Theor Appl Genet (1986) 72:264–268

An attempt at genetic transformation in chickens through cock sperm irradiation

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Received October 12, 1985; Accepted November 2, 1985
Communicated by H. Abplanalp

Summary. Two experiments were conducted to verify the real possibility for use of the "genetic transformation technique" as described by Pandey and Patchel in chickens in commercial poultry breeding. Multiple recessive and multiple dominant marker stocks were employed, as well as a tester and a donor line. Recipient tester females were first inseminated with dominant donor semen which was irradiated with doses of ^{60}Co gamma irradiation ranging from 100 to 800 Gy (control group) and 24 h later were reinseminated with unirradiated, normal semen of the recipient strain (experimental group). One "genetic transformed" chicken was found in the first experiment; no genetic transformed event occurred in the second experiment but one embryo was present in the 600 Gy irradiated group with parthenogenetic development capable of giving a live chick. One hundred Gy was observed not to be enough to destroy completely sperm fertilizing ability. An increased frequency of parthenogenetic development was found in all groups after insemination with irradiated semen. There were 11 individuals with developmental abnormalities from the total of 1264 analysed embryos which died after the 18th day of incubation. We concluded that egg transformation is a rare event in domestic fowl and further research for use of this technique in commercial poultry breeding is needed.

Key words: Domestic fowl – Semen irradiation – Genetic transformation – Parthenogenesis-Embryonic abnormalities

Introduction

It has been claimed by Pandey (1980a) and Pandey and Patchell (1982) that irradiated male gametes can be used as a vehicle for animal-to-animal genetic transformation. Genetic transformation for egg and feather colour has been obtained in the whole animal, chicken, by the use of irradiated cock sperm. By this technique recipient females were first inseminated with irradiated semen from the donor and, 24 h later, were reinseminated with unirradiated, normal semen of the recipient strain. The expression of the transferred gene was irregular and might occur either in the first generation, after insemination with irradiated semen, or in the following backcross generation. It was suggested that integration of a normally recessive, transferred gene at non-homologous sites in the recipient chromosomes might affect not only its regularity of expression but also its dominant relationship with the original maternal gene, "releasing" it from the dominance of the latter. Pandey and Patchell (1982) reported that this technique potentially offers a rapid method of improving an already established strain of an animal.

Similar reports on egg transformation in plants by the use of irradiated pollen have been reported in *Nicotiana* (Pandey 1978, 1980b; Jinks et al. 1981; Caligari et al. 1981) and in barley (Powell et al. 1983). Transformation may occur in association with parthenogenetic diploidy, or as a result of a later, second normal fertilisation by unirradiated pollen of an egg already transformed by the first pseudofertilisation. However, Sanford et al. (1984) in *Zea mays* and Chyi et al. (1984) in pea, rapeseed and apple concluded that if egg transformation occurs outside of *Nicotiana* it is a very rare event, and its frequent occurrence in *Nicotiana* must be, at best, an isolated phenomenon.

The purpose of our research was to examin the real possibility of use of this technique in poultry breeding.

Material and methods

In these trials two separate experiments were conducted. Three strains of chickens were used in the first experiment.

'Light Sussex' (LS) and 'White Plymouth Rock' (WPR) strains were used as the irradiated donors and 'New Hampshire' (NH) as the female recipient stock. LS was homozygous for the sex-linked silver plumage colour gene *S* and WPR was homozygous for the dominant autosomal plumage colour inhibitor gene *I*, autosomal dominant plumage-pattern extended black gene *E* and dominant sex-linked barring gene *B*. The NH strain was homozygous for recessive plumage colour genes *s*, *i*, *e* and *b*. With the given dominance relations, transformation of the plumage colour genes for silver and white, black and barred, respectively, could be visually identified in the F_1 progeny.

In cocks, 27 control and 56 experimental hens in the recipient NH strain were used. The pooled semen of 12 LS cocks was irradiated with 500 Gy only while the pooled semen of 12 WPR was irradiated with doses of gamma-rays varying from 100 to 500 Gy. The artificial insemination technique was used every week during the experiment.

In the second experiment another two strains of chickens were used. The 'Wyandotte Bantam Barred' (WBB) was used as the irradiated donor and the 'Rhode Island Red' (RIR) as the female recipient parent. The WBB strain was homozygous for the dominant plumage colour genes *E*, *S* and *B* and for the dominant gene for rose comb *R*. The RIR strain was homozygous for the recessive plumage colour genes *e*, *s* and *b* and for recessive single comb genes *f* and *p*. Twenty cocks, 20 control and 100 experimental hens in the recipient RIR strain were used. At the same time 40 cocks of WBB were used as a donor strain. The pooled semen of the donor strain was exposed to doses of gamma radiation ranging from 100 Gy to 800 Gy. In this experiment we also investigated all the eggs to determine possible parthenogenetic development. The eggs which were identified by candling as "clear" or dead embryos were broken and examined macroscopically for parthenogenetic development according to the method of Křiženecký et al. (1955).

We used the same procedure in both experiments. Both control and experimental groups were at first inseminated with irradiated semen of the donor strain, but the experimental group was reinseminated 24 h later with unirradiated, normal semen of the recipient strain. In order to increase the fertility this process was conducted twice a week in most replications when the WPR and WBB strain were used as donor lines. The semen was obtained by abdominal massage and irradiated with gamma rays (^{60}Co); the dose rate was 13.2 Gy/min.

The time interval between the collection of semen, semen irradiation and the insemination of hens ranged from 40 to 120 min depending on the given dose. The semen was irradiated in glass test tubes using a therapeutical radioactive cobalt apparatus GUT-400 C. The dosimetry was evaluated by a Siemens Universal Dosemeter.

Each experiment lasted about 5 months. All the birds were reared in cages and fed according to the programme of laying strains. The collected eggs were placed in a standard egg incubator and candled on the 7th and 14th day to eliminate clear eggs and dead embryos. On the 18th day of incubation the eggs with live embryos were placed in the hatchery. The frequency of developmental abnormalities was analysed in embryos that died in the hatchery by the alisarin skeletal method (Staples and Schnell 1964). Fertility, embryonal mortality and hatchability were recorded and the plumage colour of all day-old chickens was analysed. About 10% of the hatched chickens were reared and analysed at the age of 8 and 20 weeks for plumage colour. All cocks used as donors were tested on hens of recipient strains for their dominance of colour plumage genes after the conclusion of the experiment.

Results

The complete lack of fertile eggs from irradiated semen alone showed that a dose of 500 Gy destroyed the ability of sperm for normal fertilization. There was also no parthenogenetic development of embryos capable of developing into a live chick in the first experiment (Table 1). The difference between the fertility of the eggs from the crosses 'Light Sussex'×'New Hampshire' (43.3%) and 'White Plymouth Rock'×'New Hampshire' (87.6%) is due to the fact that the combination WPR× NH was inseminated and reinseminated twice a week.

Table 1. Results of the first experiment. Percentage values of clear and fertile eggs calculated from set eggs, embryonic mortality from fertile eggs and hatched chickens from set eggs are in parentheses

Insemination group	Dose in Gy	Second insemination	Eggs			Embryonic mortality from fertile eggs		No. chicks hatched (% of eggs set)	Colour of plumage of day-old chickens
			Set	Clear	Fertile	At day 7	At hatch		
LS×NH	500	–	982	982 (100)	–	–	–	–	–
LS×NH	500	NH	1,885	1,069 (56.7)	816 (43.3)	79 (9.7)	46 (5.6)	691 (36.7)	690 light red or buff, *1 silver*
WPR×NH	500	–	215	215 (100)	–	–	–	–	–
WPR×NH	500	NH	443	55 (12.4)	388 (87.6)	21 (5.4)	29 (7.5)	338 (76.3)	388 light red or buff
NH×NH	0	–	158	48 (30.4)	110 (69.4)	5 (4.5)	3 (2.7)	102 (64.6)	102 light red or buff
LS×NH	0	–	58	19 (32.8)	39 (67.2)	2 (5.1)	1 (2.6)	36 (62.1)	36 silver
WPR×NH	0	–	85	28 (32.9)	57 (67.1)	4 (7.0)	2 (3.5)	51 (60.0)	51 white with black spots

Table 2. Results of the second experiment. In all insemination groups cocks 'Wyandotte Bantam Barred' × hens 'Rhode Island Red' were used. In parentheses are percentage values as calculated in Table 1

Dose in Gy	Second insemination	Eggs			Embryonic mortality			Hatched chicks	Colour of day-old chick
		Set	Clear	Fertile	At day 7	At day 14	At hatch		
800	–	151	151 (100)	–	–	–	–	–	–
800	RIR	1,096	234 (21.4)	862 (78.6)	43 (5.0)	35 (4.1)	253 (29.4)	531 (48.4)	red
700	–	187	187 (100)	–	–	–	–	–	–
700	RIR	862	472 (54.8)	390 (45.2)	16 (4.1)	58 (14.9)	221 (56.7)	95 (11.0)	red
600	–	409	408 (99.8)	1 (0.2)	–	–	–	1 (0.24)	red
600	RIR	1,687	258 (15.3)	1,429 (84.7)	72 (5.0)	52 (3.6)	352 (24.6)	953 (56.5)	red
500	–	253	253 (100)	–	–	–	–	–	–
500	RIR	1,312	419 (31.9)	893 (68.1)	51 (5.7)	37 (4.1)	237 (26.5)	568 (43.3)	red
300	–	131	131 (100)	–	–	–	–	–	–
300	RIR	1,071	301 (28.1)	770 (71.9)	36 (4.7)	122 (15.8)	142 (18.4)	470 (43.9)	red
100	–	98	91 (92.9)	7 (7.1)	1 (14.3)	–		6 (6.1)	barred black
100	RIR	962	282 (29.3)	680 (70.7)	49 (7.2)	37 5.4)	59 (8.7)	535 (55.6)	42 barred black 493 red
WBB × RIR		134	36 (26.9)	98 (73.1)	6 (6.1)	5 (5.1)	21 (21.4)	66 (49.3)	barred black

Table 3. Analysis of parthenogenetic development in the second experiment. The percentage value are calculated from clear eggs

Dose in Gy	No. of clear eggs	No. development		Minor development (small membranes)		Advanced development (large membranes or blood vessels	
		No.	%	No.	%	No.	%
Groups inseminated with only irradiated semen (1)							
800	151	143	94.7	7	4.6	1	0.7
700	187	150	80.2	35	18.7	2	1.1
600	408	383	93.9	24	5.9	1	0.2
500	253	207	81.8	45	17.8	1	0.4
300	131	105	80.2	25	19.1	1	0.8
100	91	68	74.7	21	23.1	2	2.2
Not inseminated control group (2)							
–	210	206	98.1	4	1.9	0	0.0
Re-inseminated groups (3)							
800	234	136	58.1	51	21.8	47	20.1
700	472	302	64.0	135	28.6	35	7.4
600	258	130	50.4	21	8.1	107	41.4
500	419	243	58.0	109	26.0	67	16.0
300	301	171	56.8	83	27.6	47	15.6
100	282	163	57.8	44	15.6	75	26.6

In the reinseminated group 'Light Sussex' × 'New Hampshire' there was only one chicken present with plumage of a silver colour among 691 hatched chickens (0.14% assumed genetic transformed occurrence). No chickens with plumage colour other than red (buff) were present in the reinseminated group WPR × NH. The control matings (insemination of the recipient hens with unirradiated, normal semen of donor strains) showed the full dominancy of donor silver *S* or of colour inhibitor *I* genes in crosses LS × NH and WPR × NH. The "transformed" chicken was female and in adult age it was phenotypically similar to a pure 'Light Sussex' individual.

The dose of 100 Gy was not enough to destroy completely the fertilizing ability of the sperm as 6 chickens hatched in this no reinseminated group in the second experiment (Table 2). All other doses given completely destroyed the fertilizing ability of the sperm but one embryo with parthenogenetic development capable of developing into a live chick was present in 600 Gy group. The control matings of the donor 'Wyandotte Bantam Barred' cocks with 'Rhode Island Red' hens showed full dominance of the dominant colour genes of the WBB strain. No genetic transformed event occured among 2,617 hatched chicks in the reinseminated 300–800 Gy groups in the second experiment. However, a few chickens in later postembryonal development were stripped but this was considered to be a normal situation in some 'Rhode Island Red' individuals.

We found an increased frequency of developed blastodiscs in the eggs of all groups after insemination with irradiated semen from 'Wyandotte Bantam Barred' males when these were compared with those of the same hens to insemination. The difference, however, proportion of eggs with no developed blastodiscs was statistically significant only for 100 Gy group (Table 3). The frequency of developed blastodiscs in the reinseminated groups showed that among the so-called "clear" eggs, many were fertilized but with dead blastodiscs, in which the germ died either before being placed

Table 4. The occurrence of developmental abnormalities in dead embryos in the hatcher (second experiment)

No. of autopsied embryos	Type of malformation								
	No. of affected embryos	Head deformities	Anoph-thalmia	Microph-thalmia	Beak deformities	Achondro-plasia of the chest	Leg deformities	Dupli-cation	Reduced growth
1,264	11	5	3	3	10	4	2	2	3

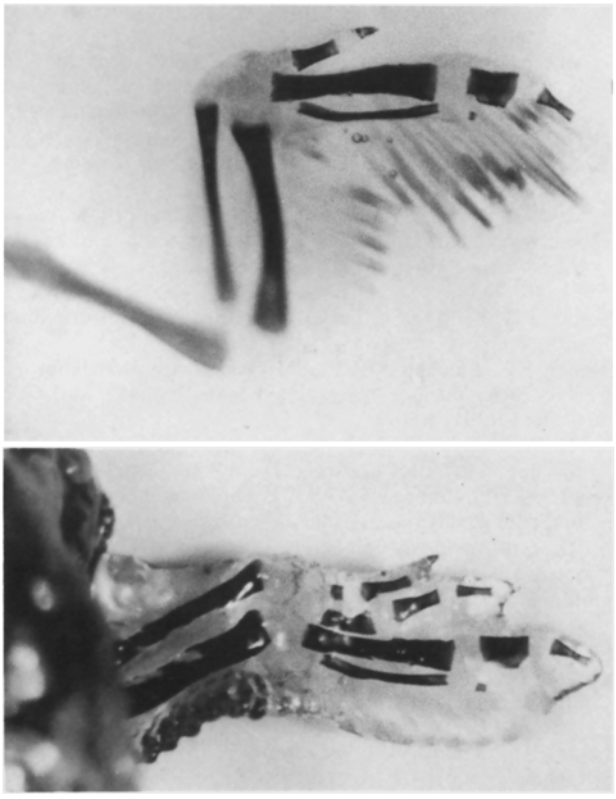

Fig. 1. *Above:* normal development of wing bones of a chicken embryo on the 19th day; *below:* observed in abnormalities an embryo developed from 600 Gy irradiated semen and the 24 h re-inseminated group (notice the duplication and the development of supernumerary phalanxes of the first fingers of the wing)

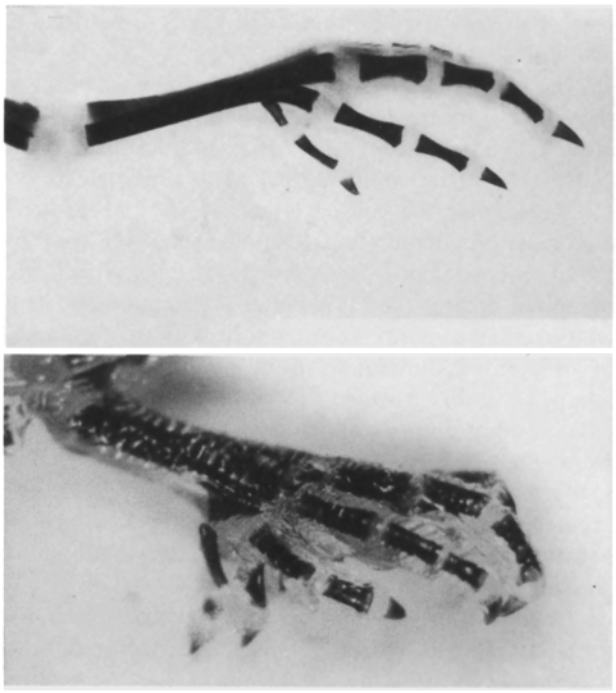

Fig. 2. *Above:* normal development of foot bones of a chicken embryo on the 19th day; *below:* observed in abnormalities an embryo developed from 600 Gy irradiated semen and the 24 h re-inseminated group (notice: the first toe of the foot is doubled with a splitting of the basic phalanx and with a supernumerary phalanx. The third toe is duplicated in all its elements)

in the incubator or the development was interrupted in the first stages.

We found 11 individuals (0.87%) with developmental abnormalities from the total number of 1,264 analysed embryos which died after the 18th day of incubation (Table 4). Beak deformities were the most frequent abnormalities. In all cases the upper beak was heavily shortened and crossed with the lower beak. We also found head deformities in the affected individuals (hernia cerebralis, acleiencephalia, one-sided and two-sided microphthalmia and anophthalmia). Achondroplasia of the chest was found in 4 individuals. Leg and wing deformities belonged to the sporadic disorders (Figs. 1 and 2).

Discussion and conclusion

Results of our experiments have shown the possibility of genetic transformation in domestic fowl by the technique described by Pandey and Patchell (1982) but the frequency of genetic tansformed chickens was, in contrast to that cited by these authors, many times lower. From the 3,646 potential transformation events screen-

ed, only 1 apparent transformation occured (0.027% frequency). Our results are similar to the results of Sanford et al. (1984) who found only 6 apparent transformed mutations from 59,000 potential transformed events (0.010% frequency) in *Zea mays*. A possible explanation of this very different rate of frequency of transformation between our and Pandey and Patchell's (1982) results might be connected with different gene expression of marker genes used in our and their experiment after their transformation as a part of the genome. Further work needs to be done to clarify this phenomenon.

The increased frequency of developed blastodiscs in the eggs of groups inseminated with irradiated semen only suggests that eggs were pseudofertilizated by 300–800 Gy irradiated sperms. The early death of blastodiscs may be caused either by the induction of dominant lethal mutations (Baumgartner 1982) or by the parthenogenetic development of eggs which was described by Sarvella (1971) after insemination of hens with irradiated sperm. The comparison of the frequency of malformed individuals from the total number of autopsied embryos which died in the hatcher, with the data of other authors, shows that the frequency found by us does not exceed the occurrence of spontaneous malformations (Romanoff and Romanoff 1972); the occurrence of certain type of developmental disorders, however, (beak deformities, duplications) were found in a higher frequency.

In conclusion it might be assumed that egg transformation caused by irradiated semen in domestic fowl is a rare event. For the use of this method in poultry breeding, further investigations concerning the doses given, classification of the gene expression after transformation, the optimum time intervals between the first and the second insemination, etc., is needed.

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