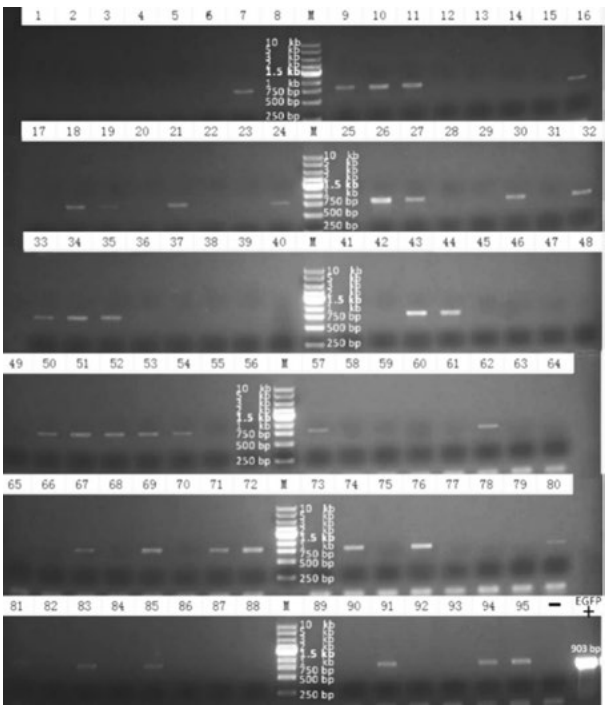

Fig. 2. Photographs of electrophoretic separation of PCR products by detecting EGFP gene sequence in the DNA of cells

In this case, the unequal transmission of the transgenic construct to the offspring of different sexes was observed and the chimeric animals carried the primary germ cells of the recipient and donors. Among the donor cells of chimeras there were both ZZ and ZW. Thus, a chimeric female could give oocytes with a W and Z ratio that did not correspond to the classical 50:50. Among her offspring there could be individuals obtained from donor ZZ, that is why one could expect a frequent transfer of Z-chromosome and a greater number of sons than daughters in chimera females.

Among the eight male chimeras, only three produced offspring. A total of 45 offspring were obtained from pregnant ducks, of which 13 were transgenic (28.9 %), including 5 (38.5 %) males and 8 females (61.5 %). Nine transgenic males did not give offspring, DNA analysis of their bodies and ejaculate confirmed their chimerism and the presence of a transgenic construct in all of them. Among the offspring obtained from two (out of three fertile) males G0, the percentage of transgenic daughters was 66.7 % and 85.7 %, and the

third male passed the transgene only to his sons.

In case of the presence of donor ZW cells in males, more daughters could be expected from such individuals (due to the fact that from the father they would receive the W-chromosome of the donor and in combination with the Z-chromosome of the mother would give a female), as well as lower fertility (because the W-chromosome of the donor from the father in conjunction with the W-chromosome of the mother gives a non-viable combination of *WW*). This can explain the presence of a greater number of daughters than sons in male chimeras as well as the reduced reproductive ability. Transmission of the transgenic construct to only sons of male #30 can indicate that the daughters with constructs in the Z-chromosome from the father do not survive.

Some data suggest that germline chimeras show significant changes in sex hormone levels in the ovaries and blood plasma, which may affect their reproductive capacity [54].

Discussion

Thus, taking into account the results of transmission of the transgenic structure from G0 to G1 siblings, we should note that all cases can be analyzed as the results of independent events, that occurred during transfection of donor blastodermal cells or recipient cells after donor cells were transferred into the embryo together with construct DNA and Lipofectamine 2000. Belonging to the offspring of donors among the offspring obtained from G0 individuals was confirmed only in 37.3 %. Comparison of our results with the data of other researchers conducted on different species of birds using different stages of construction and vector application (Table 3) shows that the majority of studies were conducted on chickens. The construct was introduced at stages X [19–21] and 14-17 HH [22, 55–57, 23, 24].

Genome editing was performed using piggyBac transposon [22, 19, 55], retroviral vector [57], lentivirus vector [29, 21], CRISPR\Cas9 Lipofectamine 2000 [23, 24]. The data shown in the table indicate that the highest yield of transgenic individuals was observed in the studies by Park, T. S., & Han, J. Y. (2012) [22], where all 6 animals were transgenic. Almost half of their siblings were transgenic, which can indicate the autosomal type of construction succession. Wang, Z.-B., Du, Z.-Q., (2018) obtained 68.7 % of surviving chickens after the introduction of the construct by using PGC, among which 59.2 % were transgenic. The transmission of the transgene to the next generation was only 1.2 % [56].

Transfection of PGCs with Lipofectamine 2000 using the CRISPR\Cas9 editing system made it possible to obtain 3.1 % of F0 animals, of which 62.5 % were transgenic [23]. Oishi, I., Yoshii, K., (2018) showed the transmission of the transgenic construct to 19.7 % of the descendants of transgenic chimeras [24]. Jordan, B. J., Vogel, S.B. (2014) showed a high survival rate of embryos (52.5 %) after direct injection into blastodisc cavity, 100 % of the surviving offspring were transgenic [19], however, none of them passed transgene to the next generation. When using direct injection of exogenous DNA into the blastodermal disc cavity, bird survival ranged from 4.8 to 62.7 %, and successful transmission to the next generation was observed in 0.77 % — 15.1 % [20, 21, 56]. Thus, compared to the direct injection

Table 3. **Effectiveness of the use of direct injections and PGC**

Species	Injected at stage	Transgenesis method	Vector	Received (G0)	Transgenic (G0)	Transgenic (G1)	By:
Chickens	14-15 HH (50-60h)	piggyBac transposon	PGC	6	6\6 **(100 %)**	228\459 **(49,7 %)**	22
Chickens	Stage X	piggyBac JetPEI	Direct injection	42\80 **(52,5 %)**	42\42 **(100 %)**	0\150	19
Chickens	14-15 HH	piggyBac Lipofectamine 2000	PGC	136\198 **(68,7)**	16\27 **(59,2 %)**	1\81 **(1,2 %)**	56
Chickens	14-15 HH	Retroviral Vector Lipofection	Direct injection	32\51 **(62,7)**	32 **(100 %)**	6\181 **(3,3 %)**	55
Chickens	15 HH	retroviral vector	Direct injection	21\74 **(28,4)**	21 **(100 %)**		57
Chickens	Stage X	FIV-lentivirus vector	Direct injection	10\208 **(4,8)**	10 **(100 %)**	4/518 **(0.77 %)**	20
quail	Stage X	lentiviral vector	Direct injection	8\80 **(10 %)**	8 **(100 %)**	19\126 **(15,1 %)**	21
Chickens	14-17 HH.	CRISPR\Cas9 Lipofectamine 2000	PGCs	8\260 **(3,1 %)**	5\8 **(62.5 %)**		23
Chickens	14-16 HH	CRISPR\Cas9 Lipofectamine 2000	PGCs	4	4 **(100 %)**	31\157 **(19,7 %)**	24
Ducks	Stage X	CRISPR\Cas9 Lipofectamine 2000	Blastodermal cells	20\200 (10.0 %)	7/20 (35.0 %)	59\197 (29.9 %)	58
Ducks	Stage X	CRISPR\Cas9 Lipofectamine 2000	Direct injection	9\300 (3.0 %)	4\9 (44.4 %)	37\102 (36.3 %)	59

of exogenous DNA into the blastodermal disc cavity, the PGC method is more successful in the transgene transmission to the next generation.

Conclusions

Transgenic duck chimeras were created using donor blastodermal cells after transfection with DNA vector and Lipofectamine 2000. To edit the target region of the duck genome, we used the CRISPR/Cas9 system with homologous directional reduction (HDR). The EGFP reporter gene was used as the transgene. Of the 200 eggs, in which the transfected blastodermal cells were introduced, 20 offspring were obtained, including 8 males and 12 females. Thus, the survival of embryos was 10 %. Among the surviving fertile were 13/20 animals G0 (65 %): 10/12 (83.3 %) females and 3/8 (37.5 %) males. The procedure of obtaining chimeras has a stronger effect on the survival and fertility of male chimeras.

From 13 of 20 birds G0, a total of 197 offspring (including 117 (59.4 %) daughters and 80 (40.6 %) sons) were received, of which 59 were EGFP-positive (30.3 %), including 10 males 16.9 %) and 49 females (83.1 %). The technique used by us can be successfully applied in further researches and at creation of a transgenic duck.

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Створення трансгенної качки *(Anas Platyrhynchos)* за використання бластодермальних клітин та методу CRISPR/CAS9

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Метою роботи була розробка методики створення трансгенних химер качок за використання донорських бластодермальних клітин після трансфекції з плазмідною ДНК та ліпофектаміном. Систему CRISPR/Cas9 з гомологічно спрямованою репарацією (HDR) використовували для редагування цільового сайту генома качки. Матеріали та методи. За використання бластодермальних клітин донорів після трансфекції з ДНК вектору з ліпофектаміном створені трансгенні химери качки. Для редагування цільової ділянки геному качки використали систему CRISPR/Cas9 з гомологічно направленою репарацією (HDR). В якості трансгена використали репортерний ген EGFP. **Висновки.** З 200 яєць, в які були введені трансфіковані бластодермальні клітини, отримали 20 нащадків, у тому числі 8 самців і 12 самок. Таким чином, виживаність ембріонів склала 10 %. Серед вижившихплідними виявилися 13/20 тварин G0 (65 %): 10/12 (83.3 %) самок та 3/8 (37.5 %) самців. Процедура отримання химер сильніше впливає на виживаність та плідність самців-химер. Від 13 з 20 птахів G0, отримали в цілому 197 нащадків (в т.ч. 117 (59.4 %) дочок і 80 (40.6 %) синів) з яких 59 були EGFP-позитивними (30.3 %), в тому числі 10 самців (16.9 %) і 49 самок (83.1 %). Використана нами методика може бути успішно застосована у подальших дослідженнях та при створенні трансгенної качки.

Ключові слова: CRISPR/Cas9, EGFP, трансгенна качка, бластодермальні клітини, химера

Создание трансгенной утки *(Anas Platyrhynchos)* с использованием бластодермальных клеток и метода CRISPR / CAS9

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Целью работы являлась разработка методики создания трансгенных химер уток с использованием донорских бластодермальных клеток после трансфекции с плазмидной ДНКи липофектамином. СистемуCRISPR/Cas9 с гомологически направленной репарацией (HDR) использовали для редактирования целевого сайта генома утки. Материалы и методы. Трансгенные химеры уток были созданы с использованием донорских бластодермальных клеток после трансфекции с векторной ДНКилипофектамином. Для редактирования целевой области генома утки мы использовали систему CRISPR/Cas9 с гомологично направленным востановлением поврежденной ДНК (HDR). В качестве трансгена использовали репортерный ген EGFP. **Выводы.** Из 200 яиц, в которые были внесены трансфицированные бластодермальные клетки, было получено 20 потомков, в том числе 8 самцов и 12 самок. Таким образом, выживаемость эмбрионов составила 10 %. Среди выживших фертильных животных было 13/20 животных G0 (65 %): 10/12 (83,3 %) самок и 3/8 (37,5 %) самцов. Процедура получения химер сильнее влияет на выживаемость и плодовитость самцов химер. Всего от 13 из 20 птиц G0 получено 197 потомков (в том числе 117 (59,4 %) дочерей и 80 (40,6 %) сыновей), из которых 59 были EGFP-положительными (30,3 %), в том числе 10 самцов 16,9 %) и 49 самок. (83,1 %). Используемая нами методика может быть успешно применена в дальнейших исследованиях и при создании трансгенной утки.

Ключевые слова: CRISPR/Cas9, EGFP, трансгенная утка, бластодермальные клетки, химера.

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