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HATCHABILITY IMPROVEMENT IN MULARDS BASED ON SELECTION OF VIABLE PRONUCLEI CARRIERS AT ARTIFICIAL INSEMINATION

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Abstract

Present state and possible solutions are discussed for the problem of hatchability improvement in mulards, interspecies hybrids of Muscovy and domestic ducks. Different techniques of semen collection from Muscovy males were studied: manual massage and temporal placement of female in a cage with a male with subsequent semen collection into an artificial vagina. It was found that the latter technique improves ejaculate volume and sperm cell motility resulting in 5 % improvement of mulard egg fertility. Recommended sperm concentration is ca. 150 million per dose; semen should be introduced into the oviduct to the depth of 4 cm. Thus, effective way of collecting sperm in musk drakes was shown for the first time. Also, we studied an influence of the breed and the line on the hatchability of mulards and the expediency of selecting carriers of genetically compatible pronuclei to increase the output of hybrid young animals when crossing musk and domestic ducks. In the crosses, the parents were musk drakes, cross Jubilee, lines Y1, Y2 and Y3 characterized by good meat qualities, and house ducks of the Bashkir color breed (high egg-laying line BC2) and the Peking breed (high fertile line A4). Trials aimed at the investigation of the effects of genetic breeds and lines on the efficiency of hybridization showed the absence of significant differences in the hatch of mulards between different genetic combinations. However, mulards from Muscovy males (line Y1, cross Yubileynyi) and Pekin females (line A4, cross Agidel 34) had 4.5-7.2 % ($P \le 0.05$-0.01) higher live bodyweight at slaughter as compared to other studied combinations. Here, a new method for selection of carriers of genetically compatible pronuclei without any violation of their genomic integrity was designed and experimentally tested. The method involves artificial insemination with a mixture of highly active semen of alien species and low-active semen of the native species (2:1). Carriers of livable pronuclei identified by this method improved the hatch of mulards by 20.5 % ($P \le 0.001$) compared to randomly taken individuals from the breeding flock. Heritability coefficient (from mothers to daughters) of mulard egg hatchability of 27 % evidences that hatchability in mulards can be improved via the selection of compatible families together with the evaluation of parents based on the productive performance in mulards.

Keywords: ducks, Muscovy ducks, Pekin ducks, mulards, selection, hatchability, hatch, livability

As is known, interspecific hybridization is common in developing new plant varieties and animal breeds, however, in poultry farming it is not yet properly widespread due to low hatching of hybrid offspring [1-3]. In the available literature, there is disparate information on hybrids of chicken or waterfowl species but mostly these are a few isolated cases having no economic value [4-6]. Poultry farming knows only one example of use of such hybrids called mulards derived from cross between female common Muscovy duck (*Cairina moshata*) and domestic duck (*Anas platyrhynchos* L.). Mulards are characterized by high viability and excellent taste and nutritive parameters of meat [7-9]. However, this hybrid had not been yet widely spread due to low hatchability of eggs. Mulard hatchability is usually 30-35 % which prevents its wide used in commercial poultry [10-12].

Egg hatchability in poultry depends on genetic specificities and farming methods [13-15], breeding [16-18], housing [19-21] and egg incubation technology [22-24].

The study we are here presenting is the first to determine optimized technique of semen collection from Muscovy males, to assess effects of breeds and lines on hatchability of mulards. Also, we estimated practical effect of selecting parents with genetically compatible pronuclei to increase the output of hybrid birds when crossing musk and domestic ducks.

Our purpose was to improve interspecies hybridization of musk and domestic ducks by providing better sperm quality and selection of parental lines and carriers of genetically compatible pronuclei to be involved in crossings.

Techniques. Experiments on interspecies hybridization were carried out on musk ducks (*Cairina moshata*) and domestic ducks (*Anas platyrhynchos* L.) (Russian Research and Technological Institute of Poultry Farming, Moscow Province; Blagovarskii Poultry Breeding Farm, Republic of Bashkortostan). Muscovy males (cross Jubilee, lines Y1, Y2, and Y3), domestic ducks of the Bashkir color breed (BC2 as maternal line of cross BC12), and Pekin females (A4 as maternal line of cross Agidel 34) were involved in crossing. The duck crosses mentioned have been created in Russia and are characterized by high reproductive and productive properties [8, 25[. Poultry feeding and keeping were in line with the accepted recommendations (Methodological guidelines for breeding of the agricultural poultry. Sergiyev Posad, 2015) and with technological design standards (Methodological Guidelines for Technological Designing of Poultry Farming Enterprises RD-APK 1.10.0504-13. Moscow, 2013).

Common technique of semen collection from Muscovy males (two groups of $n = 30$) applied included 5-fold manual massage of the abdominal part of body or temporal placement of female in a cage with a male with subsequent semen collection into an artificial vagina. Ejaculate volume, counts and activity of sperm cells (motility in points) in ejaculate were determined by commonly accepted methodology. Microscope MBR-1 (Russia) with ×400 zooming was used, and sperm cell activity was assessed by 10-point scale [26]. Pre-developed biotechnical environment which reduces the destabilizing effect of secrets of ovarian tube mucosa on alien sperm was used upon artificial insemination. Dosage per one insemination was nearly 150 million sperm cells; sperm was introduced into the ovarian tube at 4 cm depth.

One hundred eggs from each group were incubated (a Stimul IV-8 incubator, Stimul-Inc Company, Russia). For estimation of true fertilized eggs and the age of embryonic death, all eggs after incubation were opened. Incubation protocol was as generally accepted [27].

Individual carriers of viable pronuclei under interspecific crossing were identified by artificial insemination with highly-active alien semen of 9-10-point motility in mixture with low-active native semen of 4-5-point motility in 2:1 ratio. Carriers of viable pronucleus were those resulted in at least 60 % hatchability of viable chicks.

Selected Pekin female carriers of viable pronuclei were reproduced upon intraspecific mating. The obtained offspring were grown and divided into 6 breeding pens of 4 ducks each, with individually evaluated productivity. Individual caps and standard wing band set were used to control the origin of offspring obtained by incubation.

Body weight, meat quality, meat chemical composition, nutritional value, and heritability of the traits were estimated as per commonly accepted methodologies.

Statistical analysis was performed using software Statistica 10.0 (StatSoft, Inc., USA) and Microsoft Excel tools. Results were presented as mean (*M*) and standard error of mean (±SEM). Student's *t*-test was used to confirm validity of differences between the means. The values were statistically significant at P ≤ 0.05.

Results. As is clear from the data (Table 1), after 5-fold manual massage only 60 % of males had secreted sperm, while use of "decoy duck" increased their number up to 80 %. At that, obtained ejaculate was characterized by 8.5 % higher volume (P ≤ 0.0001) and a 5.5 % increase in sperm cell activity (P ≤ 0.001). The increase of volume and higher activity of sperm cells, apparently, are due to additional amount of reproductive gland secretion in ejaculate. Clearly, this promoted higher fertility of sperm cells introduced into ovarian tube of ducks line A4, which were used in hatchability tests to assess quality of the Muscovy sperm obtained by different methods.

1. Comparative analysis of semen fertility in Muscovy males (*Cairina moshata*) of Jubilee cross line Y1 depending on the technique of semen collection (*M*±SEM)

Parameter	Manual massage	"Decoy duck"
Assessed number of Muscovy males	30	30
Males who secreted semen	18	24
Ejaculate volume, cm^3	0.28±0.03	0.33±0.04
Number of sperm cells, bill/cm^3	2.38±0.07	2.42±0.06
Sperm cell activity, points	9.00±0.06	9.50±0.40
Number of inseminated Pekin ducks	50	50
Eggs incubated	100	100
Egg fertility, %	80.0±3.7	85.0±4.2
Egg hatchability, %	40.0±5.1	42.4±5.8
Mulards hatched, %	32.0±2.7	36.0±3.1

Mulards were produced by artificial insemination without affecting the integrity of the bird's genome. Male parents were Muscovy males (cross Yubileynyi, lines Y1, Y2, and Y3) characterized by good meat properties. Female parents were domestic Bashkir color breed ducks (BC2, the maternal line of cross BC12) with high egg hatchability, and also Pekin ducks (A4, the maternal line of cross Agidel 34) with high fertility. We have compared outcomes of interspecific mating depending on the bird's genotype, i.e. breed and line (Table 2).

2. Incubation of eggs from crossing Muscovy (*Cairina moshata*) males of Jubilee cross line with domestic ducks (*Anas platyrhynchos* L.) of various breeds and lines (*M*±SEM)

Parameter	Line of Muscovy ducks (♂)		
	Y1	Y2	Y3
Bashkir color breed (line BC2) (♀)			
Eggs incubated	100	100	100
Eggs fertilized, %	82,0±3,4	80,0±4,2	81,0±3,4
Egg hatchability, %	43,9±2,6	42,5±3,1	43,2±3,7
Hatchability of hybrid offspring, %	36,0±1,9	34,0±1,8	35,0±2,1
Pekin breed (line A4) (♀)			
Eggs incubated	100	100	100
Eggs fertilized, %	81,0±3,5	82,0±4,1	80,0±3,6
Egg hatchability, %	43,2±4,2	43,9±4,2	42,5±3,8
Hatchability of hybrid offspring, %	35,0±3,6	36,0±3,4	34,0±3,0

As followed from the obtained data, no valid differences were found in incubation outcomes for eggs from various crossing combinations. Hybrid offspring differed only in plumage color and meat body type at slaughter age. Mulards reproduced from combination ♂line Y1 (Muscovy ducks) × ♀line A4 (Pekin duck, cross Agidel 34) were superior to other combinations in body weight at slaughter age by 4.5-7.2 % (P ≤ 0.05-0.01). Accordingly, it is feasible to use Muscovy males of line Y1 (cross Yubileyniy) and Pekin females of line A4 (cross Agidel 34) for marketable products in commercial breeding of mulards.

Indicidual carriers of viable pronuclei at interspecific crossing were identified based on methodology developed within the scope of the conducted research. That is, high-active alien semen mixed with low-active own semen in specific proportion (2:1) was used for artificial insemination. Finally, 30 Pekin ducks of line A4 (cross Agidel 34) have been selected. Insemination of these birds by sperm of Muscovy males of line Y1 (cross Jubilee) led to mulard hatchability of at least 60 %, and for several laying birds this indicator reached 70-75 %. Estimation of incubation properties of the individuals, which were selected as carriers of viable pronuclei, in offspring (F_1) from interspecific crossings line ♂Y1 (Muscovy ducks) × ♀line A4 (Pekin duck, cross Agidel 34) showed that hatchability of hybrid birds significantly (by 20.5 %) exceeded the control, where randomly selected ducks from the pedigree stock were inseminated. Herewith, no valid differences between the birds groups by fertility and survivability have been observed. Thus, in view of the number of incubated eggs n = 200 in each group of selected birds, fertility comprised 80.5±4.4 vs. 81.5±4.3 % in the control, hatchability comprised 69.6±4.2 vs. 42.9±3.8 % in the control, and mulard hatchability comprised 56.0±3.8 vs. 35.5±3.1 % in the control.

Coefficient of mother-daughter heritability of hatchability for hybrids in line A4 was 27 %. The comparatively low heritability gives evidence of feasibility to select combining families of domestic ducks with assessment of the offspring quality.

It should also be noted that produced interspecific hybrids ♂line Y1 (Muscovy males) × ♀line A4 (Pekin female, cross Agidel 34) was characterized by high viability. Survivability from 1 day age to slaughter age reached 95.5-99.0 %. Body weight, meat properties (yield of chest and leg muscles), chemical composition of meat (water, protein, fat, ash, calcium, and phosphorus) and its caloric value at slaughter age in interspecific hybrids were in line with interim indicators as compared to original forms. Herewith, organoleptic indicators of meat in mulards were characterized by specific and high taste properties.

Therefore, semen sampling from Muscovy males requires temporary placement of Muscovy female in cage with male with further collection of ejaculate in artificial vagina. For production of mulards, it is feasible to use males of line Y1 (Jubilee cross) as father line, and line A4 (cross Agidel 34) as maternal line. It was proposed to increase mulard hatchability by selection of genetically compatible pronuclei. Presented data indicate that reproductive properties of Muscovy and domestic ducks could be improved by creation of the specialized breeding forms to increase mulard hatchability by up to 70 %.

REFERENCES

1. Takashima Y., Mizuma Y. Studies on the chicken guail hybrids. *Japanese Poultry Science*, 1981, 18(5): 267-272 (doi: 10.2141/jpsa.18.267).
2. Steklenev E.P. V sbornike: *Voprosy gibridizatsii kopytnykh* [In: Hebridization in ungulates]. Moscow, 1990 (in Russ.).
3. Roiter Ya.S. *Tsesarki. Rukovodstvo po soderzhaniyu i razvedeniyu* [Growing and breeding of Guinea fowl]. Moscow, 2014 (in Russ.).
4. Serebrovskii A.S. *Izbrannye trudy po genetike i selektsii kur* [Selected works on hen genetics and breeding]. Moscow, 1976 (in Russ.).
5. Davtyan A.D. *Vosproizvodstvo i iskusstvennoe osemenenie sel'skokhozyaistvennoi ptitsy* [Reproduction and artificial insemination in poultry]. Sergiev Posad, 1999 (in Russ.).
6. Roiter Ya.S. *Ptitsevodstvo*, 2010, 2: 22-23 (in Russ.).
7. fon Lyutits Kh. *Gusi i utki* [Geese and ducks]. Moscow, 2003 (in Russ.).
8. Roiter Ya.S. *Gusi i utki. Rukovodstvo po razvedeniyu i soderzhaniyu* [Geese and ducks: a guide for groeing and breeding]. Moscow, 2011 (in Russ.).
9. Onyango E.M. Adeola O.J. Inositol hexaphusphate increases musin loss from the digestive tract of ducks. *Anim. Phusiol. Anim. Nutr.*, 2012, 96(3): 416-420.
10. Goryachko N., Kos'yanenko S. *Ptitsevodstvo*, 1992, 12: 3-5 (in Russ.).
11. Feeney K.M. Parish J.L. Targeting mitotic chromosomes: a conserved mechanism to ensure viral genome persistence. *Proc. Biol. Sci.*, 2009, 276(1662): 1535-1544 (doi: 10.1098/rspb.2008.1642).

12. Kos'yanenko S. *Ptitsevodstvo*, 2013, 7: 33-36 (in Russ.).
13. Omjola A.B. Carcass and organoleptic characteristics of duck meat as influenced by breed and sex. *International Journal of Poultry Science*, 2007, 6(5): 329-334 (doi: 10.3923/ijps.2007.329.334).
14. Excoffier L. Hofer T., Foll M. Detecting loci under selection in a hierarchically structured population. *Heredity*, 2009, 103(4): 285-298 (doi: 10.1038/hdy.2009.74).
15. Kos'yanenko S.V. *Izvestiya Natsional'noi akademii nauk Belarusi. Seriya agrarnykh nauk*, 2011, 1: 66-68 (in Russ.).
16. Chen Y., Zhang Y., Huang Z.Y., Xu Q., Zhu Z., Tong Y., Yu Q., Ding J., Chen G. Molecular characterization, expression patterns and subcellular localization of RiCi-1 in the Jinding Duck (*Anas platyrhynchos domesticus*). *Developmental & Comparative Immunology*, 2013, 41(4): 766-777 (doi: 10.1016/j.dci.2013.07.018).
17. Shastar Y., Rodehutscord M. A review of the role of magnesium in poultry nutrition. *World's Poultry Sci. J.*, 2015, 71(1): 125-139 (doi: 10.1017/S0043933915000112).
18. Fisinin V.I., Egorov I.A. *Ptitsa i ptitseprodukty*, 2015, 3: 27-29 (in Russ.).
19. Stanishevskaya O. Temperature regime of incubation as a tool for enhancement of breeding efficiency for improved meat properties in broiler chicken. *Proc. XIII European Poultry Conference*. Tours, France, 2010: 740.
20. Melesse A., Maak S., Pingel H., Lengerken G.V. Assessing the thermotolerance potentials of five commercial layer chicken genotypes under long-term heat stress environment as measured by their performance traits. *J. Anim. Prod. Adv.*, 2013; 3(8): 254-264 (doi: 10.5455/japa.20120929125835).
21. Wei S., Zeng X., Han C., Liu H., Xu H. Research progress in the importance of inoculation temperature for duck egg hatching and poultry production. *World's Poultry Sci. J.*, 2016, 72(4): 847-853 (doi: 10.1017/S0043933916000672).
22. Stanishevskaya O.I. *Zootekhniya*, 2010, 3: 4-5 (in Russ.).
23. Artemov D.V. *Problemy biologii produktivnykh zhivotnykh*, 2014, 1: 5-20 (in Russ.).
24. Dyadichkina L.F. *Diagnostika prichin embrional'noi smertnosti sel'skokhozyaistvennoi ptitsy* [Embryonic death in poultry — detection of the causes]. Sergiev Posad, 2016 (in Russ.).
25. Roiter Ya.S., Kutushev R.R. *Ptitsevodstvo*, 2003, 2: 6-11 (in Russ.).
26. Davtyan A.D., Konopleva A.P., Volkonskaya T.N., Andreeva A.A., Trokholis T.N. *Rekomendatsii po iskusstvennomu osemeneniyu sel'skokhozyaistvennoi ptitsy* [Artificial insemination in poultry]. Sergiev Posad, 2008 (in Russ.).
27. Fisinin V. I., Dyadichkina L.F., Goldin Yu.S., Poznyakova N.S., Melekhina T.A., Danilov R.V., Gupalo I.M., Roiter L.M., Vedenkina I.V., Shinkarenko L.A., Vorontsov A.N., Bosov D.Yu., Afonin V.V. *Rukovodstvo. Tekhnologiya inkubatsii yaits sel'skokhozyaistvennoi ptitsy* [Guidance on egg incubation in poultry]. Sergiev Posad 2016, 90 (in Russ.).