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THE CULTURE OF EMBRYOS OF DOMESTIC MINIATURE FOWL IN THE SURROGATED EGGSHELLS

A. Rudolf, M. W. Lis, K. Głodek, J. W. Niedziółka

Department of Poultry and Fur Animal Breeding and Animal Hygiene,
Agricultural University of Kraków

The aim of this work was to comparison of chick embryo development of miniature hen and broiler cultures. At the experiment the Perry'88 culture embryo in surrogated eggshells with self-modification was used. The eggs of domestic miniature fowls and parental flock of Ross 308 broiler chicken were used as the donor. The recipient eggshells were taken out from the eggs of commercial layers.In the described experiment embryos of domestic miniature fowl and broiler chicken embryos cultured in surrogated eggshells showed differences in development, however none of embryos hatched. The first top of embryos mortalization was observed between stage S3 and S13 (25-30% of embryos) for miniature fowls while for broilers between S3 and S8 (23-35% of embryos). Next critical period was observed between S17 and S21. In this period 12-40% of miniature fowl embryos and 20% of broilers embryos finished development. In the described experiment the broiler chicken embryos were not able to develop longer than to 9th day of incubation and mortalized graduate between S25 - S35. The miniature fowl embryos characterized of better survival and some of them developed to 11th day of incubation (S37). However, 8th - 9th day of incubation (stages S34-S35) seemed to be a crucial period for embryos from this group.

Concluding, the embryos of miniature fowls seem to be less sensitive on manipulation and better tolerate culture in surrogated eggshells than broilers embryo therefore they can become comfortable experimental model in biology

Key words: SURROGATED EGGSHELLS, CHICK EMBRYO, MINIATURE HENS, IN VITRO CULTURE

Development of avian embryo becomes outside the mother body, in the chemical stable environment of egg and in limited contact by foetal membranes and egg shell with external factor. This causes that the avian egg is the useful model in the experimental biology [18]. In the many study the comfortable access to embryo (e.g. for surgical manipulation, transfer of primordial germ cells) is necessary [16, 17], therefore the methods of in vitro culture of quail and hens embryos have been perfected since 70th years of 20 century [3, 9, 10, 14]. Initially the avian germ were cultured in glass and plastic pans (dishes) but hypoglycemia, uncorrect arrangement of foetal membranes, disturbs of albumin absorption was observed and in consequences embryos mortalized [15, 19]. These defects of method were eliminated by culture of embryos in surrogated eggshell [1, 9, 12, 15]. Method of culture embryo in surrogated eggshells (method CESES) consists on transfer of content of donor-egg to the eggshell-recipient. The volume of eggshell recipient in method CSES should be bigger about 1/3 as donor eggshell, therefore eggshell of donor and eggshell recipient are issued from differ species of poultry e.g. quail – hen, hen – turkey [5, 6, 8, 11].

At the study on reproduction biology of birds the one model species is not worked out, although in the experiments Japanese quail and White Leghorn hen are used most often. In this context it seems that the domestic miniature fowls can become useful laboratory animals. These fowl are characterizes by low body weight (c. a. 1 kg) high resistance and feeding requirement. The size of egg of miniature hens is half lower than Leghorn egg. Therefore it seemed interesting to examine of development of embryos of miniature fowl in the culture of surrogated eggshell.

Materials and methods

The eggs of domestic miniature fowls (n = 100 eggs/repetition, weight of egg 35,9±3,21 g) and parental flock of Ross 308 broiler chicken (control, n=100 eggs/repetition, weight of egg 61,8±11,35 g) (Fig. 1) were used as the donor in the experiment in two repetitions. The recipient eggshells for the culturing of embryos of domestic miniature fowls and for control were taken out from the eggs at weight 60,0±9,42 g and 70,2±6,65g of commercial layers, respectively.

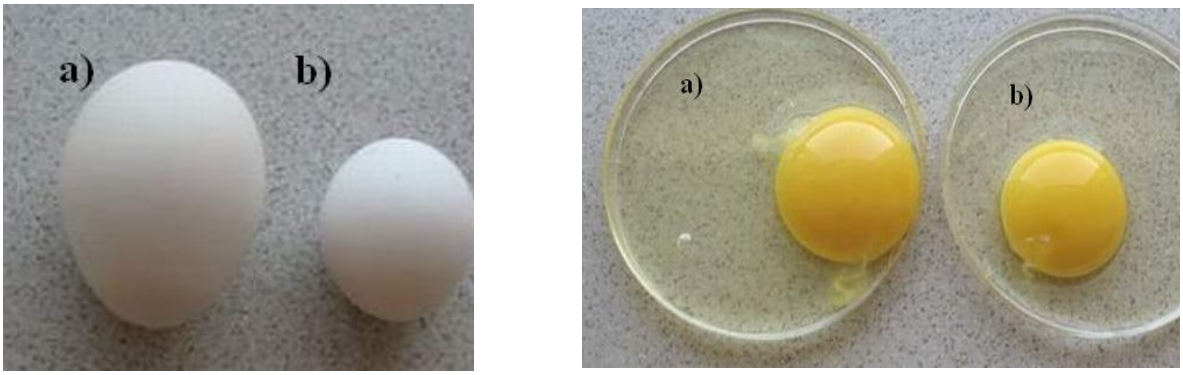


Fig. 1. The size comparison of parental flock of Ross 308 broiler chicken' (a) and domestic miniature fowls' (b) of hatching egg and its content

At the experiment the Perry'88 method CESES [12] with self-modification was used. The recipient eggshell (RES) was disinfected with 70 % ethanol. The window in the big end of egg was made and the albumin and the yolk were removed. Next, RES was cut smooth to 2/3 height (Fig. 2), the external surface of it was disinfected by 10 % $KMnO_4$ solution and its internal surface was washed with sterile distilled water. The prepared RESs were put on the lignin misted by distilled water and remained to later manipulation. Before embryo transfer RES's were set to incubator on 15–20 minutes and heated in 37,6ºC and 80–90 % relative humidity (RH) (Fig. 3).

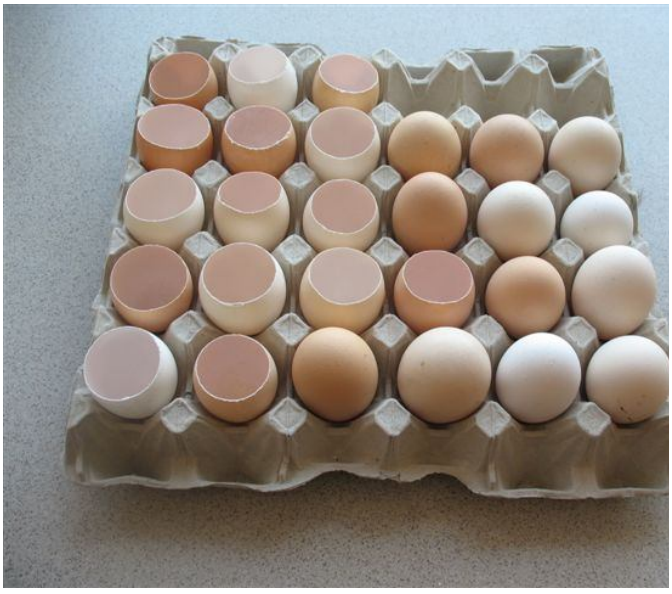


Fig. 2. Prepared surrogated eggshells

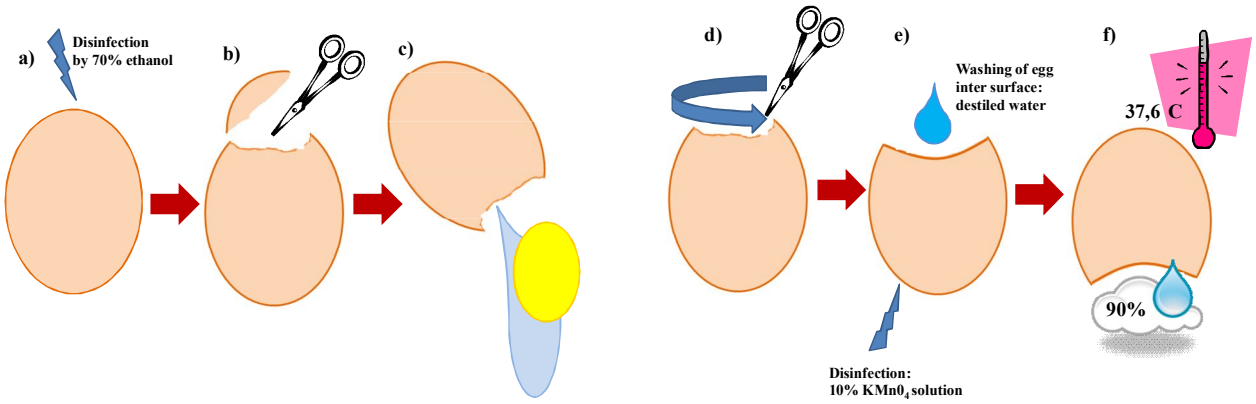


Fig. 3. The preparation of the recipient eggshell. (a) eggshell disinfection, (b) egg windowing, (c) albumin and the yolk removing, (d) eggshell smoothing, (e) disinfection of its internal surface and (f) heating

The eggshell of no-incubated, donor egg (DE) was disinfected by 70 % ethanol, opened cautionary in big end and fertilization of germ disc and quality of yolk was estimated. Next, the content of DE was pulled to RES in one, quick and caution move. Transferred albumin and yolk took up about ¾ internal space of RES (Fig. 4). The prepared CESES was cautionary sealed by Parafilm M® and moreover film border was protected by medical tape Viscoplast Polopor® (Fig. 5, and 6).

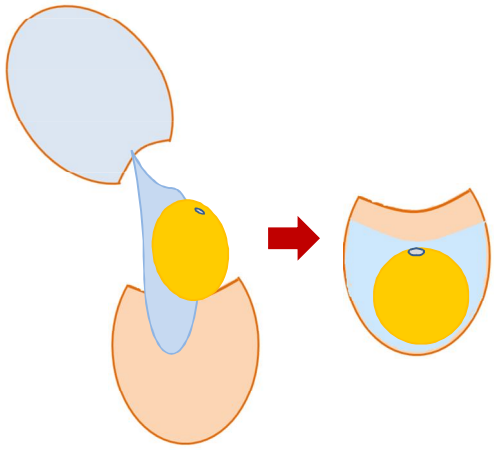


Fig. 4. Transfer of the content of donor egg to recipient eggshell

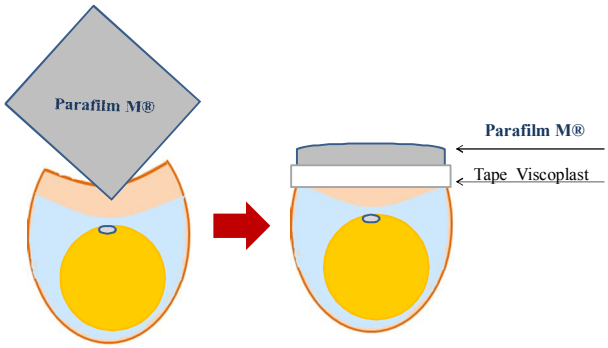
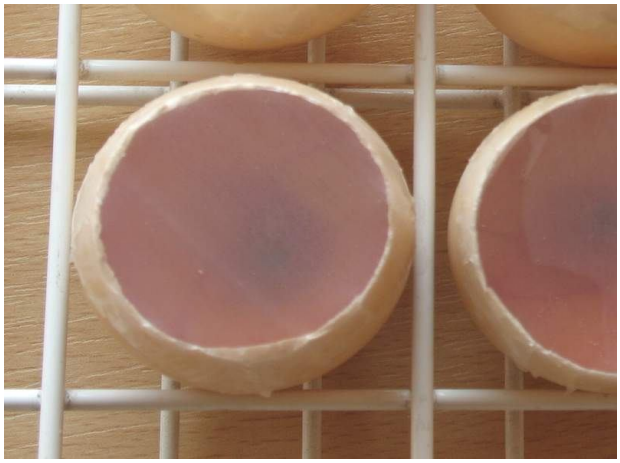


Fig 5. Protection of recipient eggshell (surrogated eggshell) with pull content of donor egg

The prepared CESESs were put on the trays, set into incubator Masalles 65 Digit® and incubated in temperature 37,6 °C and RH 60 %. During incubation the trays were leaned at 45 °C and rotated at 90 °C every one hour.

Course of development of CESESs were monitored every 24 hours. The died cultures were removed from incubator and developmental stage [4] of mortalized embryo was estimated. Difference between domestic miniature fowl CESESs and chicken embryos CESESs in distribution of embryo mortalization at following developmental stages were examined by χ^2 test while frequency of embryos death cases in particular stages were compared by μ test, with using SigmaStat 2,03 (USA).

Fig. 6. The 5th day old embryo in surrogated eggshells



Results and discussion

In the described experiment embryos of domestic miniature fowl and broiler chicken embryos cultured in surrogated eggshells showed the differences in development, however none of embryos hatched. The one of reasons was that many embryos could not continue the development after transfer to surrogated eggshell. These cases occurred 22,2–38,4 % in the group of broiler chicken embryos and 10,0–23,0 % in the group miniature fowls. Moreover, very numerous germs died during early stages of embryogenesis. The first top of embryos mortalization was observed between stage S3 and S13 (25–30 % of embryos) for miniature fowls while for broilers between S3 and S8 (23–35 % of embryos) (Tab. 1, Fig. 7).

Table 1

The mortalization distribution of domestic miniature fowl and broiler chicken embryos cultured in surrogated eggshells at following developmental stages [4]

Stage of embryogenesis /day of incubation	Embryos of Domestic Miniature Fowl		Embryos of Broiler Chick	
	Repetition 1 (n=63)	Repetition 2 (n=89)	Repetition 1 (n=70)	Repetition 2 (n=60)
S3/E1	0,0 ^{B}	12,5 ^{AD}	0,0 ^{B}	23,2 ^{C}
S7/E2	11,1 ^{A}	12,5 ^{AD}	14,3 ^{AD}	0,0 ^{B}
S8/E2	0,0 ^{B}	0,0 ^{B}	21,4 ^{C}	0,0 ^{B}
S11/E2	9,5 ^{A}	0,0 ^{B}	0,0 ^{B}	0,0 ^{B}
S12/E2	9,5 ^{A}	0,0 ^{B}	0,0	0,0 ^{B}
S13/E3	0,0 ^{B}	25,0 ^{C}	0,0 ^{B}	0,0 ^{B}
S17/E3	20,7 ^{C}	0,0 ^{B}	0,0 ^{B}	0,0 ^{B}
S18/E3	0,0 ^{B}	0,0 ^{B}	0,0 ^{B}	19,2
S20/E3	0,0 ^{B}	0,0 ^{B}	14,3 ^{AD}	0,0 ^{B}
S21/E4	9,5 ^{A}	12,5 ^{AD}	7,1 ^{A}	0,0 ^{B}
S24/E4	9,5 ^{A}	12,5 ^{AD}	0,0 ^{B}	0,0 ^{B}
S25/E5	0,0 ^{B}	0,0 ^{B}	0,0 ^{B}	19,2 ^{CD}
S26/E5	0,0 ^{B}	0,0 ^{B}	7,1 ^{A}	0,0 ^{B}
S27/E5	0,0 ^{B}	0,0 ^{B}	0,0 ^{B}	19,2 ^{CD}
S28/E6	0,0 ^{B}	0,0 ^{B}	7,1 ^{A}	0,0 ^{B}
S29/E6	0,0 ^{B}	0,0 ^{B}	7,1 ^{A}	0,0 ^{B}
S30/E7	0,0 ^{B}	0,0 ^{B}	0,0 ^{B}	19,2 ^{CD}
S31/E7	0,0 ^{B}	12,5 ^{AD}	0,0 ^{B}	0,0 ^{B}
S32/E8	0,0 ^{B}	0,0 ^{B}	7,1 ^{A}	0,0 ^{B}
S34/E8	0,0 ^{B}	12,5 ^{AD}	7,1 ^{A}	0,0 ^{B}
S35/E9	20,7 ^{C}	0,0 ^{B}	7,1 ^{A}	0,0 ^{B}
S37/E11	9,5 ^{A}	0,0 ^{B}	0,0 ^{B}	0,0 ^{B}

Note: ABCD — values marked various litter differ significantly ($P \leq 0,05$)

Next critical period was observed between S17 and S21. In this period 12–40 % miniature fowl embryos and 20 % broilers embryos finished development [1] account that about 15 % embryos cultured in vitro mortalized during first three days of incubation, mostly in stages S 17 and

E 18. Early embryos death is probably caused by distribution of homeostasis of germ and damage of egg structure. This explanation can confirm results of experiments with in ovo injection, which induct that sensitiveness of avian embryo on manipulation is very high during the first 3–4 days of development and later gradually decrease [2]. Moreover, the highest mortality is always observed immediately after in ovo injection [7].

In the described experiment the broiler chicken embryos were not able to develop longer than to 9th day of incubation and mortalized graduate between S 25–S 35 (Tab 1). The miniature fowl embryos characterized with better survival and some of them developed to 11th day of incubation (S 37). However, 8th–9th day of incubation (stages S 34–S 35) seemed to be crucial period for embryos from this group (Tab 1, Fig 7).

The death of embryos took place at the stage of embryogenesis when chorionallantoic membrane finished growth and became only one respiratory organ of embryo [13]. Borwornpinyo i wsp (2005) attended that only optimal gas exchange provides obtain the satisfying results in surrogated eggshells culture while formation of embryonic membranes could be disturbed during this procedure [14].

Concluding, the embryos of miniature fowls seem to be less sensitive on manipulation and better tolerate culture in surrogated eggshells than broilers embryo therefore they can become comfortable experimental model in biology. However, the method of culture of them need to be required.

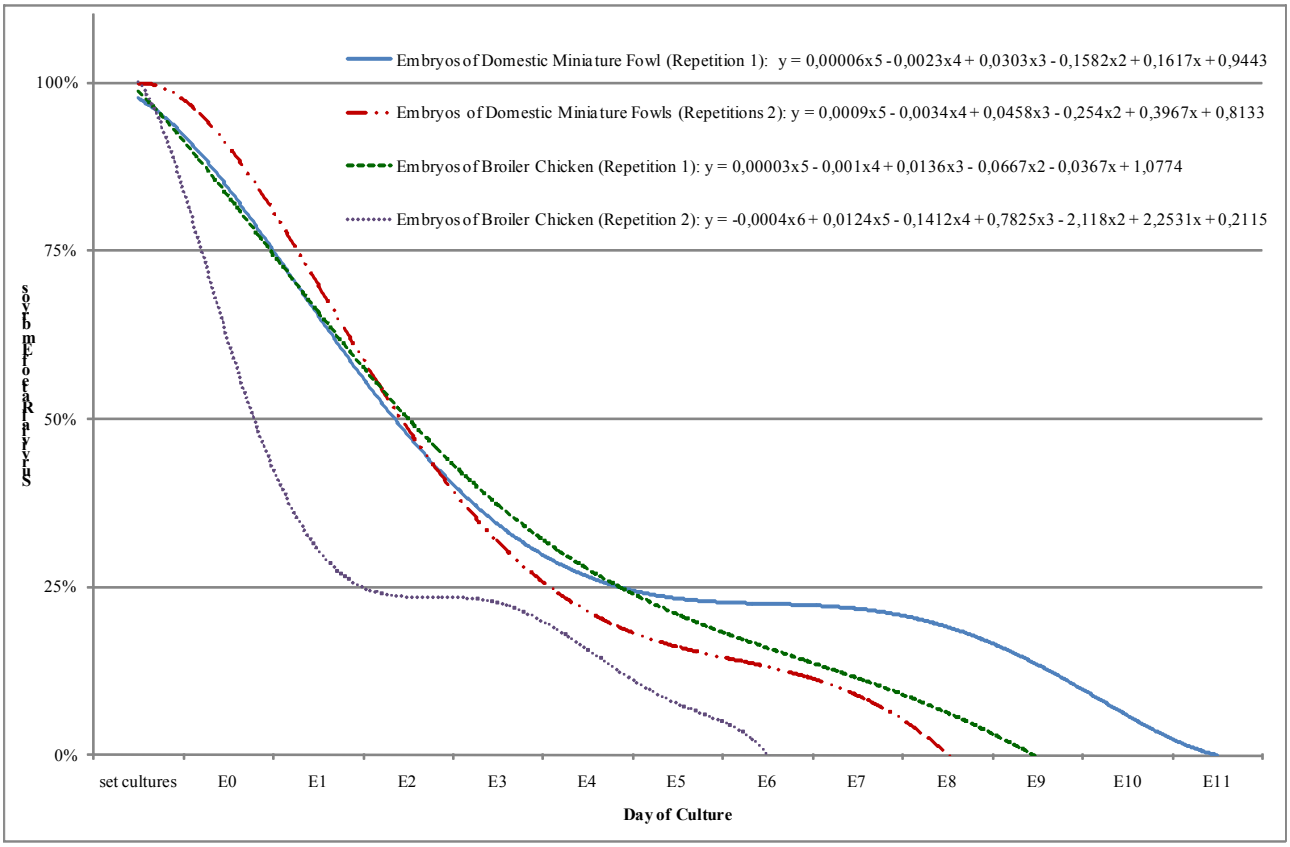


Fig. 7. Survival of domestic miniature fowl and broiler chick embryos cultured in surrogated eggshells at following developmental stages

Conclusions

1. Embryos of domestic miniature fowl cultured in surrogated eggshells lived until later developmental stage than broiler chicken embryos.

2. Much of the embryos, regardless of breed, after moving to surrogated eggshells did not continue development.

3. Survival of domestic miniature fowl and broiler chick embryos cultured in surrogated eggshells were different. A critical period for miniature fowl embryos was observed at 8-9th day of incubation while for broiler chick embryos it was at 4-6th day of incubation.

А. Рудольф, М. В. Ліс, К. Глодек, Й. В. Нєдзюлка

КУЛЬТУРА ЕМБРІОНІВ ДОМАШНЬОЇ МІНІАТЮРНОЇ ПТИЦІ У СУРОГАТНИХ ЯЙЦЯХ

Р е з ю м е

Мета роботи — порівняти розвиток ембріона пташенят мініатюрної курки і бройлера. У експерименті використали ембріон Perry'88 культури сурогатних яєць з самомодифікацією. Яйця місцевих мініатюрних птиць і материнського виду бройлера Ross 308 використали як донора. Яйця-реципієнти отримали від комерційних кур-несучок. Ембріони домашньої мініатюрної птиці та ембріони курчат-бройлерів розвивались по-різному, але ні одне курча не вилупилося. Пік смертності ембріонів спостерігався між стадіями S 3 і S 13 (25–30 % ембріонів) для мініатюрної птиці, а для бройлерів між стадіями S 3 і S 8 (23–35% ембріонів). Наступний критичний період — S 17 та S 21. У цей період припинили розвиток 12–40 % ембріонів мініатюрної птиці та 20 % ембріонів бройлерів. У описаному досліді ембріони курчат-бройлерів розвивались лише до 9 дня інкубації і гинули між S 25–35. Ембріони мініатюрної птиці виживали краще і деякі з них розвивалися до 11 дня інкубації (S37). Таким чином, 8 і 9 день інкубації (стадії S 34–35) були критичними для ембріонів цієї групи. Можна зробити висновок, що ембріони мініатюрної птиці менш чутливі до маніпуляції і краще приживаються в сурогатних яйцях ніж ембріони бройлерів, тому можуть бути зручною біологічною моделлю.

А. Рудольф, М. В. Лес, К. Глодэк, Й. В. Недзюлка

КУЛЬТУРА ЭМБРИОНОВ ДОМАШНЕЙ МИНИАТЮРНОЙ ПТИЦЫ В СУРРОГАТНЫХ ЯЙЦАХ

А н н о т а ц и я

Целью этой работы было сравнить развитие эмбриона птенцов миниатюрной курицы и бройлера. В эксперименте использовали эмбрион Perry'88 культуры суррогатных яиц с самомодификацией. Яйца местных миниатюрных птиц и материнского вида бройлера Ross 308 использовали в качестве донора. Яйца-реципиенты получили от коммерческих кур-несушек. Эмбрионы домашней миниатюрной птицы и эмбрионы цыплят-бройлеров развивались по-разному, но ни один цыпленок не вылупился. Пик смертности эмбрионов наблюдался между стадиями S3 и S13 (25–30 % эмбрионов) для миниатюрной птицы, а для бройлеров между стадиями S3 и S8 (23–35 % эмбрионов). Следующий критический период — S17 и S21. В этот период прекратили развитие 12–40% эмбрионов миниатюрной птицы и 20 % эмбрионов бройлеров. В описанном опыте эмбрионы цыплят-бройлеров развивались лишь до 9 дня инкубации и погибали между S25 — S35. Эмбрионы миниатюрной птицы выживали лучше и некоторые из них развивались до 11 дня инкубации (S 37). Таким образом, 8 и 9 день инкубации (стадии S34–s35) были критическими для эмбрионов этой группы. Можно сделать вывод, что эмбрионы миниатюрной птицы менее чувствительны к

манипуляции и лучше приживаются в суррогатных яйцах чем эмбрионы бройлеров, потому могут быть удобной биологической моделью.

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Рецензент: головний науковий співробітник лабораторії живлення ВРХ, доктор біологічних наук, професор Янович В. Г.