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Impact of biotechnological transgenesis procedures on duck productivity

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Abstract. The use of poultry as a unique model of biological research was characterised by a high level of efficiency, however, methods for creating transgenic ducks, complicated by the structure of waterfowl eggshells, are of low efficiency. The purpose of the study was to determine the influence of various biotechnological procedures for creating transgenic ducks on their productive qualities and reproductive ability to identify the optimal method for creating transgenic poultry for further

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use in scientific, research, or economic purposes. Weighting, morphometric and statistical analysis of productive traits were used during the study. 40 ducks (4 experimental groups of animals and about 3,000 of their eggs) were studied. The lowest value of the egg productivity index was obtained in the group created by busulfan injection (79.5±11.8%), the highest – in the group created by sperm-mediated gene transfer (91.8±2.3%), the group of direct injection of transgenic construct – 89.0±2.0%, which indicates that this biotechnological method of introducing transgenic construct did not have a clear effect on this indicator. The weight of ducks in different experimental groups ranged from 1,323.50±65.36 g (using the sperm-mediated gene transfer) to 1,608.08±94.76 g (in the group created using busulfan). Ducks that received direct injections had an average weight of 1,480.42±35.01 g. In the control group, the average weight at sexual maturity was 139.5±9.67 g, in the busulfan group – 148.2±13.13 g, in the direct injection group – 143.16±7.25 g, and in the sperm-mediated gene transfer group – 140.67±13.13 g. It was found that the method of injection into the embryo of a recipient sterilised with busulfan and the introduction of donor blastodermal cells negatively affect the reproductive qualities of ducks. The practical significance of the study lies in the fact that as a result of the analysis of the productivity of ducks obtained by various methods of transgenesis, it was determined that the most effective of the evaluated methods is the transfection of DNA of the transgenic construct with sperm (Sperm-mediated gene transfer, SMGT)

Keywords: egg size; egg weight; egg productivity; injections; blastodermal chimera; busulfan

Introduction

Due to its compact size, simple rearing conditions, zootechnical manipulations (artificial insemination, automated feeding, egg collection) that do not require highly qualified personnel, reproductive potential, generation interval, and embryo development without the mother's body, the bird can be used in a wide range of applied and fundamental research (Kim *et al.,* 2023). A significant advantage of a bird that serves as an object of transgenesis is the possibility of using its short generation interval, reproduction rate, and high feed conversion rate, which significantly exceeds the capabilities of other species (Ziaei & Amini, 2020). As noted by J.S. Park *et al.* (2020), transgenesis and cloning methods have become convenient tools for creating highly productive animals, animal development models, diseases, animal bioreactors, and producers of valuable biologically active drugs. As of 2023, transgenesis has become a routine procedure that allows creating an animal producer of unique high-molecular compounds in the pharmaceutical and pharmacological industries, an animal model of evolution (and hereditary diseases (Hamernik*,* 2019*;* Kim *et al*., 2020; Dehdilani *et al*., 2022). J. Lee *et al.* (2020) suggest that the first and most effective method of obtaining transgenic animals was to make changes to the zygote genome, which is successfully used on mammals, but is not effective in similar manipulations with the bird genome, which is due to the differences in the reproductive systems of mammals and birds, the uniqueness of the development of the embryo in the egg.

The creation of transgenic poultry is carried out using three main methods: 1) sperm-mediated gene transfer (SMGT) 2) injection of a DNA vector under the embryo cavity of a newly laid egg; 3) introduction of transfected blastomeres into the embryo (Kwon *et al.,* 2018*;* Konoval *et al*., 2019; 2021). The creation of transgenic poultry by injection is complicated by the lack of transparency of its egg, the presence of a large yolk, and the uniqueness of the reproductive system of the Avis class (Ibrahim &

Stadnicka, 2023). The wide pores of the shell of waterfowl cause infection of embryos in eggs opened for manipulation and prevent the success of the formation of the duck germ chimera, reducing the effectiveness of intervention procedures in embryonic development. This, according to T.L. Saunders (2023), significantly complicates the creation of appropriate conditions for carrying out the necessary biological procedures and has an impact on the efficiency of various methods of introducing transgenic constructs. SMGT is one of the effective methods for obtaining transgenic animals from the period when it was established that sperm can be used as a vector for delivering a gene to an egg. The advantages of SMGT are related to its simplicity, efficiency, speed, and cheapness.

An urgent problem in the study of biotechnological transgenesis procedures is their impact on the productive qualities of created ducks. The consequences of chimerisation and transgenesis, and their possible impact on the productivity of chimera offspring, are still poorly understood. The purpose of the study is to determine the effect of biotechnological transgenesis procedures (busulfan injection, direct injection of a transgenic construct into the subgerminal cavity of embryos at stage X, sperm transfection) on the productive qualities and reproductive ability of ducks.

Literature Review

In order to create a transgenic animal, when all the cells of its body carry foreign genetic information, the transfer of genetic constructs is carried out in the earliest periods of embryogenesis, starting with the zygote, so injecting genetic constructs into the nucleus of an egg is the most common method for creating transgenic animals. Successful transgenesis can be considered if the introduced genetic construct is present in every cell of the created organism, or the ability of this organism to produce gametes with the target genetic construct, which will allow obtaining offspring with the desired genotype. However, the reproductive system of poultry complicates the process of transgenesis compared to mammals. The duck oocyte has a one-day maturation period. The large size of poultry oocytes makes them vulnerable to damage when used in biotechnological manipulations. Normal embryonic development involves the presence of three membranes – protein, subcutaneous, and shell. The fertilised egg begins to break up in the oviduct (protein section), which leads to the presence of 40-60 thousand blastomeres in the newly laid egg, worsening the level of effectiveness of transgenesis procedures (Kim *et al.*, 2023).

Among other methodological approaches to transgenesis that are more effective for poultry is the creation of transgenic chimeras by transplanting early embryonic cells of donors after delivery of the target gene construct to their nuclei. Delivery of plasmid DNA to embryonic cells is one of the most difficult problems of non-viral transfer of exogenous genes. Synthetic peptides or non-cationic lipids (liposomes) are used to increase the efficiency of plasmid DNA transfer, which reduces cytotoxicity and increases resistance to inactivation by intracellular mechanisms, since minimal loss of pluripotent cells can negatively affect the normal development of the body (Longmuir *et al.*, 2001). Various strategies for creating a genetically modified transgenic poultry described above are currently being proposed, including the use of viral vectors in stage X embryos, and transfer of exogenous genes by injection into zygotes or embryonic stem cells, however, these methods have been little used in poultry farming, as the transmission of the transgene to subsequent generations using these methods is not stable. Another strategy for transgenesis in poultry is the cultivation of primordial germ cells in vitro (Han & Park, 2018).

The experience of creating blastodermal chimeras is described by J.N. Petitte *et al.* (1990). In the study, blastoderm cells were isolated from chicken embryos of a line with black

feathers corresponding to the recessive allele at locus 1 and transferred to chicken embryos with white feather pigment. As a result, 6 embryos out of 53 had black feathers. That is, 11.3% of the embryos were phenotypic chimeras by feather colour. One embryo with black feathers survived to hatch and was crossed to analyse the offspring, resulting in two chicks out of 719 with black plumage. This proves the possibility of using blastodermal cells to create a germ line and transmit it in generations.

The reproductive potential of poultry can be influenced by factors such as the breed of ducks, the ratio of males and females in the group, and housing conditions (for example, temperature). Reproductive capacity and fertilisation also depend on the ability of females to ovulate, the absence of pathological conditions of the reproductive system, and the provision of an appropriate environment for egg formation and development (Yakubu, 2013). The quality and quantity of sperm deposited by males is also an important factor for obtaining a high fertility rate. In addition, a significant factor of influence is the age of ducks; it affects the reproductive characteristics of both males and females (Morduzzaman *et al.*, 2015).

Hatchability is one of the most important indicators for successful productive poultry farming. Factors that affect hatchability include: belonging of the duck to the breed or breed line, the weight of the laid egg, and the conditions of its incubation (Narahari *et al.*, 1991; Abd El-Hack & Hurtado, 2019). Incubation conditions can be called optimal if the hatchability is at the maximum level, this is ensured by regulating the microclimate indicators, such as temperature and humidity, sufficient air exchange (ventilation), and periodic changes in the slope of eggs. Before laying eggs for incubation, an ovoscopy is performed to extract unfertilised eggs. Disinfection of eggs and incubators has a positive effect on hatchability (Liptoi & Hidas, 2006).

The foetal mortality rate characterises the viability of embryos in fertilised duck eggs. Embryonic mortality is divided into early and late, depending on the incubation period at which the embryo died. The foetal mortality rate is influenced by several environmental, technological, and genetic factors. Thus, to date, there is data on the methods of creating transgenic poultry and the productive characteristics of ducks, however, the influence of the method of creating transgenic poultry on its productive characteristics has not been established.

Materials and Methods

The object of the study was the productivity of ducks (Anas platyrhynchos), which belonged to the Shan partridge (Shanma) and Shaoxing breeds, created using different biotechnological transgenesis procedures. The subject of the study was biotechnological methods for creating transgenic poultry. The study was conducted at the Department of Genetics, Breeding and Biotechnology of Animals of NUBIP of Ukraine and the Guowei research farm of the Zhejiang Academy of Agrarian Sciences. All studies were conducted in compliance with bioethical principles for vertebrate animals (Directive 2010/63/EU…2010).

In this study, 40 ducks were divided into 4 experimental groups and about 3,000 eggs obtained from the birds under study were investigated. The control group consisted of Shaoxing ducks. To establish a group of ducks created by busulfan injection, the Shaoxing breed was used as recipients and the Shanma breed as donors. The Shanma breed was used in studies of the productivity of ducks obtained by direct injection of a transgenic construct into the subgerminal cavity. Sperm transfection was performed with Shaoxing ducks. The control group consisted of 10 animals, a group of ducks created using busulfan – 5 animals, a group of individuals obtained using direct injections of transgenic constructs into the subgerminal cavity – 6 animals, a group of ducks hatched from eggs, whose mothers were artificially inseminated with sperm-mediated gene transfer

from donor ducks – 19 individuals. All the ducks under study were kept in the same conditions, using a cage type of housing with constant access to water and feed. The study was conducted for 142 days. Body weight was determined individually with an accuracy of 10 g for all ducks aged 41 to 61 weeks.

The average weight and size of eggs were determined daily. The length (L) and breadth (B) of the eggs are measured with an accuracy of 0.1 mm using a caliper.

Eggs were weighed using an electronic scale (JM-a 20001) with an accuracy of 0.1 g. The egg shape index was calculated using the equation:

$$SI = 100 \times d/D, \quad (1)$$

where d – transverse (small) diameter, mm; D – longitudinal (large) diameter, mm.

The egg productivity index was calculated using the equation:

$$EPI = D/AE \; x \; 100, \quad (2)$$

where D – number of days of the study period of the test duck, starting from the first day of egg production (laying the first egg); AE – total number of eggs obtained for the entire period of egg production from the duck under study, starting from the first day of egg production (laying the first egg).

The obtained data was statistically processed on a computer using the MS Excel 2016 spreadsheet processor using descriptive statistics and the F-criterion.

Results and Discussion

The results of the analysis of productive traits of the control group of ducks are presented in Table 1. The egg production of the control group, live weight of the birds, and the age of sexual maturity of the animals under study were consistent with the data obtained in previous studies of the Shaoxing breed (Chepiha *et al.,* 2017).

Table 1. *Duck performance in the control group*

Indicator	M±m	Cv±mCv
Egg productivity (during the week)	*6.01±0.209	29.9±0.054
Egg productivity index (142 days), %	87.5±4.53	16.4±0.090
Live weight, g	1,422.40±57.00	12.7±0.079
Age of sexual maturity, days	139.5±9.67	21.8±0.104
Egg weight, g	70.6±0.198	9.20±0.006
Egg length, cm	6.05±0.056	3.65±0.042
Egg breadth, cm	4.52±0.053	3.89±0.044
Egg shape index, %	*75.7%±0.3	0.7±0.018

Source: compiled by the authors

The data on the performance of ducks created by busulfan treatment of Shaoxing recipient embryos with Shanma donor embryos are shown in Table 2. According to Table 2, egg productivity of ducks (during the week) was within the same indicator of the Shaoxing breed. The egg productivity index was somewhat inferior to the control data. Live weight was within the limits typical of the Shaoxing breed. The age of sexual maturity was somewhat inferior to the control. By egg weight, the animals had indicators that are characterised by a larger range of variability than the control group. The egg productivity index (for 142 days) of the duck group created using busulfan was lower than that of the control group. This may indicate a negative impact of the procedure for sterilising donor embryos.

Table 2. *Productivity of a group of ducks created by busulfan injection*

Indicator	M±m	Cv±mCv
Egg productivity (during the week)	6.4±0.268	27.0±0.077
Egg productivity index (142 days), %	79.5±11.8	32.8±0.181
Live weight, g	*1,608.08±94.76	13.2±0.114
Age of sexual maturity, days	148.2±13.13	19.8±0.140
Egg weight, g	***71.4±0.157	5.07±0.071
Egg length, cm	6.26±0.134	4.93±0.070
Egg breadth, cm	4.53±0.041	2.09±0.045
Egg shape index, %	75.2%±0.3%	0.8±0.028

Note: statistical significance: *p<0.05; **p<0.01; ***p<0.001
Source: compiled by the authors

Table 3 shows the data obtained on the productivity of a group of ducks created by injecting DNA of the transgenic construct into the germinal cavity of a freshly laid egg. Egg production (during the week) in this group had no statistically significant differences from other groups. However, the egg productivity index (for 142 days) was statistically significantly different from the other groups. The eggs of this group weighed less than the group created by busulfan injections.

Table 3. *Productivity of a group of ducks created by direct injection of transgenic construct DNA into the subgerminal cavity of embryo*

Indicator	M±m	Cv±mCv
Egg productivity (during the week)	6.50±0.11	16.55±0.03
Egg productivity index (142 days), %	**89.0±2.0	9.22±0.022
Live weight, g	*1,480.42±35.01	10.03±0.023
Age of sexual maturity, days	143.16±7.25	21.47±0.049
Egg weight, g	***69.3±0.141	9.05±0.021
Egg length, cm	6.02±0.008	10.41±0.025
Egg breadth, cm	4.51±0.016	3.90±0.009
Egg shape index, %	***74.66±0.1	4.54±0.011

Note: statistical significance: *p<0.05; **p<0.01; ***p<0.001
Source: compiled by the authors

Table 4 shows the performance indicators of a group of ducks obtained by using sperm-mediated gene transfer. Egg production (during the week) in this group was the highest. The egg productivity index (for 142 days) of this group was the highest among all groups. The live weight of the group's poultry was slightly lower than that of other groups. A comparison of the data obtained shows that ducks in this group had the lowest age of onset of sexual maturity among the experimental groups. The egg weight of this group was inferior to other groups. Egg length in animals of this group was the smallest, egg breadth was also inferior to similar indicators of other groups.

Table 4. *Productivity of a group of ducks obtained by sperm-mediated gene transfer*

Indicator	M±m	Cv±mCv
Egg productivity (during the week)	*6.61±0.15	12.12±0.029
Egg productivity index (142 days), %	91.8±2.3	5.57±0.013
Live weight, g	*1,323.50±65.36	11.04±0.025
Age of sexual maturity, days	140.67±13.13	20.87±0.048
Egg weight, g	67.0±1.990	8.08±0.019
Egg length, cm	5.93±0.11	5.10±0.012
Egg breadth, cm	4.44±0.04	2.40±0.006
Egg shape index, %	*74.91±0.1	5.03±0.012

Note. Statistical significance: *p<0.05; **p<0.01; ***p<0.001
Source: obtained by the authors

Based on the data obtained by the authors of the current study and the calculations made, the influence of each of the methods for obtaining experimental animals on the performance indicators of their groups separately and on productivity in general was compared. The number of eggs laid by a female over a certain period of time is an indicator of her egg productivity, or egg production, and is the main feature, valuable for breeding selection and a decisive indicator, not only of the poultry egg, but also of the meat productivity. This is due to the fact that egg productivity determines the productive and reproductive qualities of the female, that is, ultimately, the amount of product received from one individual. This indicator is influenced by factors such as the age of ducks, breed, feeding and temperature conditions in the premises where animals are kept, etc., which is consistent with the results obtained by A.M. King'Ori (2011). In the current study, all experimental animals were kept in the same conditions. Egg production is genetically determined, as a high level of polymorphism of genes that affect poultry egg production is currently known (Huang & Lin, 2011). The influence of the genotype of animals on their egg productivity cannot be excluded, which may have caused fluctuations in the coefficients of variation obtained in this study in some groups, which actually reached 30% of the maximum level of biological variability characteristic of samples of unrelated individuals.

The data of the control group of ducks correspond to the performance indicators of their breed (Shaoxing) and the results of previous studies by the authors (Chepiha *et al*., 2019; Konoval *et al*., 2019; 2021*)*. Comparison of the results of the analysis of the control group and ducks obtained by busulfan injection indicates that there are no statistically significant differences between the groups in egg productivity (within a week). Table 3 shows data on the productivity of a group of ducks created by direct injection of a transgenic construct into the subgerminal cavity. Comparison with the control group showed differences in egg productivity index and egg weight. In terms of egg productivity during the week and egg production index, the group created by sperm-mediated gene transfer was better compared to other experimental groups. However, statistically significant differences were obtained only between the control group and the group obtained by using sperm-mediated gene transfer.

One of the most significant indicators that characterise trends in the growth and development of poultry is live weight. Live weight refers to quantitative characteristics and is determined by hereditary characteristics; balanced feeding and keeping conditions of poultry play an important role in the severity of hereditary

potential. Poultry with normal body weight have fewer problems with productive and reproductive functions than poultry with insufficient or excessive body weight for their age. Poultry growth is a complex biological process that occurs due to a combination of the interaction of genotype and environmental conditions. Normal growth is characterised by the formation of body weight, typical in size and shape for a given species, breed, line, and cross.

The results of the current study were as follows: for the control group – 1,422.40±57.00 g; for the group created with busulfan – 1,608.08±94.76 g; for the direct injection group – 1,480.42±35.01 g; for the group obtained by sperm-mediated gene transfer – 1,323.50±65.36 g. Evaluating the productivity of this indicator, it can be concluded that the negative effect of busulfan in the corresponding group is not observed in comparison with the indicators of egg productivity. In contrast, this group of ducks had a higher live weight than animals obtained by sperm-mediated gene transfer. It can be assumed that the live weight of ducks obtained by using busulfan was higher, since they had a lower egg productivity index. The animals obtained by DNA transfection of the construct and injections also statistically significantly differed in live weight. For the groups created by other methods, there was also no significant deviation of this indicator compared to the control group, which means that the biotechnological processes used do not have a significant impact on this indicator. The growth and weight gain of poultry have a complex structure and depend on genetic and environmental factors. These factors are species, sex, breed, care, and feeding, which is confirmed by R.G. Campbell *et al*. (1985). While growth is characterised by weight gain, development is characterised by changes in the functions, structure, and shape of tissues and organs in the body. The sex of birds also affects live weight. The effect of sex on poultry growth becomes more apparent with age. Although the growth of waterfowl varies by species, it is usually faster in males than in females. Providing poultry with nutrients and minerals, the cost and type of feed, bird health, well-being and environmental issues are important factors for ensuring normal growth and development of animals (Darmani Kuhi *et al.,* 2010). Another important aspect is the duck housing system, the impact of which is highlighted in the study by K. Önk *et al.* (2018)

The mean age of sexual maturity was 139.5±9.67 days for the control group, 148.2±13.13 days for the busulfan-treated group, 143.16±7.25 days for the direct injection group, and 140.67±13.13 days for the sperm-mediated gene transfer group. Again, a negative effect of busulfan on the reproductive ability of ducks can be observed, which is expressed in the later onset of sexual maturity of ducks compared to the control and other groups. Normal indicators of the age of sexual maturity in other groups indicate that there is no significant effect of transgenesis procedures on the physiological development and reproductive ability of the duck's body. Egg weight is the second most important breeding trait, which is of the greatest economic importance in the production of egg products. Weight is one of the indicators of egg quality, can indicate the suitability of eggs for incubation and has an impact on hatchability. This indicator is influenced by: duck breed, temperature conditions, and feeding (Okruszek *et al.,* 2006).

The average egg weight index is within the breed standard for all groups except the group created by sperm-mediated gene transfer, but there is no direct relationship between weight loss and the biotechnological procedure used. This indicator may be influenced by the age of ducks (Chepiha *et al.,* 2017). It is also important to keep in mind that when comparing the data obtained in this study regarding the average egg weight with the data obtained in other studies, it is necessary to consider the interbreeding difference. The difference in this

indicator between different breeds is described in the study by A. Okruszek *et al.* (2006). Also in this study, the following was given: the difference between ducks in different weeks of egg production. Egg quality indicator, which is determined by the ratio of the transverse diameter to the longitudinal one and expressed as a percentage – the egg shape index. The egg shape index score was normal in all the groups studied, regardless of the method of their creation. Despite large fluctuations in the average annual index of egg shape from individual laying hens (from 67 to 83%), the average coefficient of variability of the trait is small and varies mainly in the limits of 3.6-5.4%.

Normal indicators of the shape index for light cross ducks are 70%, heavy – 71%; for musk ducks – 72%. The egg productivity index is the optimal indicator for characterising the egg production of the ducks under study. The best result, as noted above, was obtained from the group created by sperm-mediated gene transfer (91.8±2.3%), the direct injection group of the transgenic construct also showed a fairly high performance indicator (89.0±2.0%), which indicates that this biotechnological method of introducing the transgenic construct did not have a clear effect on this indicator. The lowest value of the egg productivity index was obtained in the group created by busulfan injection (79.5±11.8%), so it can be assumed that the data obtained are related to the effect of the 4-butandiol dimethanesulfonate (busulfan), which inhibits the division of primary germ cells, sterilising the recipients' organisms before the injection of donor blastoderm cells and, thus, affecting the subsequent egg productivity of poultry.

The shape of eggs is practically not related to the features of feeding and keeping poultry. However, this trait is influenced by the age of ducks, their breed, and the week of the cycle when the egg-laying is examined. Special attention should be paid to reducing the shape variability that was characteristic of large eggs at the end of the egg-laying cycle, which have a variable shape and are prone to breaking. The hatchability of duck eggs within the normal shape index is almost identical, so selection for a certain standard egg shape should be introduced. Notably, this indicator may be influenced by other factors (Abd El-Hack *et al.,* 2019*)*. For example and analysis, data obtained in studies that examined this criterion of economic productivity of ducks and the factors that affect it can be cited. Thus, the study by I. Ismoyowati *et al.* (2020) examined the expression of growth hormone, the genes responsible for follicle-stimulating hormone receptors, the genes of prolactin, ovoinhibitor, melatonin receptor, and insulin-like growth factor-2 in the ovary, and found polymorphisms associated with egg-laying traits in the Jinding and Youxian duck breeds. This study confirms the genetic determinism in the manifestation of this trait. The groups of animals investigated by the researchers could be polymorphic in genes whose polymorphism is associated with the egg production of ducks. Other factors that have been studied can also be attributed to the influence of egg productivity and overall productivity factors, namely: breed, age of ducks, and temperature conditions in the room where the ducks are kept. In the current study, all animals were in the same room, were the same age, belonged to two breeds at the same time, or were chimeras of these two breeds, which could also affect their productive traits.

Summarising, it is worth noting that the results of the study of 4 groups of animals, one of which was control, and 3 experimental, indicate a high level of polymorphism within each of the groups, which may be conditioned by the influence of genes associated with the productivity of ducks. All the animals under study were equally descended from groups of animals belonging to two breeds that were polymorphic in their quantitative trait genes. Therefore, it is possible that the influence of this particular polymorphism was higher than the influence of the factor of the method of obtaining the

animals under study in terms of their productivity. Thus, the results confirm the need for careful individual selection of source material at the stage of preparing experiments that are complex in execution and long in duration of monitoring. Therefore, the question of creating duck lines with an increased frequency of homozygous loci (as is customary in studies, for example, with mouse and chicken lines) with a low level of internal linear polymorphism remains relevant. The creation of lines with a high frequency of homozygous loci can be time-consuming and unexpected in terms of results, given the presence of genetic burden and the effect of multiple allelism by genes of quantitative traits and the interaction of non-allelic genes on animal productivity.

All the animals under study were F_0 (in case of creating chimeras) or F_1 (when using sperm-mediated gene transfer) and descended from siblings, descendants of groups of ducks of the same origin. The starting material was egg embryos obtained from a group of ducks (P) that produced 50 eggs in one day, which were used in experiments to create transgenic ducks. In total, more than 2,000 newly laid eggs were used in the previous experiment, which ultimately produced only 36 female ducks and 19 male ducks, some of which were infertile (mostly males) and did not produce offspring. The main experimental losses were associated with embryonic mortality due to infection of embryos after violating the integrity of the eggshell of the recipient embryo when creating a window in a duck egg that has broad spores. The simplest and most successful method for obtaining transgenic offspring was the method of sperm-mediated gene transfer. It allowed the DNA of the construct to enter the oocyte together with the sperm, which helped to avoid experimental interference in the development of the embryo, and also gave hope that the organisms obtained would not be mosaics due to the presence of a transgenic construct in all their cells. This expectation was not met, possibly due to the rapid fragmentation of blastomeres and cell division during embryonic development, when embedding the construct in the duck genome was carried out for some time only in some blastomeres. All this indicates that there are a number of questions that should be addressed in the future when using transgenic constructs as a tool for creating animals with unique properties.

Conclusions

Data analysis and comparison of performance indicators of different groups of ducks indicate a high efficiency of the sperm-mediated gene transfer method compared to other transgenesis methods used on ducks. This method has demonstrated advantages in efficiency, ease of use, and non-traumatic effects on the embryo. The lowest value of the egg productivity index was obtained in the group created by busulfan injection (79.5±11.8%), the highest – in the group created by sperm-mediated gene transfer (91.8±2.3%). The direct injection group of the transgenic design has an egg productivity index of 89.0±2.0%, which indicates that this biotechnological method of introducing the transgenic design did not have a clear effect on this indicator. The live weight of ducks of different groups ranged from 1,323.50±65.36 g (sperm-mediated gene transfer) to 1,608.08±94.76 g for the group created using busulfan. Ducks in the direct injection group had an average weight of 1,480.42±35.01 g. The control group, on average, acquired sexual maturity at the age of 139.5±9.67 days, the busulfan group – 148.2±13.13 days, the direct injection group – 143.16±7.25 days, the sperm-mediated gene transfer group – 140.67±13.13 days. The biotechnological process using direct injections of transgenic constructs does not affect the productive capacity of the duck, but the procedure itself is associated with high risks of infection in the embryo, and, as a result, with low rates of embryonic survival. Studies of ducks obtained from exposure of recipient embryos to busulfan followed by injection of cells from intact donor

embryos revealed the most severe damage to the reproductive system of ducks, in particular egg production and reproductive capacity, which could be the result of sterilisation of the recipients, namely, the action of an alkylating agent on their primary germ cells for the purpose of sterilisation by affecting the progenitor cells of the recipient's future germ cells before the introduction of donor blastodermal cells. However, no significant effect on the physical fitness of the duck was found. In the future, it is advisable to investigate the impact of biotechnological procedures for obtaining transgenic ducks on their offspring in subsequent generations, considering the presence or absence of a transgenic design and its intergenerational transmission.

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Conflict of Interest

None.

References

[1] Abd El-Hack, M.E., Hurtado, C.B., Toro, D.M., Alagawany, M., Abdelfattah, E.M., & Elnesr, S.S. (2022). Impact of environmental and incubation factors on hatchability of duck eggs. *Biological Rhythm Research*, 53(1), 79-88. doi: 10.1080/09291016.2019.1628394.

[2] Campbell, R.G., Karunajeewa, H., & Bagot, I. (1985). Influence of food intake and sex on the growth and carcase composition of Pekin ducks. *British Poultry Science*, 26(1), 43-50. doi: 10.1080/00071668508416785.

[3] Chepiha, A.M., Kostenko, S.O., Svyrydenko, N.P., Doroshenko, M.S., Kyrienko, A.Yu., Korol, P.V., Konoval, O.M., Lu, L., Bu, S., Huang, C., & Li, L. (2018). Monitoring of eggs productivity of the Shaoxing breed ducks of different age. *Scientific Bulletin of the National University of Life and Environmental Science of Ukraine. Series "Technology of Production and Processing of Animal Husbandry Products"*, 289, 77-85.

[4] Darmani Kuhi, H., Porter, T., López, S., Kebreab, E., Strathe, A.B., Dumas, A., Dijkstra, J., & France, J. (2010). A review of mathematical functions for the analysis of growth in poultry. *World's Poultry Science Journal*, 66(2), 227-240. doi: 10.1017/S0043933910000280.

[5] Dehdilani, N., Yousefi Taemeh, S., Goshayeshi, L., & Dehghani, H. (2022). Genetically engineered birds; pre-CRISPR and CRISPR era. *Biology of Reproduction*, 106(1), 24-46. doi: 10.1093/biolre/ioab196.

[6] EUs Directive 2010/63/EU on the protection of animals used for scientific purposes. (2010). Retrieved from https://norecopa.no/legislation/eu-directive-201063/.

[7] Hamernik, D.L. (2019). Farm animals are important biomedical models. *Animal Frontiers*, 9(3), 3-5. doi: 10.1093/af/vfz026.

[8] Han, J.Y., & Park, Y.H. (2018) Primordial germ cell-mediated transgenesis and genome editing in birds. *Journal of Animal Science and Biotechnology,* 9, article number 19. doi: 10.1186/s40104-018-0234-4.

[9] Huang, J.F., & Lin, C.C. (2011). Production, composition, and quality of duck eggs. In *Improving the safety and quality of eggs and egg products* (pp. 487-508). New Delhi: Woodhead Publishing.

[10] Ibrahim, M., & Stadnicka, K. (2023). *The science of genetically modified poultry.* Retrieved from https://www.researchgate.net/publication/367138368_The_science_of_genetically_modified_poultry.

[11] Ismoyowati, I., Suswoyo, I., Rosidi, R., Mugiyono, S., & Hidayat, N. (2020). The effect of environmental factor, population and age of duck on egg production. *Animal Production,* 22(2), 118-125. doi: 10.20884/1.jap.2020.22.2.51.

[12] Kim, G.D., Lee, J.H., Song, S., Kim, S.W., Han, J.S., Shin, S.P., Park, B.C., & Park, T.S. (2020). Generation of myostatin-knockout chickens mediated by D10A-Cas9 *nickase*. *The FASEB Journal*, 34(4), 5688-5696. doi: 10.1096/fj.201903035r.

[13] Kim, Y.M., Woo, S.J., & Han, J.Y. (2023) Strategies for the generation of gene modified avian models: Advancement in avian germline transmission, genome editing, and applications. *Genes*, 14(4), article number 899. doi: 10.3390/genes14040899.

[14] King'Ori, A.M. (2011). Review of the factors that influence egg fertility and hatchability in poultry. *International Journal of Poultry Science*, 10(6), 483-492. doi: 10.3390/genes14040899.

[15] Konoval, O., Korol, P., Tabaka, P., Kostenko, S., Lizhi, L., Chepiha, A., Doroshenko, M., Drahulian, M., Xingchen, B., Xuetao, H., & Liumeng, L. (2019). Generation of transgenic ducks by CRISPR/Cas9-mediated gene insertion combined with the sperm-mediated gene transfer (SMGT). *Biopolymers and Cell*, 35(6), 427-436. doi: 10.7124/bc.000A16.

[16] Konoval, O.M., Korol, P.V., Kostenko, S.O., Tabaka, P.P., Lizhi, L., Chepiha, A.M., Doroshenko, M.S., Sydorenko O.V., Dzhus P.P., Svyrydenko, N.P., Lytvynenko, T.V., Xingchen, B., Xuetao, H., Li, L., Drahulian, M.V., Kostyuk, E.R., & Filipova, P.O. (2021) Efficacy of blastodermal cells and CRISPR/CAS9 method in the creation of transgenic duck (*Anas Platyrhynchos*). *Biopolymers and Cell*, 37(4), 289-302. doi: 10.7124/bc.000A5B.

[17] Kwon, M.S., Koo, B.C., Kim, D., Nam, Y.H., Cui, X.S., Kim, N.H., & Kim, T. (2018). Generation of transgenic chickens expressing the human erythropoietin (hEPO) gene in an oviduct-specific manner: Production of transgenic chicken eggs containing human erythropoietin in egg whites. *PLoS ONE*, 13(5), article number e0194721. doi: 10.1371/journal.pone.0194721.

[18] Lee, J., Kim, D., & Lee, K. (2020). Current approaches and applications in avian genome editing. *International Journal of Molecular Sciences*, 21(11), article number 3937. doi: 10.3390%2Fijms21113937.

[19] Liptoi, K., & Hidas, A. (2006). *Investigation of possible genetic background of early embryonic mortality in poultry*. Retrieved from https://www.tandfonline.com/doi/abs/10.1079/WPS2005101.

[20] Longmuir, K.J., Haynes, S.M., Dickinson, M.E., Murphy, J.C., Willson, R.C., & Waring, A.J. (2001). Optimization of a peptide/non-cationic lipid gene delivery system for effective microinjection into chicken embryo *in vivo*. *Molecular Therapy Methods & Clinical Development*, 4(1), 66-74. doi: 10.1006/mthe.2001.0418.

[21] Morduzzaman, M., Bhuiyan, A.K.F.H., Rana, M., Islam, M.R., & Bhuiyan, M.S.A. (2015). Phenotypic characterization and production potentials of Nageswari duck in Bangladesh. *Bangladesh Journal of Animal Science*, 44, 92-99. doi: 10.3329/bjas.v44i2.26007.

[22] Narahari, D., Mujeer, K.A., Ahmed, M., Asha Rajini, R., & Sundararasu, V. (1991). Factors influencing the hatching performance of duck eggs. *British Poultry Science*, 32(2), 313-318. doi: 10.1080/00071669108417355.

[23] Okruszek, A., Książkiewicz, J., Wołoszyn, J., Kisiel, T., Orkusz, A., & Biernat, J. (2006). Effect of laying period and duck origin on egg characteristics. *Archives Animal Breeding*, 49(4), 400-410. doi: 10.5194/aab-49-400-2006.

[24] Önk, K., Sarı, M., Gürcan, I.S., & Işık, S.A. (2018). Live weight and body measurements of male and female native ducks raised in different raising systems. *Revista Brasileira de Zootecnia*, 47, article number e20170084. doi: 10.1590/rbz4720170084.

[25] Park, J.S., Lee, K.Y., & Han, J.Y. (2020). Precise GE in poultry and its application to industries. *Genes,* 11(10), article number 1182. doi: 10.3390/genes11101182.

[26] Petitte, 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(1), 185-189. doi: 10.1242/dev.108.1.185.

[27] Saunders, T.L. (Ed.). (2023). *Transgenesis: Methods and Protocols*. Berlin: Springer.

[28] Yakubu, A. (2013). Characterisation of the local Muscovy duck in Nigeria and its potential for egg and meat production. *World's Poultry Science Journal,* 69(4), 931-938. doi: 10.1017/S0043933913000937.

[29] Ziaei, S.M., & Amini, M. (2020). Investigation of waste in livestock and poultry industry and methods to improve feed conversion ratio in it. *The Journal of Environment, Agriculture and Biological Sciences*, 2(4), 5-12.

Вплив біотехнологічних процедур трансгенозу на продуктивність качок

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Анотація. Використання птиці як унікальної моделі біологічних досліджень відзначилось високим рівнем ефективності, однак, методи створення трансгенних качок, ускладнені структурою шкаралупи яйця водоплавних птахів, мають низьку ефективність. Метою роботи було визначення впливу різних біотехнологічних процедур створення трансгенних качок на їх продуктивні якості та репродуктивну здатність для виділення оптимального методу створення трансгенної птиці для подальшого використання в наукових, дослідних або господарських цілях. Під час проведення дослідження використовували зважування, морфометричний та статистичний аналіз продуктивних ознак. Досліджено 40 качок (4 дослідні групи тварин та близько 3000 їх яєць). Найнижче значення індексу яєчної

продуктивності отримано в групі, створеній за допомогою ін'єкції бусульфану (79,5 11,8 %), найвище – у групі, створеній методом трансфекції сперми (91,8±2,3 %), група прямої ін'єкції трансгенної конструкції – 89,0±2,0 %, що свідчить про те, що даний біотехнологічний метод введення трансгенної конструкції не мав явного впливу на даний показник. Вага качок в різних експериментальних групах варіювалася від 1323,50±65,36 г (з використанням методу трансфекції) до 1,608.08±94,76 г (в групі, створеній за допомогою бусульфану). Качки, які отримували прямі ін'єкції, мали середню вагу 1,480.42±35,01 г. У контрольній групі середня вага при досягненні статевої зрілості становила 139,5±9,67 днів, в групі бусульфану –148,2±13,13 днів, в групі прямих ін'єкцій – 143,16±7,25 днів, а в групі трансфекції – 140,67±13,13 днів. Встановлено, що метод ін'єкції в ембріон реципієнта, стерилізованого бусульфаном, та введення донорських бластодермальних клітин негативно впливає на репродуктивні якості качок. Практичне значення дослідження полягає в тому, що в результаті аналізу продуктивності качок, отриманих різними методами трансгенозу, визначено те, що найефективнішим з оцінених є трансфекція ДНК трансгенної конструкції зі спермою (Sperm-mediated gene transfer, SMGT)

Ключові слова: розмір яйця; маса яйця; яєчна продуктивність; ін'єкції; бластодермальна химера; бусульфан