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EFFECT OF INTRODUCING DUCK AND TURKEY DNA ON EMBRYONIC DEVELOPMENT OF FAYOUMI CHICKS

Ahmed Nagwa A.[1], S.A. Riad[1], A.M.M. Ali[2], A.E. El-Wardany[2] and B.A. Ali[2]

1- Department of Animal Production, Faculty of Agriculture, University of Cairo, Giza, Egypt, 2- Genetic Engineering & Biotechnology Division, National Research Center, Cairo, Egypt

SUMMARY

A number of 2263 Fayoumi eggs were used to introduce DNA from either duck or turkey into chicken embryonic cell to determine its effect on the embryonic development. Dosages were 0.68, 1.68, 2.60 and 3.34 ug DNA from duck/egg and 1.03 and 2.60 ug DNA from turkey/egg. Results declared that normal eggs gave the best hatchability (84.8%) and the lowest total embryonic mortality (15.2%), total aberration (6.8%) and haploid value (3.6%). Both drilling eggs and injected solvent solution into them damaged many cells of the embryo.

Although inserting any type of DNA into eggs caused negative effects on hatchability it stimulated hatch body weight and significantly decreased embryonic mortality at 21 days of incubation.

The negative effects on hatchability, total embryonic mortality and aberrations were more drastic by using DNA from turkey than that from duck. These effects exceeded gradually by increasing the dose of foreign DNA. Numerical changes were more frequently than structural ones.

Keywords: Foreign DNA, chicken eggs, embryo, hatchability, chromosomal aberrations

INTRODUCTION

Gene transfer has been played an integral part in

genetic diversity and evolution of species. Interesting attempts were made to produce hybrids between different avian species. Insemination was used to cross chicken males with Japanese quail females (Wilcox and Clark,1961) and chicken males with turkey females (Harada and Buss, 1981).

Recently, new techniques has been developed to introduce genetic material into growing cell embryo and then observe the consequences of gene expression during embryonic development.

The feasibility of introducing cloned recombinant DNA into animal genomes by micro injection of early embryos has been cleared demonstrated in mouse (Gordon et al., 1980), drosophila (Gazaryan et al.,1984), poultry (Shuman and Shoffner, 1985), rabbits, sheep and pigs (Hammer et al., 1985) as well as microorganisms (Ali et al., 1988).

Shuman and Shoffner (1985) declared that gene transfer may have several advantages, not only increasing the genetic variation by the introduction of genetic material directly into the genome, but also transcending the limitations of sexual reproduction and permitting gene flow between vastly different organisms. Therefore, recombinant DNA and gene transfer may give the breeder a new tool to discovery desirable genes that may lead to improvement and solve breeding problems.

This work is an attempt to introduce DNA either from duck or turkey into chicken embryonic cell to determine its effect on embryonic development.

MATERIALS AND METHODS

The experiment work was carried out in the Poultry Research Farm, Faculty of Agriculture, Cairo University, Genetic Engineering and Biotechnology Division, National Research Center, during January to July 1994. A number of 2263 Fayoumi eggs were used to insert either duck or turkey DNA into chicken cells to determine its effect on the embryonic development.

Eggs were collected daily, stored for a week, fumigated and incubated at 99.5 F. After three days (72 hours) of incubation, cleared eggs were kept out. Remaining fertile eggs were divided randomly into three groups allotted to the source of foreign DNA injected

with, as follows:

1- Duck DNA= Eggs were injected with DNA extracted from duck brain.
2- Turkey DNA= Eggs were injected with DNA extracted from turkey brain.
3- Control= Eggs without injection with DNA.

Each group was classified to sub-groups according to the DNA dosage. Eggs for the first group (Duck DNA) were injected with 0.68, 1.68, 2.60 and 3.34 ug DNA/egg. Eggs for the second group (Turkey DNA) were injected with 1.03 and 2.60 ug DNA/egg. Eggs for the third group (Control) were divided as non drilled, drilled eggs of a hole of 2 mm over the air cell then sealed with wax and drilled eggs of a hole of 2 mm over the air cell, injected with solvent solution (0.1x) SSC (sodium chloride sodium citrate), then sealed with wax.

All eggs were candled at 18th day of incubation. Those without evidence of live embryonic development were broken out and examined to estimate degenerated eggs and the age of dead embryos using the developmental status proposed by Lillie (1952). Remaining eggs were placed back and kept in the incubator four days more. Hatchability and embryonic mortality were calculated. Hatched chicks were wing-banded and individually weighted.

High molecular weight DNA was extracted from the brain by the method of Bendich and Bolton (1967). Purity of DNA was determined by the method of Charles (1970). Insertion of DNA into the chicken embryos was made by the method of Salter et al. (1986).

Ten embryos were harvested 24 hours after treatment, injecting 70 ul of 0.05% colcemid (ciba) two hours before harvest. Administration was carried out by the method of Bloom et al. (1972) and Stock et al. (1972). solid tissue preparations were modified in one treatment by fixation in 1:1 instead of 3:1 mixture of ethanol and acetic acid (El-Wardany and Bakir, 1988). Approximately 50 metaphase spreads considering only five largest pairs of chromosomes will constitute about 50% of the nuclear DNA/cell (Bloom,1978). Chromosomal aberrations were recorded by bright-field microscopy.

Chi-square analysis were used for embryonic mortality,hatchability and chromosomal aberrations. Statistical analysis for hatch weight contained the

analysis of variance using GLM model of SAS (1990) and the Duncan multiple range test (1955).

RESULTS AND DISCUSSION

The mean values of hatchability and hatch body weight are presented in Table (1). It can be observed that the overall mean percentage of hatchability was 52.2%. This value was better than 38% that reported by Bosselman et al. (1990) who microinjected of REV vectors into unincubated chicken embryo blastoderms.

Table 1. Effect of DNA injection on hatchability and hatch body weight

Treatment	No. fertile eggs	Hatchability fertile eggs %	Hatch weight (gm) ± SE
Overall mean	1908	52.2	30.8
Control eggs:			
Nun-drilled	250	84.8**	30.3b ± 2.7
Drilled	228	69.3	30.1b ± 3.0
Drilled & solvent	198	66.2	30.4b ± 2.5
Duck DNA dosage:			
0.68	151	70.9**	30.2b ± 3.0
1.68	317	58.7**	30.5b ± 2.8
2.60	265	47.9**	31.0ab± 3.0
3.34	237	46.8**	31.5a ± 3.0
Turkey DNA dosage:			
1.03	166	24.1**	31.6a ± 3.0
2.60	96	1.01[1]	31.81[1]

** Significant ($P < 0.01$) from the third control group.
a,b Means within the same column having different letters differ ($P<0.05$).
1 Value of one bird that was kept out from the analysis.

Concerning the controls, results showed that hatchability was significantly higher in the first group (84.8%) than that in the second (69.3%) and the third one (66.2%). The significant difference of 15.5% between the first and the second control groups might be due to the drilling, while the difference (18.6%) between the

first and the third ones might be due the injection of the solvent solution as well as the drilling of eggs. Rolon and Buhr (1992) found that drilling a hole of 2 mm through the egg shell above the air cell at the first day of incubation significantly decreased the hatchability.

Regarding the DNA injected eggs, a depression in hatchability was observed in all experimental groups, with an exception of the 0.68 ug duck DNA/eggs. Using this low dosage resulted in 13.9% lower hatchability than the first control group but 1.6% and 4.7% higher hatchability than the second and the third control ones, respectively. On the other hand, using the higher dosages 1.68, 2.60 and 3.34 ug duck DNA/egg decreased the hatchability by 26.1%, 36.9% and 22.5% compared with the second control group and by 7.5%, 18.3% and 19.4% in comparison with the third control group, respectively. Using 1.03 and 2.60 ug turkey DNA reduced the hatchability by 60.7% and 83.8% compared with the first control group and by 42.1% and 65.2% in comparison with the third control group, respectively. These result could refer to the inserting duck or turkey DNA in the chicken eggs and caused adverse effect on hatchability which increased by increasing the inserted dosage of the foreign DNA. This adverse effect was more drastic with DNA from turkey than that from duck.

Significant differences in hatch weight among the experimental groups was observed (Table 1). It can be observed that although inserting high dosage of DNA into eggs decreased hatchability, it stimulated the hatch body weights. That is may be due to the successful expression of the responsible growth genes in chicken embryos (Shuman and Shoffner, 1985).

Concerning the down color, all hatched chickens had typically the Fayoumi pattern, with exception of nine mutants. All of them appeared with the injection of duck DNA. One chick from 0.68 and six chicks were from 1.68 had quite black duck color down. The other two chicks were from 2.60 and had plumage at their toes. Similar result was obtained by Leroy and Benoit (1966) who reported extensive changes in the quantity and distribution of melanin pigment visible in chicken plumage after injecting blood from guinea-fowl into Rhode Island Red chickens. Moreover, Benoit et al., (1966) reported that hereditary modifications of

morphological characteristics in Pekin ducks following the injection of DNA from Khaki Campbell ducks.

The percentage of embryo deaths occurred during the incubation period is presented in Table 2. It can be observed that 33.8% from the total embryonic mortality was degenerated within the control groups, the degenerated value increased significantly by 12% as a result of drilling eggs while it increased 20.1% because of the action of solvent solution beside drilling eggs. Percent of degeneration was exceeded by about 10-30% in duck groups and 35% in turkey groups (except 2.60) compared with the third control ones.

Table 2. Effect of DNA injection on embryonic mortality during the incubation period

Treatments	T.E.M.,%	Deg.,%	Embryonic mortality, % day						
			3	6	9	12	15	18	21
Overall mean	47.8	33.8	4.6	2.2	2.8	3.9	8.9	10.1	33.7
Control eggs:									
Non drilled	15.2**	5.3**	2.6	2.6	0.0	0.0	10.5	7.9*	71.1**
Drilled	30.7	17.3**	0.0	0.0	5.9	0.0	15.6*	14.3*	46.9**
Drilled& solvent	33.8	25.4	8.9	4.4	0.0	1.5	8.9	18.1	32.8
Duck DNA dosage									
0.68	29.1**	57.0**	0.0	0.0	4.6	11.3	8.9	2.4*	15.8**
1.68	41.3**	46.1**	6.8	0.0	3.1	1.5	6.1*	10.7*	25.7**
2.60	52.1**	55.9**	3.6	2.0	0.8	0.8	0.0	14.4*	22.2**
3.34	53.2**	35.8**	7.9	3.2	3.9	8.6	12.8*	5.6*	22.2**
Turkey DNA dosage									
1.03	75.9**	61.1**	3.2	2.4	4.0	1.6	5.5*	2.4*	19.8**
2.60	99.0**	0.0	8.6	4.2	3.2	10.2	12.2*	14.8*	46.8**

T.E.M., % Total embryonic mortality,%
Deg., % Degenerated, %
* Significant (P<0.05) from the third control group.
** Significant (P<0.01) from the third control group.

Results in Table 2 showed that the overall mean percentage of embryonic mortality at 3 days of incubation was 4.6%. This value was lower than that reported by Thorne et al. (1991) who found that it was 11.9% in layer lines and 16.4% in broiler ones. This early embryonic mortality may be occurred as a result of genetically unbalanced chromosome arrangement in embryos (Wagner et al., 1981) or the incidence of abortive membranes that an embryo was lacking (Thorne et al., 1991).

Concerning the distribution of embryo deaths at

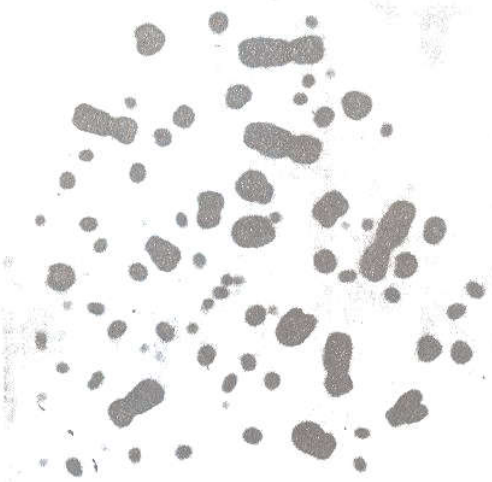
designated ages, there were no significant differences in early mortality at 3, 6 and 9 days of incubation. However, the critical significant mortality peak in all groups was late at 18 and 21 days of incubation. It can be observed that introducing any type and dose of DNA (exept 2.6 Turkey DNA dosage) into chicken embryo cells was decreased significantly the embryonic mortality at 21 day of incubation in comparing with control groups (Table 2). The majority of embryonic mortality at late days of incubation reflected the incidence of malposition embryos which failed to hatch. Jaenisch (1988) stated in mice that mutant strains had an embryonic lethal phenotype which included defects in limbs formation, transmission distortion and disturbance of kidney function.

Table 3. Effect of DNA injection on chromosomal aberrations

Treatment	Total aberrations	Numerical type %			Structural type %	
		Mono-somic	Hap-loid	Trip-loid	Break	Centromeric attenuation
Overall mean	17.7	2.0	13.9	0.5	0.3	0.8
Control eggs:						
Non drilled	6.8**	1.2	3.6**	0	0.8	1.2
Drilled	11.2**	2.0	5.6**	0.4	0.8	1.2
Drilled+solvent	23.2	1.6	21.6	0	0	0
Duck DNA dosage:						
0.68	12.0**	1.6	8.4**	0.4	0.4	1.2
1.68	17.2*	2.0	14.8**	0	0	0
2.60	13.2**	1.2	11.2**	0	0	0.8
3.34	18.8*	0.8	18.0 *	0	0	0
Turkey DNA dosage:						
1.03	20.8*	2.0	14.8**	2.0	0.4	1.6
2.60	27.2*	4.0	21.6	0.4	0.4	0.4

** Significant ($P < 0.01$) from the third control group.
* Significant ($P < 0.05$) from the third control group.
When the observed value was Zero, data cannot be analysed.

The aberrant metaphase score at 24 hours after treatment are listed in Table 3. and Plates. 1 and 2. The overall mean percentage of total aberration was 17.7% consisting of 2.0% monosomic, 13.9 haploid, 0.5% triploid, 0.3% break and 0.8% centromeric attenuation. Thorne et al. (1991) obtained only 12.5% abnormal chromosomes in broiler and layer lines, which consisted



(a)

(b)

(C)

Plat. 1. Metaphase cells with numerical aberrations:
a) Triploid (3N), b) Haploid (1N) and
c) Monosomic (2N-1).

Plat. 2. Metaphase cells: a) Normal diploid (2N=78), with structural aberrations: b) Break and c) Centromeric attenuation.

of 24.1% haploids, 33.9% hapbloid-euploid, 21.4% triploid, 14.3% diploid-polyploid, 3.6% aneuploid, 1.8% tetraploid and 0.9% translocation.

The numerical changes were more frequently at all treatments than structural ones. Haploids accounted to be the highest numerical changes in all groups than monosomics and triploids. Bitgood and Shoffner (1990) reported that most forms of heteroploidy were lethal during early embryonic development. Klein and Saar (1992) found in a goose selected line that two third of all aberrant embryos included haploid cells.

The normal eggs showed the lowest total embryonic mortality (15.2%), total aberration (6.8%) and haploid value (3.6%). The corresponding values were 30.7%, 11.2% and 5.6 % for drilled eggs being 33.8, 23.2 and 21.6 for the solvent injected eggs (Table 2 and 3). It is intersting to observe that introducing foreign DNA (except 2.6 turkey DNA dosage) was significantly lower total aberration and haploid values than in the third control group. Ali et al. (1988) reported that there is a role of repetitious DNA sequences in the evolution of inbred characteristics.

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تأثير حقن د.ن.١ الغريب المستخلص من البط والرومى على النمو الجنينى فى الدجاج الفيومى

نجوى عبد الهادى احمد[١] - سوزان احمد رياض[٢] - عاصم محمد محمد على[٢] - عفاف السيد الوردانى[٢] - باهى احمد على[٢]

١- قسم الإنتاج الحيوانى - كلية الزراعة - جامعة القاهرة ، ٢- المركز القومى البحوث

استخدم فى هذا البحث ٢٢٦٣ بيضة فيومى حقنت بـ د٠ن١٠ المستخلص من البط والرومى لتحديد تأثيره على النمو الجنينى فى الدجاج الفيومى٠ قسم البيض الى مجاميع كالتالى ٣ معاملات كنترول الاولى بيض غير معامل والثانية بيض مثقوب والثالثة مثقوب ومحقون بمادة SSC (مذيب الـ د٠ن١٠) و٤ معاملات حقن فيها البيض بالـ د٠ن١٠ المستخلص من البط بالجرعات الاتية٠,٦٨ ، ١,٦٨، ٢,٦٠، ٣,٣٤ ميكروجرام/ بيضة ومعاملتان حقن فيها البيض بالـ د٠ن١٠ المستخلص من الرومىبالجرعات الاتية١,٠٣، ٢,٦٠ميكروجرام /بيضة واغلق البيض المثقوب فى جميع المعاملات بالشمع اوضحت النتائج ان البيض الغير مثقوب الطبيعى اعطى اعلى نسبة فقس (٨٤,٨٪) واقل نسبة نفوق جنينى (١٥,٢٪) واقل شذوذ كروموسومى (٦,٨٪) بينما ثقب البيض او حقنة بمذيب الـ د٠ن١٠ ادى الى تلف عديد من الخلايا الجنينية ٠

ادخال الـ د٠ن١٠ الغريب ادى الى تقليل نسبة الفقس بينما ادى الى زيادة معنوية فى وزن الجسم كما أدى إلى نقص معنوى فى نسبة النفوق الجنينيى عند ٢١ يوم من التفريخ ٠ كان تأثير استخدام د٠ن١٠ من الرومى اسوأ من استخدام د٠ن١٠ من البط على نسبة الفقس والنفوق الجنينى الكلى وزاد هذا التأثير السيء تدريجيا باستخدام جرعات عالية من د٠ن١٠ كما لوحظ ان التغيرات العددية فى الكروموسومات كانت اكثر من التغيرات فى التركيب الكروموسومى٠