



Journal homepage: <https://animalscience.com.ua/en>

*Animal Science and Food Technology*, 12(4), 5-17

Received: x.xx.2021 Revised: x.xx.2021 Accepted: x.xx.2021

UDC 636.5.082.597.034.453

DOI: 10.31548/animal2021.04.001

## Productivity of germinative duck chimaeras and their descendants

Maria Doroshenko<sup>1</sup>, Svitlana Kostenko<sup>1</sup>, Petro Korol<sup>2</sup>, Alona Chepiha<sup>3</sup>, Olena Sydorenko<sup>2</sup>, Pavlyna Dzhus<sup>2</sup>, Natalia Svyrydenko<sup>1</sup>, Tamara Lytvynenko<sup>1</sup>, Oksana Konoval<sup>4</sup>, Lizhi Lu<sup>5</sup>, Polina Filipova<sup>1</sup>, Regina Oleynik<sup>1</sup>, Liumeng Li<sup>6</sup>, Maria Drahulian<sup>1</sup>

<sup>1</sup>National University of Life and Environmental Sciences of Ukraine  
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine

<sup>2</sup>Institute of Animal Breeding and Genetics and a. M. V. Zubets,  
National Academy of Sciences of Ukraine  
08321, 1 Pogrebnyaka Str., Kyiv region, Chubynske, Ukraine

<sup>3</sup>Private Joint Stock Company "On The Production Of Insulin "INDAR"  
02099, 5 Zroshuvalna Str., Kyiv, Ukraine

<sup>4</sup>Ukrainian Laboratory of Quality and Safety of Agricultural Products of National  
University of Life and Environmental Sciences of Ukraine  
08162, 7 Mashynobudivna Str., Kyiv region, Chabany, Ukraine

<sup>5</sup>Zhejiang Academy of Agricultural Sciences  
310058, Zijingang Campus, Hangzhou, China

<sup>6</sup>Zhejiang Guowei Technology Co. LTD,  
310058, Zhejiang, Hangzhou, China

**Abstract.** The relevance of the study is conditioned upon the necessity to explore the possible influence of chimerisation on the productivity of germinative duck chimaeras and their descendants. To obtain duck chimaeras, the method described by Aige-Gil & Simkiss and Tagirov was applied. Shanma duck embryos were used as recipients and Shaoxin duck embryos homozygous for the plumage colour gene (wild type) were used as donors. To evaluate the egg production of germinative chimaeras of ducks, the analysis of experimental animals and their control counterparts was performed. Analysis of the age of sexual maturation (laying the first egg) indicates that the chimaeras matured later. While in the control group the average age of puberty was 139±9 days, in the chimaera group it was 148±13 days. Thus, it can be concluded that in this

---

### Suggested Citation:

Doroshenko, M., Kostenko, S., Korol, P., Chepiha, A., Sydorenko, O., Dzhus, P., Svyrydenko, N., Lytvynenko, T., Konoval, O., Lu, L., Filipova, P., Oleynik, R., Li, L., & Drahulian, M. (2021). Productivity of germinative duck chimaeras and their descendants. *Animal Science and Food Technology*, 12(4), 5-17.

\*Corresponding author

experiment chimaeras matured later than control animals, which may be related to the effect of busulfan during the sterilisation of recipient embryos. The average live weight of ducks in the control group was lower, and the group itself was more united. Thus, in control ducks, the weight was  $1422.40 \pm 57.00$  g, and in chimaeras –  $1608.80 \pm 94.76$  g. The advantage of chimaeras over the control group in terms of live weight may be related to the fact that the control group consisted of recipients of the Shanma breed. Egg production of ducks for the entire research period was  $87.5 \pm 0.05\%$  (control) and  $79.5 \pm 0.12\%$  (busulfan). The weight of eggs in ducks of two groups for the entire period was:  $70.62 \pm 0.199$  g (control) and  $71.15 \pm 0.157$  g. Morphometric parameters of eggs of the researched groups of ducks: average values of egg length –  $6.056 \pm 0.0564$  cm (control) and  $6.269 \pm 0.1341$  cm (busulfan); egg width –  $4.520 \pm 0.0053$  cm (control) and  $4.529 \pm 0.004$  cm (busulfan). There were no statistical intergroup differences in the morphometric parameters of the eggs of the research groups. Analysis of the productivity of daughters of germinative duck chimaeras demonstrates that, in general, the chimerisation of parents did not affect the productivity of their daughters. The analysis of the productivity of the group of daughters obtained from chimeric animals demonstrates that, by most indicators, this group occupies an intermediate place between the groups whose breeds served as donors and recipients. The method author of the research uses to obtain chimaeras is of practical value for the conservation of genetic resources

**Keywords:** germinative chimaera, Shaoxing duck, shanma, busulfan, egg productivity of ducks

## 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 & Petite, 2004; Kagami, 2016). The methods of cloning and transgenesis have become a routine tool for designing animal models of development (Kathleen *et al.*, 2010), diseases (Ogilvie *et al.*, 2017), bioreactors and producers of valuable biologically active drugs (Petite & Mozdziak, 2002; Pavlou & Reichert, 2004), highly productive aquaculture objects (Devlin *et al.*, 2009). However, using the conventional technique for microinjection of foreign DNA into the pronucleus of a fertilised egg, which is well-developed for many mammalian species

(Gordon & Ruddle, 1981), encounters difficulties when involved with birds (Perry, 1988; Love *et al.*, 1994). The development 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 frequently used in mammals, is practically impossible for birds since fertilisation occurs in the infundibulum of the reproductive tract and can be polyspermic (Mozdziak & Petite, 2004). Therefore, manipulation of the zygote proved difficult to use in the development of a transgenic bird (Love, 1994). Over the past decades, some alternative strategies have been developed to obtain transgenic poultry by using chimeric animals established by the transfer of blastodermal cells.

Primordial germ cells are successfully used to establish 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, currently, the efficiency of transgenic poultry in many cases remains very low, and the technique of using ducks to establish transgenic birds is practically not developed (Sztań *et al.*, 2012).

### Analysis of Recent Researches and Publications

Nowadays, the duck (*Anas platyrhynchos* Linnaeus, 1758) is an understudied 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 appealing means for the synthesis of therapeutic proteins since the sterile content of eggs is protected by a hard eggshell. Busulfan is used to suppress cell proliferation. Injection of busulfan into the subgerminal cavity is one of the methods that increase the number of donor cells during the development of chimaeras (Aige-Gil & Simkiss, 1991; Tagirov, 2010).

However, the methods for developing germinative duck chimaeras encounter difficulties associated with the structure of the eggshell in waterfowl; the consequences of chimerisation and its potential influence on the productivity of chimaera 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 chimaeras was

previously explored (Doroshenko *et al.*, 2018). For the analysis of survival, Korol *et al.* (2021) used embryos obtained using various methods of introducing the DNA.

To evaluate the egg productivity of daughters from germinative chimaeras (males), a study was performed on three groups of ducks with different origins. Analysis of the productivity of daughters from germinative duck chimaeras demonstrated that, in general, the chimerisation 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 author of the research used to obtain chimaeras can be successfully used on ducks 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 chimaeras will reduce the risks of a decrease in the genetic diversity of birds (Doroshenko *et al.*, 2021).

Thus, the productivity of daughters from drake chimaeras was explored. However, the egg productivity of female chimaeras remains understudied. This work is devoted to this issue.

### Materials and Methods of Research

All experiments with animals were performed according to 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 chimaeras was used a method such as the production of somatic and embryonic chimaeras 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. Sterilisation 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 fertilised 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<sub>2</sub>HPO<sub>4</sub>; 1.8 mM KH<sub>2</sub>PO<sub>4</sub>; 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 chimaeras.* Shanma duck embryos were used as recipients, and Shaoxing duck embryos, homozygous for the plumage

colour 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 eggshell. 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. It 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 explored. 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 measured 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 chimaeras ( $F_0$ ) were obtained. For the first time, to obtain blastodermal chimaeras 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 designed for developing germinative duck chimaeras using busulfan injections. It has been demonstrated that duck embryos are more sensitive to busulfan than hens embryos. Injection of busulfan at a concentration of 300 ng/egg results in the mortality of 95.0-96.3% 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 chimaeras was 65-77.8%.

Analysis of the age of puberty (laying of the first egg) indicates that the chimaeras matured later. If the average age of puberty in the control group was  $139.5 \pm 9.67$  days, then in the group of chimaeras –  $148.2 \pm 13.13$  days. Thus, the author of the research can attest that in this experiment, the chimaeras matured later than the control animals, which may be due to the effect of busulfan in the sterilisation of recipient embryos. After the onset of puberty in this generation, the author analysed the live weight in two groups of ducks aged from 41 to 61 weeks.

**Table 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 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 chimaeras – 1608.08±94.76 g. The predominance of chimaeras over the control group in terms of live weight may be explained by the fact that the control group consisted of recipients of the Shanma breed. This breed is characterised by egg productivity and is lighter than the Shaoxing breed (embryos of this breed served as donors). Thus, donor cells could be affected by the weight gain of chimaeras. The author's previous study demonstrated 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 chimaeras – 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 using busulfan, correspond to similar indicators of both the control group and the offspring of male germinative chimaeras.

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

In previous studies, the mean values of egg production per month in the investigated ducks were 27.52±0.84 % in the group of daughters from the Shanma breed drake, 27.47±0.61% in the group of daughters from Shaoxing breed drake and 27.73±0.53 eggs in the group of daughters from germinative chimaeras (Doroshenko *et al.*, 2021). It corresponded to approximately 91.56-92.4%.

Thus, the sterilisation 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 impaired, 50.43% of the eggs after artificial insemination were unfertilised. Therewith, the percentage of fertilised 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).

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 weight of eggs in experimental ducks is normal, as 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. Notably, according to this indicator, the egg weight (71.21 g) in the group of ducks of the 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. Results similar to the previous ones concerning the egg production of daughters from chimaera drake were obtained.

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. In addition, a similar feature was 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 chimaeras of ducks demonstrated that, in general, the chimerisation of their parents did not affect the performance of daughters. An analysis of the productivity of a group of daughters obtained from chimeric animals demonstrates 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 the previous studies on the productivity of Shaoxing and Shanma ducks, the association of duck productivity with age (Chepiga *et al.*, 2017), egg colour (Chepiga *et al.*, 2017) and microsatellite loci (Chepiga *et al.*, 2018) were presented.

Hypothetically, the procedure for obtaining chimeric offspring cannot affect the productive qualities of their offspring. It is known that reproductive chimaeras can have reduced fertility and be sterile (Doroshenko *et al.*, 2017). However, the descendants of chimaeras are not connected with the process of chimerisation of their parents. The descendants of donors have the properties of donors and the descendants of recipients – recipients, respectively, since the chimerisation procedure does not affect hereditary information. Using busulfan as an agent

that inhibits the proliferation of recipient cells can cause a mutagenic effect, but teratogenic effect and high embryonic mortality were observed. Possibly, the chimerisation procedure affects the survival of primary germ cells and, thus, cell selection occurs at the early stages of development. The data obtained may indicate the necessity of further investigation of the effect of chimerisation procedures on the first generation of chimaeras and their descendants.

Notably, the populations investigated by authors are not pure breeding lines but polymorphic at the loci of quantitative traits, which could affect the results of the studies.

### Conclusions

The method used to obtain chimaeras can be successfully applied in ducks to preserve genetic resources. Analysis of the age of puberty (laying of the first egg) indicates that the chimaeras matured later. If in the control group the average age of puberty was 139.5±9.67 days, then in the group of chimaeras – 148.2±13.13 days. Thus, the author of the research can attest that in this experiment, the chimaeras matured later than the control animals, which may be due to the effect of busulfan in the sterilisation 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 chimaeras 1608.80±94.76 g. The predominance of chimaeras over the control group in terms of live weight may be explained by the fact that the control group consisted of recipients of the 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. Results similar to the previous ones concerning the egg production of daughters from chimaera drake were obtained.

Preservation of frozen germ cells of rare bird species and native bird breeds with the prospect of their reproduction using germinal chimaeras 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., & Petite, 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] Petite, J.N., & Mozdziaik, 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] Sztań, N., Patakine' Várkonyi, E., Liptó'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]. *Biotechnologiya*, 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 biotechnological 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 hermitative 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] Petite, J.N., Clark, M.E., Liu, G., Verrinder Gibbins, A.M., & Etches, R.J. (1990). Production 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 chimer 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.

## Продуктивність гермінативних химер качок та їхніх нащадків

Марія Сергіївна Дорошенко<sup>1</sup>, Світлана Олексіївна Костенко<sup>1</sup>,  
Петро Вікторович Король<sup>2</sup>, Альона Михайлівна Чепіга<sup>3</sup>,  
Олена Василівна Сидоренко<sup>2</sup>, Павлина Петрівна Джус<sup>2</sup>,  
Наталія Петрівна Свириденко<sup>1</sup>, Тамара Валентинівна Литвиненко<sup>1</sup>,  
Оксана Миколаївна Коновал<sup>4</sup>, Ліжи Лу<sup>5</sup>, Поліна Олександрівна Філіпова<sup>1</sup>,  
Регіна Святославівна Олійник<sup>1</sup>, Ліуменг Лі<sup>6</sup>, Марія Валеріївна Драгулян<sup>1</sup>

<sup>1</sup>Національний університет біоресурсів і природокористування України  
03041, вул. Героїв Оборони, 15, м. Київ, Україна

<sup>2</sup>Інститут розведення і генетики тварин імені М.В.Зубця НААН України  
08321, вул. Погребняка, 1, Київська обл., Бориспільський р-н, с. Чубинське, Україна

<sup>3</sup>Приватне акціонерне товариство «По виробництву інсулінів «Індар»  
02099, вул. Зрошувальна, 5, Київ, Україна

<sup>4</sup>Українська лабораторія якості і безпеки продукції АПК, НУБіП України  
08162, вул. Машинобудівників, 7, Києво-Святошинський р-н, с.м.т. Чабани, Україна

<sup>5</sup>Чжецзянська академія сільськогосподарських наук  
310021, Чжецзянь, Ханчжоу, Китай

<sup>6</sup>Zhejiang Guowei Technology Co. LTD  
311800, Чжецзянь, Ханчжоу, Китай

**Анотація.** Актуальність дослідження зумовлена необхідністю вивчення можливого впливу химеризації на продуктивність гермінативних химер качок та їхніх нащадків. Для отримання химер качок застосували метод, описаний Aige-Gil & Simkiss та Тагіровим. Як реципієнтів використовували ембріони качок Шанма, а донорів – ембріони качок Шаосінь, гомозиготні за алелем гена кольору оперення (дикий тип). 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 складала реципієнти породи Шанма. Яєчність качок за весь період дослідження становила  $87,5 \pm 0,05\%$  (контроль) і  $79,5 \pm 0,12\%$  (бусульфан). Маса яєць у качок двох груп за весь період становила:  $70,62 \pm 0,199$  г (контроль) і  $71,15 \pm 0,157$  г. Морфометричні показники яєць досліджуваних груп качок: середні значення довжини яйця –  $6,056 \pm 0,0564$  см (контроль) та  $6,269 \pm 0,1341$  см (бусульфан); ширина яєць –  $4,520 \pm 0,0053$  см (контроль) і  $4,529 \pm 0,004$  см (бусульфан). Статистичних міжгрупових відмінностей за морфометричними параметрами яєць досліджуваних груп не було. 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, становить практичну цінність для збереження генетичних ресурсів

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