UDC 636.5.082.597.034.453 https://doi.org/10.31548/animal2021.04.001

PRODUCTIVITY OF GERMINATIVE DUCK CHIMERAS AND THEIR DESCENDANTS

M. S. DOROSHENKO,
Graduate student, Department of Genetics, Breeding and Biotechnology of Animals*
https://orcid.org/0000-0002-7647-4750
E-mail: mariyamgart@i.ua
S. O. KOSTENKO,
Professor, Department of Genetics, Breeding and Biotechnology of Animals
https://orcid.org/00000-0002-7816-3374
E-mail: svitlanakasijan@ukr.net
National University of Life and Environmental Sciences of Ukraine
P. V. KOROL,
Graduate student, Department of Genetics and Biotechnology of Animals*
https://orcid.org/0000-0002-3866-4246
E-mail: svbrj@ukr.net
Institute of Animal Breeding and Genetics nd. a. M. V. Zubets of National Academy of Agrarian Science of Ukraine
A. M. CHEPIHA,
PhD, Biotechnologist
https://orcid.org/0000-0002-7810-2002
E-mail: alenachepiga@ukr.net
Private Joint Stock Company On the Production of Insulin "INDAR"
O. V. SYDORENKO,
the Head of Animal Genetic Resources Department
https://orcid.org/0000-0003-2429-9361
E-mail: sydorenkoolena@ukr.net
P. P. DZHUS,
the Head of Breeding Beef Cattle Laboratory
https://orcid.org/0000-0002-4808-0260
E-mail: cvic_ua@ukr.net
Institute of Animal Breeding and Genetics nd. a. M. V. Zubets of National Academy of Agrarian Science of Ukraine
N. P. SVYRYDENKO,
PhD, Assistant Professor, Department of Genetics, Breeding and Biotechnology of Animals
https://orcid.org/0000-0003-0958-2038
E-mail: svyrydenko@nubip.edu.ua
T. V. LYTVYNENKO,
PhD, Assistant Professor, Department of Genetics,

Breeding and Biotechnology of Animals
https://orcid.org/0000-0002-0405-3367
E-mail: tv-litv@ukr.net
National University of Life and Environmental Sciences of Ukraine
O. M. KONOVAL,
Postdoctoral Researcher
https://orcid.org/0000-0003-4955-8040
E-mail: oxanakonoval@ukr.net
Institute of Animal Husbandry and Veterinary Science, Zhejiang Academy of Agricultural Sciences (Hangzhou, China)
Ukrainian Laboratory of Quality and Safety of Agricultural Products of National University of Life and Environmental Sciences of Ukraine
L. LU,
Professor, Institute of Animal Husbandry and Veterinary Science,
https://orcid.org/0000-0001-9701-131X
E-mail: lulizhibox@163.com
Zhejiang Academy of Agricultural Sciences (Hangzhou, China)
R. S. OLEYNIK,
Student, Faculty of Livestock Raising and Water Bioresources*
https://orcid.org/0000-0002-6958-8178
E-mail: regina.oleynik@icloud.com
P. A. FILIPOVA,
Student, Faculty of Livestock Raising and Water Bioresources*
https://orcid.org/0000-0002-1184-1468
E-mail: philipovapolin@gmail.com
National University of Life and Environmental Sciences of Ukraine
L. LI,
Senior engineer in genetic breeding and general manager
https://orcid.org/0000-0002-6604-241X
E-mail: liliumengok@126.com
Zhejiang Guowei Technology Co. LTD, Zhejiang (Hangzhou, China)
M. V. DRAHULIAN,
PhD, Biotechnologist
https://orcid.org/0000-0003-1512-0104
E-mail: m.drahulian@gmail.com
Rinderunion Baden-Württemberg Genetik GmbH

Abstract. *The consequences of chimerization and its possible influence on the productivity of chimera offspring remain poorly understood. The objects of research were ducks (Anas platyrhynchos) of Shanma (Shan partridge duck) and Shaoxing breeds kept at the Zhuji Guowei Poultry Development Co, Ltd, China. The study was conducted in the poultry genetics laboratory*

* Scientific supervisor – Doctor of Biological Sciences, Professor S. O. Kostenko

of the Zhejiang Academy of Agricultural Sciences at a duck farm of Zhejiang Generation Biological Science and Technology Co., Ltd. (Zhejiang Province, China). To create chimeras of ducks, the method described by Aige-Gil & Simkiss (1991) and Tagirov (2010) was used. Blastodiscs were isolated from freshly hatched fertilized eggs using a filter paper ring. Shanma duck embryos were used as recipients and Shaoxing duck embryos, homozygous for plumage color gene allele (wild type), were used as donors. Busulfan (SigmaAldrich, United States) was used as a chemical agent that suppresses a division of primary germ cells in recipient embryos. An opening in the eggshell (window) of recipients (Shanma breed) was made between the blunt and sharp end of eggs. This reduced the distance between an injector and an embryo needle. The recipients were incubated for 8–10 hours at a temperature of 38 °C. After incubation of recipient eggs for 8 hours, the windows were opened in them. Busulfan was injected into the subgerminal cavity of the embryo with a micropipette (1.5–3 μl of liquid). After busulfan injection, the empty cavity was filled with nutrient medium (RPMI-1640) supplemented with antibiotics (ampicillin, streptomycin), the opening was closed by plastic wrap and adhesive tape. The eggs were incubated at a reduced temperature (+32 °C) for 24 hours to prolong the duration of busulfan action on primary germ cells. More than 50% of embryos died in the first 2–3 days after the beginning of incubation. Head and neck disorders were observed in 1.2% of embryos. Busulfan injection at a concentration of 300 ng per egg led to 95.0–96.3% mortality of duck embryos, a concentration of 150 ng per egg – 33.3–75.3%, a concentration up to 75 ng per egg – 18.75–38.5%. Analysis of the age of puberty (laying of the first egg) indicates that the chimeras matured later. If the average age of puberty in the control group was 139 ± 9 days, in the group of chimeras – 148 ± 13 days. Thus, we can attest that in our experiment, the chimeras matured later than the control animals, which may be due to the effect of busulfan in the sterilization of recipient embryos. The average weight of ducks in the control group was lower, and the group itself was more consolidated. Thus, the control ducks weighed 1422.40 ± 57.00 g, the chimeras – 1608.80 ± 94.76 g. The predominance of chimeras over the control group in live weight may be due to the fact that the control group consisted of recipients of Shanma breed. Egg production of ducks for the entire study period was 87.5 ± 0.05% (control) and 79.5 ± 0.12% (busulfan). The weight of eggs in ducks from two groups for the entire period was 70.62 ± 0.199 g (control) and 71.15 ± 0.157 g. The egg morphometric parameters in studied groups of ducks: the average values of egg length – 6.056 ± 0.0564 cm (control) and 6.269 ± 0.1341 cm (busulfan); egg width – 4.520 ± 0.0053 cm (control) and 4.529 ± 0.004 cm (busulfan). There were no statistical intergroup differences in the morphometric parameters of the eggs from the studied groups. In fact, we obtained results similar to the previous ones, which concerned the egg production of daughters from chimera drakes.

Keywords: *germinative chimera, Shaoxing duck, Shanma, busulfan, duck egg productivity*

Introduction.

Due to its high reproductive potential, short intergeneration interval. and embryonic development outside the mother's body, poultry provides unique opportunities for its use in fundamental and applied biological research (Mozdziak & Petitte, 2004; Kagami, 2016). The methods of cloning and transgenesis have become a routine tool for creating animal models of development (Kathleen et al., 2010), diseases (Ogilvie et al., 2017), bioreactors and producers of valuable biologically active drugs (Petitte & Mozdziak, 2002; Pavlou & Reichert, 2004), highly productive

aquaculture objects (Devlin et al., 2009). However, the use of the conventional technique for microinjection of foreign DNA into the pronucleus of a fertilized egg, which is well developed for many mammalian species (Gordon & Ruddle, 1981), faces difficulties when applied to birds (Perry, 1988; Love et al., 1994). The creation of a transgenic bird is complicated by the structure of its opaque egg cell with a large yolk and the unique reproductive system of this class. Direct microinjection of DNA into the oocyte, which is often used in mammals, is practically impossible for birds since fertilization occurs in the infundibulum of the reproductive tract and can be polyspermic (Mozdziak & Petitte, 2004). Therefore, manipulations with the zygote turned out to be difficult for their use when creating a transgenic bird (Love, 1994). Over the past decades, some alternative strategies have been developed to obtain transgenic poultry through the use of chimeric animals created by the transfer of blastodermal cells.

Primordial germ cells are successfully used to create transgenic birds (Ginsburg & Eyal-Giladi, 1987) and as a tool for preserving the genetic resources of local breeds (Kagami et al., 1997; Kino et al., 1997; Yi-Chen Chen et al., 2019). However, to date, the efficiency of transgenic poultry in many cases remains very low, and the technique of using ducks to create transgenic birds is practically not developed (Szta´n et al., 2012).

Analysis of recent researches and publications.

Today, the duck (*Anas platyrhynchos* Linnaeus, 1758) is a poorly studied scientific (breeding) object in comparison with the species *Gallus gallus domesticus*, *Coturnix coturnix* but one of the most economically promising poultry species. A duck can secrete a lot of protein in the oviduct and can regularly produce eggs over a 20–24-hour cycle, which is a very attractive means for the synthesis of therapeutic proteins since the sterile content of eggs is protected by a hard egg shell. Busulfan is used to suppress cell proliferation. Injection of busulfan into the subgerminal cavity is one of the methods that increases the number of donor cells during the creation of chimeras (Aige-Gil & Simkiss, 1991; Tagirov, 2010).

However, the methods for creating germinative duck chimeras face difficulties associated with the structure of the egg shell in waterfowl; the consequences of chimerization and its possible influence on the productivity of chimera offspring remain poorly understood (Sawicka et al., 2011). Transgenic animals are almost not inferior to their non-transgenic counterparts (Korol et al., 2019). The effect of the reproductive season on the sperm productivity of germinative drake chimeras was previously studied (Doroshenko et al., 2018). For the analysis of survival, Korol et al. (2021) used embryos obtained using various methods of introducing the DNA.

In order to assess the egg productivity of daughters from germinative chimeras (males), a study was carried out on three groups of ducks with different origins. Analysis of the productivity of daughters from germinative duck chimeras showed that, as a whole, the chimerization of their parents did not affect the performance of daughters. An analysis of the productivity (egg production, pieces, length, width, egg weight, and shape index) in a group of daughters obtained from chimeric animals indicates that, according to most indicators, this group occupies an intermediate position between the groups whose breeds served as donors and recipients. The method we have used to obtain chi-

meras can be successfully used on ducks in order to preserve genetic resources. Preservation of frozen germ cells of rare bird species and native bird breeds with the prospect of their reproduction using germinal chimeras will reduce the risks of a decrease in the genetic diversity of birds (Doroshenko et al., 2021).

Thus, the productivity of daughters from drake chimeras was investigated. However, the egg productivity of female chimeras remains unexplored. This work is devoted to this issue.

Materials and methods of researches.

All experiments with animals were carried out in accordance with the provisions of the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" (1986).

The objects of research were ducks (*Anas platyrhynchos*) of Shanma (Shan partridge duck, Shan Ma duck) and Shaoxing breeds kept at the Zhuji Guowei Poultry Development Co, Ltd (China). The study was conducted in the poultry genetics laboratory of the Zhejiang Academy of Agricultural Sciences at a duck farm of Zhejiang Generation Biological Science and Technology Co., Ltd. (Zhejiang Province, China).

To obtain duck chimeras, we used a method such as the production of somatic and embryonic chimeras in chickens by transferring early blastodermal cells (Petitte et al, 1990; Tagirov, 2010) with changes in time according to the embryonic development of the duck. Sterilization of duck embryos was done with busulfan (Aige-Gil & Simkiss, 1991; Tagirov, 2010). To identify the offspring of chimeric donors, the microsatellite analysis of the parents was used (Kostenko et al., 2017).

Isolation of blastodermal cells. Blastodiscs were isolated from freshly hatched fertilized eggs using a filter paper ring (Lucas & Jamroz, 1961). The obtained embryos were washed twice from the yolk in a phosphate-buffered saline (PBS) solution (170 mM NaCl; 3.4 mM KCl, 4 mM Na_2HPO_4; 1.8 mM KH_2PO_4; pH 7.2). Then, 10–12 embryos were transferred into 1 ml of PBS containing 0.25% trypsin and 0.04% ethylenediamine-tetraacetate (EDTA), and incubated for 10 minutes at 37 °C, then pipetted with a Pasteur pipette and centrifuged for 10 s at 1500 rpm/min. The pellet was resuspended in 1 ml of RPMI 1640 nutrient medium containing 10% fetal calf serum. The cell suspension was concentrated by centrifugation for 10 s at 1500 rpm, followed by the removal of 0.7 ml of the supernatant, and then the cells were resuspended again in the medium that remained.

Obtaining duck chimeras. Shanma duck embryos were used as recipients and Shaoxing duck embryos, homozygous for the plumage color gene allele (wild type), were used as donors. Donor cells were injected into the subgerminal cavity of recipients with a micropipette (outer diameter 50–70 μm) through a round opening (window) with a diameter of 0.7 cm in the egg shell. Each embryo was injected with 3–4 μl of the suspension, which contained 600–1000 donor cells. The opening in the egg was covered with a piece of thin plastic wrap, which was glued to the shell with protein and then sealed on top with a larger adhesive tape. Busulfan (SigmaAldrich, United States) was used as a chemical agent that suppresses the division of primary germ cells in recipient embryos.

Preparation of recipient eggs. An opening in the eggshell (window) of the recipients (Shanma breed) was made between the blunt and sharp ends of the

eggs. This reduced the distance between the injector and the embryo needle. The eggs from recipients were incubated for 8–10 hours at a temperature of 38 °C.

Preparation of busulfan solution. Busulfan was dissolved immediately before use in 10% dimethyl sulfoxide (DMSO), diluted with 3–5 µl of RPMI 1640 nutrient medium. The concentrations used were 300 ng/egg, 150 ng/egg, and 75 ng/egg.

Busulfan treatment. After incubation of recipient eggs for 8 hours, the windows were opened in them. Busulfan was injected into the subgerminal cavity of the embryo with a micropipette (1.5–3 µl of liquid). After busulfan injection, the empty cavity was filled with nutrient medium (RPMI-1640) supplemented with antibiotics (ampicillin, streptomycin), the opening was closed with plastic wrap and adhesive tape. The eggs were incubated for 24 hours at a reduced temperature (+32 °C) to prolong the duration of busulfan action on the primary germ cells.

Experimental and control animals were kept in individual cages in the same room with constant access to water and food. The egg productivity of 5 experimental animals, which were obtained as a result of their treatment in the embryonic period with busulfan (chimera group), and 10 control animals was studied. A total of 1617 eggs were examined in 142 days in the period from December 13, 2016, to May 3, 2017.

The body weight was determined individually with an accuracy of 10 g for all ducks aged from 41 to 61 weeks.

Average egg weight and size were determined every day. The egg length (L) and width (W) were measured with an accuracy of 0.1 mm with a vernier caliper.

Eggs were weighed on a JM-A 20001 electronic balance with an accuracy of 0.1 g. The egg shape index (SI) was calculated using the formula:

$$SI = W/L \times 100 \quad (1)$$

The obtained data were statistically processed on a computer by a spreadsheet processor "MS Excel 2010" using descriptive statistics and the F-test for two samples for deviation procedures (Zhelyazkov & Tsvetanova, 2002).

Results of the research and their discussion.

As a result of the experiments, animal chimeras (F_0) were obtained. For the first time, to obtain blastodermal chimeras of ducks, busulfan (1,4-butanediol dimethanesulfonate) was used as an agent that suppresses the development of primary germ cells, an alkylating agent whose mechanism of action is based on cross-linking of DNA strands, as a result of which the replication process is disrupted. A method was developed for creating germinative duck chimeras using busulfan injections. It has been shown that duck embryos are more sensitive to busulfan than chick embryos. Injection of busulfan at a concentration of 300 ng/egg leads to 95.0–96.3% mortality of duck embryos. More than 50% of embryos died in the first 2–3 days after the beginning of incubation. Head and neck disorders were observed in 1.2% of embryos. When using busulfan at a concentration of 150 ng/egg, a mortality rate of 33.3–75.3% was observed. A decrease in a concentration up to 75 ng/egg led to 18.75–38.5% embryonic mortality.

The assessment of duck chimerism by means of the analysis of microsatellite loci and analysis of the phenotype indicates that the efficiency of obtaining germinative duck chimeras was 65–77.8%.

Analysis of the age of puberty (laying of the first egg) indicates that the chimeras matured later. If the average age of puberty in the control group was 139.5 ±

9.67 days, then in the group of chimeras – 148.2 ± 13.13 days. Thus, we can attest that in our experiment, the chimeras matured later than the control animals, which may be due to the effect of busulfan in the sterilization of recipient embryos. After the onset of puberty this generation, we analyzed the live weight in two groups of ducks aged from 41 to 61 weeks.

The average live weight of ducks in the control group was lower and the group itself was more consolidated. Thus, the control ducks weighed 1422.40 ± 57.00 g and chimeras – 1608.08 ± 94.76 g. The predominance of chimeras over the control group in live weight may be due to the fact that the control group consisted of recipients of Shanma breed. This breed is characterized by the egg direction of productivity and is lighter than Shaoxing breed (embryos of this breed served as donors). Thus, donor cells could be affected by the weight gain of chimeras. Our previous study showed that the average live weight of daughters from Shanma drakes was 1554.20 ± 23.54 g, in the group of daughters from Shaoxing drakes – 1505.47 ± 17.06 g, and a group of daughters from germinative chimeras – 1535.69 ± 17.34 g (Doroshenko et al., 2021).

Thus, the data of the average live weight of ducks obtained as a result of biotechnological procedures associated with the use of busulfan, correspond to similar indicators of both the control group and the offspring of male germinative chimeras.

The egg production index in ducks for the entire study period was 87.5 ± 4.53% (control) and 79.5 ± 11.8% (busulfan).

In our previous research, the average values of egg productivity per month in studied ducks were 27.52 ± 0.84% in the group of daughters from Shanma drakes, 27.47 ± 0.61% in the group of daughters from Shaoxing drakes, and 27.73 ± 0.53 eggs in the group of daughters from germinative chimeras (Doroshenko et al., 2021). This corresponded to approximately 91.56–92.4%.

Thus, the sterilization of recipient embryos could have an impact on egg production. One of the experimental duck egg production index was only 34.92%. The reproductive ability of this chimeric duck was also impaired, 50.43% of the eggs after artificial insemination were unfertilized. At the same time, the percentage of fertilized eggs in the initial population was 87.5 ± 3.032 – 92.5 ± 2.414%, depending on the age of the ducks (Chepiha et al., 2017).

1. Average indicators in the control and experimental groups of ducks

Rate	Control group		Busulfan group	
	M ± m	Cv ± mCv	M ± m	Cv ± mCv
Egg production index (142 days), %	87.5 ± 4.53**	16.4 ± 0.090	79.5 ± 11.8	32.8 ± 0.181
Live weight of ducks, g	1422.40 ± 57.00	12.7 ± 0.079	1608.08 ± 94.76	13.2 ± 0.114
Puberty age, days	139.5 ± 9.67	21.8 ± 0.104	148.2 ± 13.13	19.8 ± 0.140
Egg weight, g	70.6 ± 0.198***	9.20 ± 0.006	71.4 ± 0.157	5.07 ± 0.071
Egg length, cm	6.05 ± 0.056	3.65 ± 0.042	6.26 ± 0.134	4.93 ± 0.070
Egg width, cm	4.52 ± 0.053	3.89 ± 0.044	4.53 ± 0.041	2.09 ± 0.045
Egg shape index, %	75.7 ± 0.3	0.7 ± 0.018	75.2 ± 0.3	0.8 ± 0.028

Note: Statistical significance at * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

The egg weight in ducks of two groups for the entire period was 70.6 ± 0.198 g (control) and 71.4 ± 0.157 g ($p < 0.001$).

In general, it can be noted that the average egg weight in experimental ducks is normal because according to the standard in Shaoxing ducks, it should be 62–68 g and becomes relatively stable (69–73 g) at the end of egg laying.

Thus, according to the results of our previous analysis of physical and morphological parameters in Shaoxing ducks, it was reliably established that the average weight of eggs with a green shell is greater than white eggs (71.43 ± 0.208 g and 68.52 ± 0.415 g; $p < 0.01$) (Chepiha et al., 2017).

The egg weight is one of the main indicators affecting their quality. It should be noted that according to this indicator, the egg weight (71.21 g) in the group of ducks of Shanma breed unambiguously prevailed in comparison with other groups of ducks. But the eggs of chimeric animals were significantly larger (69.94 g) than the eggs of Shaoxing ducks (69.12 g). The range of egg weights for various duck breeds is 60–90 g (Gorski et al., 1998; Adamski, 2005; Rahman, 2010; Xia et al., 2019). For example, the maximum egg weight in the Longyan breed ducks was 65.2 g in the period from 23 to 57 weeks of age and 66.9 g in the period from 41 to 57 weeks of age (Huang & Lin, 2011).

The egg morphometric parameters of the studied duck groups: the average values of egg length were 6.056 ± 0.0564 cm (control) and 6.269 ± 0.1341cm (busulfan); egg breadth – 4.520 ± 0.0053 cm (control) and 4.529 ± 0.004 cm (busulfan). There were no statistical intergroup differences in the morphometric parameters of the eggs of the studied groups. In fact, we obtained results similar to the previous ones, which concerned the egg production of daughters from drake chimeras.

Thus, the average values of egg length were in the range of 5.98 ± 0.022 cm, 6 ± 0.02 cm, and 6.06 ± 0.02 cm according to the experimental groups. A similar feature was also observed for the egg width – 4.55 ± 0.01 cm, 4.8 ± 0.01 cm, 4.49 ± 0.01 cm. Analysis of the productivity of daughters from germinative chimeras of ducks showed that, as a whole, the chimerization of their parents did not affect the performance of daughters. An analysis of the productivity of a group of daughters obtained from chimeric animals indicates that, according to most indicators, this group occupies an intermediate position between the groups whose breeds served as donors and recipients (Doroshenko et al., 2021).

The egg index of the two studied groups (control – 0.758 and busulfan – 0.748) did not have statistically significant differences.

The index of egg in the 1st group showed slightly higher values compared to the values in the 2nd and 3rd groups, but the difference in values was not statistically confirmed (Doroshenko et al., 2021).

In our previous studies on the productivity of ducks of Shaoxing and Shanma breeds, we showed a relationship between the productivity of ducks with age (Chepiha et al., 2017), egg color (Chepiga et al., 2017), and microsatellite loci (Chepiga et al., 2018).

Hypothetically, the procedure for obtaining chimeric offspring cannot affect the productive qualities of their offspring. It is known that reproductive chimeras can have reduced fertility and also be sterile (Doroshenko et al., 2017). However, the descendants of chimeras are not directly related to the process of chimerization of their parents. The descendants of donors have the properties of donors and the descendants of recipients – recipients, respectively, since the chimerization procedure does not affect hereditary information. The use of busulfan

as an agent that inhibits the proliferation of recipient cells can cause a mutagenic effect, however, we observed a teratogenic effect and high embryo mortality. It is possible that the chimerization procedure affects the survival of the primary germ cells and thus the selection of cells occurs at the early stages of development. Our data may indicate the need for further study of the effect of chimerization procedures on the first generation of chimeras and their descendants.

It should be noted that the populations studied by us are not pure lines, but polymorphic at the loci of quantitative traits, which could affect the results of our studies.

Conclusions.

The method we have used to obtain chimeras can be successfully used in ducks in order to preserve genetic resources. Analysis of the age of puberty (laying of the first egg) indicates that the chimeras matured later. If in the control group the average age of puberty was 139.5 ± 9.67 days, then in the group of chimeras – 148.2 ± 13.13 days. Thus, we can attest that in our experiment, the chimeras matured later than the control animals, which may be due to the effect of busulfan in the sterilization of recipient embryos. The average live weight of ducks in the control group was lower and the group itself was more consolidated. Thus, the control ducks weighed 1422.40 ± 57.00 g and the chimeras 1608.80 ± 94.76 g. The predominance of chimeras over the control group in live weight may be due to the fact that the control group consisted of recipients of Shanma breed. The egg production of ducks for the entire study period was 87.5 ± 0.05% (control) and 79.5 ± 0.12% (busulfan). The egg weight in ducks of two groups for the entire period was 70.62 ± 0.199 g (control) and 71.15 ± 0.157 g ($p < 0.001$). The egg morphometric parameters of the studied duck groups: the average values of egg length were 6.056 ± 0.0564 cm (control) and 6.269 ± 0.1341 cm (busulfan); egg width – 4.520 ± 0.0053 cm (control) and 4.529 ± 0.004 cm (busulfan). There were no statistical intergroup differences in the morphometric parameters of the eggs of the studied groups. In fact, we obtained results similar to the previous ones, which concerned the egg production of daughters from drake chimeras.

Preservation of frozen germ cells of rare bird species and native bird breeds with the prospect of their reproduction using germinal chimeras will reduce the risks of a decrease in the genetic diversity of birds.

References

1. Kagami, H. (2016). Perspectives on avian stem cells for poultry breeding. Animal Science, 87, 1065-1075.
2. Mozdziak, P. E., & Petitte, J. N. (2004). Status of transgenic chicken models for developmental biology. Developmental dynamics: an official publication of the American Association of Anatomists, 229(3), 414-421.
3. Tjørve, K. M. C., & Tjørve, E. (2010). Shapes and functions of bird-growth models: how to characterise chick postnatal growth. Zoology, 113(6), 326-333.
4. Ogilvie, L. A., Kovachev, A., Wierling, C., Lange, B. M., & Lehrach, H. (2017). Models of models: a translational route for cancer treatment and drug development. Frontiers in oncology, 7, 219. https://doi.org/10.3389/fonc.2017.00219
5. Pavlou, A. K., & Reichert, J. M. (2004). Recombinant protein therapeutics – success rates, market trends and values to 2010. Natural Biotechnology, 22, 1513-1519.
6. Petitte, J. N., & Mozdziak, P. E. (2014). Production of transgenic poultry. In Transgenic animal technology. (pp. 335-357). Elsevier.
7. Devlin, R. H., Sakhrani, D., Tymchuk, W. E., Rise, M. L., & Goh, B. (2009). Domestication and growth hormone transgenesis cause similar changes in gene expression in coho

salmon (Oncorhynchus kisutch). Proceedings of the National Academy of Sciences of the United States of America 106. 3047-3052.

8. Gordon, J. W., & Ruddle, F. H. (1981). Integration and stable germ line transmission of genes injected into mouse pronuclei. Science, 214(4526), 1244-1246.
9. Love, J., Gribbin, C., Mather, C., & Sang, H. (1994). Transgenic birds by DNA microinjection. Biotechnology, 12, 60- 63.
10. Perry, M. M. (1988). A complete culture system for the chick embryo. Nature, 331, 70-72.
11. Ginsburg, M., & Eyal-Giladi, H. (1987). Primordial germ cells of the young chick blastoderm originate from the central zone of the area pellucida irrespective of the embryo-forming process. Development, 101, 209-219.
12. Kagami, H., Tagami, T., Matsubara, Y., Harumi, T., Hanada, H., Maruyama, K., ... & Naito, M. (1997). The developmental origin of primordial germ cells and the transmission of the donor-derived gametes in mixed-sex germline chimeras to the offspring in the chicken. Molecular Reproduction and Development: Incorporating Gamete Research, 48(4), 501-510.
13. Kino, K. B., Pain, B., Leibo, M., Cochran, M., Clark, M. E., & Etches, R. J. (1997). Production of chicken chimeras from injection of frozen-thawed blastodermal cells. Poultry Sciences, 76, 753-760.
14. Chen, Y. C., Lin, S. P., Chang, Y. Y., Chang, W. P., Wei, L. Y., Liu, H. C., ... & Wu, S. C. (2019). In vitro culture and characterization of duck primordial germ cells. Poultry Science, 98, 1820-1832.
15. Szta´n, N., Patakine´ Va´rkonyi, E., Lipto´i, K., & Barna, J. (2012). Baromfifajok embriona´lis sejtjeinek kezele´se´vel szerzett tapasztalatok [Observations of embryonic cell manipulations in different poultry species]. Magy. Allatorvosok, 8, 475-481.
16. Aige-Gil, V., & Simkiss, K. (1991). Sterilisation of avian embryos with busulphan. Research in veterinary science, 50(2), 139-144.
17. Tagirov, M. T. (2010). Poluchenie himergermintativnoj linii ptic [Production of Chimerkingtint Bird Line]. Biotechnologya, 3(2), 82-88.
18. Lucas, A. M., & Jamroz, C. (1961). Atlas of Avian Hematology. Washington. D. C: U. S. Department of Agriculture.
19. Sawicka, D., Brzezińska, J., & Bednarczyk, M. (2011). Cryoconservation of embryonic cells and gametes as a poultry biodiversity preservation method. Folia biologica (Kraków), 59(1-2), 1-5.
20. Korol, P. V., Kostenko, S. O., Konoval, O. M., Lu, L., & Li, L. (2019). Egg productivity of EGFP-transgenic ducks. Animal science and food technology, 10(3), 20-26.
21. Korol, P. V., Kostenko, S. O., Konoval, O. M., Doroshenko, M. S., Lu, L., Chepiha, A. M., ... & Drahulian, M. V. (2021). Optimization of semen preparation biothechnological procedures for DNA transfection with lipofectamine. Biodiversity, ecology and experimental biology, 23(1), 52-58.
22. Korol, P. V., Kostenko, S. O., Konoval, O. M., Doroshenko, M. S., Lu, L., Chepiha, A. M., ... & Drahulian, M. V. (2021). Transgenesis biotechnological procedures influence of domestic duck embryos survival. Scientific Reports of NULES of Ukraine, 4(92), 1-19. doi: 10.31548/dopovidi2021.04.006
23. Korol, P. V., Kostenko, S. O., Konoval, O. M., Doroshenko, M. S., Lu, L., Chepiha, A. M., ... & Filipova, P. (2021). Efficacy of blastodermal cells and CRISPR/CAS9 method in the creation of transgenic duck (Anas Platyrhynchos). Biopolymers and Cell, 4(37), 289-302.
24. Doroshenko, M. S., Kostenko, S. A., Sviridenko, N. P., Litvinenko, T. V., Konoval, O. N., Lu, L., ... & Li, L. (2021). Duck hermintative chimer offsprings productivity. Innovations in animal husbandry and safety of animal products. Achievements and outlooks, 287-294.
25. European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes. (1986). COETSER 1 (18 March 1986). Retrieved from http://www.worldlii.org/int/other/treaties/COETSER/1986/1.html
26. Petitte, J. N., Clark, M. E., Liu, G., Verrinder Gibbins, A. M., & Etches, R. J. (1990). Pro-

duction of somatic and germline chimeras in the chicken by transfer of early blastodermal cells. Development, 108, 185-189.
27. Doroshenko, M. S., Chepiga, A. M., Kostenko, S. O., Korol, P. V., Konoval, O. M., Lu, L., ... & Li, L. (2018). Influence of reproductive season on sperm productivity germinative chemer of spleen. Breeding and genetics of animals, 55, 187-195.
28. Kostenko, S., Konoval, O., Chepiha, A., Korol, P., Doroshenko, M., Lu, L., ... & Li, L. (2017). Genetic diversity and population structure of Shaoxing and Shanma ducks breeds by microsatellite loci. Scientific Bulletin of NULES of Ukraine, 271, 110-121.
29. Chepiha A. M., Kostenko S. O., Konoval O. M., Lu L., Bu X., Huang L., Huang X., & Li L. (2017). Monitoring of eggs productivity of the Shaoxing breed ducks of different age. Scientific reports of NULES of Ukraine, 0(6(70)). doi: 10.31548/dopovidi2017.06.011
30. Chepiha, A. M., Kostenko, S. O., Korol, P. V., Doroshenko, M. S., Konoval, O. M., Lu, L., ... & Li, L. (2017). Analysis of physical and morphological parameters and incubation ability of eggs in Shaoxing ducks of different ages. Breeding and genetics of animals, 54, 119-126.
31. Shaoxing Ducks [S]: DB 33068/T 02.1–2012.–Zhuji: Zhuji Quality and Technique Supervision Bureau, 2012. – (National Standard of China). 40.
32. Zhelyazkov, E., & Tsvetanova, Y. (2002). Manual of Genetics. Stara Zagora, 175-195.
33. Xia, W. G., Chen, W., Abouelezz, K. F. M., Azzam, M. M. M., Ruan, D., Wang, S., ... & Zheng, C. T. (2019). Estimation of calcium requirements for optimal productive and reproductive performance, eggshell and tibial quality in egg-type duck breeders. Animal, 1-9.
34. Adamski, M. (2005). The effect of genotype on egg morphological and physical traits of ducks during the first laying period. The works of The Commission of Agricultural and Biological Sciences BTN, 55, 13-24.
35. Rahman, M. M., Khanr, M. J., Aiam, M.S., Islam, M. A., & Ranaa, M. J. (2010). Egg quality characteristics of three genotypes of duck reared in the coastal area of Bangladesh. Journal of the Bangladesh Society for Agricultural Science and Technology, 97-102.
36. Gorski, J., Pietkiewicz, M., & Witak, B. (1998). Evaluation of egg quality in meat ducks. Science Journals PTZ Breeding Journal, 36, 349-356.
37. Huang, J. F., & Lin, C. C. (2011). Production, composition, and quality of duck eggs. Council of Agriculture, Taiwan Woodhead Publishing Limited.
38. Chepiha, A. M., Kostenko, S. O., Korol, P. V., Konoval, O. M, Lu, L., Bu, X., ... & Li, L. (2017). Analysis of physical-morphological indicators of eggs of different color in the shaoxing breed of ducks. Collection of scientific works "Visnyk of Zaporizhzhya National University. Biological Sciences", 2, 34-41.
39. Chepiga, A. M., Kostenko, S. A, Svyrydenko, N. P., Doroshenko, M. S., Kyrienko, A., Korol, P. V., ... & Lu, L. (2018). Microsatellite analysis of the population of the rock with different levels of egg productivity. Scientific bulletin of NUBIP. Series technology of production and processing of livestock products, 289, 77-85.

Дорошенко М. С., Костенко С. О., Король П. В., Чепіга А. М., Сидоренко О. В., Джус П. П., Свириденко Н. П., Литвиненко Т. В., Коновал О. М., Лу Л., Філіпова П. А., Олійник Р. С., Лі Л., Драгулян М. В. (2021). ПРОДУКТИВНІСТЬ ГЕРМІНАТИВНИХ ХИМЕР КАЧОК ТА ЇХНІХ НАЩАДКІВ. *ANIMAL SCIENCE AND FOOD TECHNOLOGY, 12(4): 5-17. https://doi.org/10.31548/animal2021.04.001*

Анотація. *Для аналізу можливого впливу химеризації на продуктивність гермінативних химер качок та їхніх нащадків проаналізували яєчну продуктивність та живу масу гермінативних качок, їхніх дочок та аналогів. Об'єктами дослідження були качки (Anas platyrhynchos) порід Шанма (Shan partridge duck) та Шаосінь, що утримуються на качиній фермі Zhuji Guowei Poultry Development Co., Ltd, Китай. Дослідження проводили в лабораторії генетики птиці Чжецзянської Академії аграрних наук та качиній фермі компанії Zhejiang Generation Biological Science and Technology Co., Ltd. Для отримання химер качок застосували метод, описаний Aige-Gil & Simkiss (1991) та Тагіровим (2010). Бластодиски виділяли зі щойнознесених запліднених яєць за допомогою кільця з фільтрувального паперу. Як реципієнтів використовували ембріони качок Шанма, а донорів – ембріони качок Шаосінь, гомозиготні за алелем гена кольору оперення (дикий тип). Бусульфан (SigmaAldrich, США) використовували як хімічний агент, що пригнічує поділ первинних статевих клітин ембріонів-реципієнтів. У реципієнтів (порода Шанма) між тупим і гострим кінцями яєць було зроблено отвір (вікно) у яєчній шкаралупі. Це зменшило відстань між інжектором і голкою ембріона. Яйця реципієнтів інкубували впродовж 8–10 годин при температурі 38 °С. Після інкубації яєць реципієнтів упродовж 8 годин вікна в них були відкриті. Бусульфан вводили в підембріональну порожнину за допомогою мікропіпетки (1,5–3 мкл рідини). Після введення бусульфану порожнину яйця заповнювали культуральним середовищем (RPMI-1640) з антибіотиками (ампіцилін, стрептоміцин), отвір закривали поліетиленовою плівкою та клейкою стрічкою. Яйця інкубували при зниженій температурі (+32 °С) упродовж 24 годин щоби продовжити тривалість дії бусульфану на первинні статеві клітини. Понад 50% ембріонів загинули впродовж перших 2–3 діб після початку інкубації. Порушення голови та шиї спостерігалися в 1,2% ембріонів. Введення бусульфану в концентрації 300 нг/яйце призводить до 95,0–96,3% смертності качиних ембріонів, у концентрації 150 нг/яйце – 33,3–75,3%, у концентрації до 75 нг/яйце – 18,75–38,5%. Для того, щоб оцінити яєчну продуктивність гермінативних химер качок був проведений аналіз експериментальних тварин та їх контрольних аналогів. Аналіз віку статевого дозрівання (відкладання першого яйця) свідчить про те, що химери дозріли пізніше. Якщо в контрольній групі середній вік статевого дозрівання становив 139 ± 9 діб, то в групі химер – 148 ± 13 діб. Отже, можна засвідчити, що в нашому експерименті химери дозріли пізніше, ніж контрольні тварини, що може бути пов'язано з дією бусульфану при стерилізації ембріонів-реципієнтів. Середня жива маса качок контрольної групи була нижчою, а сама група була більш згуртованою. Так, у контрольних качок вага складала 1422,40 ± 57,00 г, у химер – 1608,80 ± 94,76 г. Перевага химер над контрольною групою за живою масою може бути пов'язана з тим, що контрольну групу складали реципієнти породи Шанма. Яєчність качок за весь період дослідження становила 87,5 ± 0,05% (контроль) і 79,5 ± 0,12% (бусульфан). Маса яєць у качок двох груп за весь період становила: 70,62 ± 0,199 г (контроль) і 71,15 ± 0,157 г. Морфометричні показники яєць досліджуваних груп качок: середні значення довжини яйця – 6,056 ± 0,0564 см (контроль) та 6,269 ± 0,1341 см (бусульфан); ширина яєць – 4,520 ± 0,0053 см (контроль) і 4,529 ± 0,004 см (бусульфан). Статистичних міжгрупових відмінностей за морфометричними параметрами яєць досліджуваних груп не було. Фактично ми отримали результати, подібні до попередніх, які стосувалися несучості дочок химер селезня. Аналіз продуктивності дочок гермінативних химер качок свідчить,*

що загалом химеризація батьків не вплинула на продуктивність їхніх дочок. Аналіз продуктивності групи дочок, отриманих від химерних тварин, свідчить, що за більшістю показників ця група займає проміжне місце між групами, чиї породи слугували донорами та реципієнтами. Метод, який ми використовували для отримання химер, можна успішно використовувати в качок для збереження генетичних ресурсів. Збереження заморожених статевих клітин рідкісних видів птахів та місцевих порід птиці з перспективою подальшого розмноження за допомогою гермінтативних химер знизить ризик зменшення їх генетичного різноманіття.

Ключові слова: *гермінативна химера, качка шаосінь, шанма, бусульфан, яєчна продуктивність качок*