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CONSTRUCTION OF NEW TRANSGENIC QUAIL'S POPULATION USING INOCULATION METHOD.

By

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Abstract: *Birds are of great interest for a variety of research purposes, and effective methods for manipulating the avian genome would greatly accelerate progress in fields that rely on birds as model systems for biological research, such as developmental biology and behavioral neurobiology. Inoculation method has used as an effective method for producing transgenic quails that express the genomic DNA and/or Open Reading Frame "ORF" of growth hormone gene driven by Hubbard broiler breeder's chickens or Muscovy ducks. Two generations of quails carrying intact transgenes were produced, validating the use of the inoculation procedure for large-scale production of transgenic flocks. Inoculation of Muscovy duck gene at level 40ng resulted in increase of the hatchability by 20% approximately in the 2nd generation. There are no significant differences due to treatments on fertility or mortality percentages. Expression was sufficient to allow morphological changes to be perceptible for many treated quails. Specific transgenic expression at high levels provides a powerful tool for biological research and opens new avenues for genetic manipulation in quails.*

INTRODUCTION

Genetic engineering, or as commonly referred to recombinant deoxyribonucleic acid (DNA) technology, provides greater promise for the improvement of chicken. The technology is now sufficiently advanced so that chicken can be endowed with almost any gene or cluster of genes of interest to produce genetically-engineered chicken expressing desired requirements. Japanese quail have been used successfully as a model system for poultry in genetic selection and other research **(Baumgartner, 1994)**. Although the traditional method of gene transfer is effective to retrieve specific traits, then by using recurrent selection in successive back-crossing

the desired genes can be transferred although it is laborious and time consuming **Shoffner (1990).**

Transgenic technology is the process of introducing foreign, exogenous DNA not from either parents and usually from another species into the embryonic cells of newly fertilized egg to produce transgenic bird **(Buins, 1993)**. The strategy for modifying chicken genome to produce genetically engineered (transgenic) chickens is essentially the same like those adapted for plants **(Wagih and Wagih, 1999; Wagih, 2000)**. Transgenic animals have been used as models for studying gene functions and for producing very marketable proteins. Microinjection has been the most studied form of transgenic production in mammals, but is considerably more difficult to apply to poultry **(Li *et al.* 1995)**.

Recently, gene transfer into chicken cells is performed by four major systems including transfection of avian sperm **(Nakanishi and Iritani, 1993)**, development of germ-line chimeras by using primordial germ cells **(Watanabe *et al.*, 1994)** and blastodermal cells **(Eyal-Giladi and Kochav, 1976; Bosselman *et al.*, 1989)**, and development of embryonic stem cell lines **(Zhu *et al.*, 2005)**. inoculation of genetic material into fertilized egg, lipofection, electroporation, and the virus-mediated method. Each system has its own features and applicability as presented by **Yasugi and Nakamura (2000)**. The different approaches and their relative successes have been discussed extensively with the most successful methods described thus far being based on the use of viruses as gene transfer vectors **(Zajchowski and Etches 2000, D'Costa *et al.* 2001, Ivarie 2003, Mozdziak and Petitte 2004, Sang 2004)**. Although the avian transgenic technology has been fraught with technical difficulties, and transgenic chickens expressing reporter genes have only recently been developed **Mozdziak and Petitte (2004)**. Direct DNA injection of freshly laid chicken eggs is much more technically difficult compared with mammals because a fertile freshly laid chicken egg contains approximately 50,000–60,000 cells when it is laid **(Spratt and Haas, 1960)**. The technique currently available depends on the transfer of genetic material to animal genomes, and is now generally applied to the characterization of certain variants of species whose genome has been altered by the transfer of gene of foreign DNA into the pronuclear of zygotes and chicken embryos. DNA and/or genes could become integrated into the genome and was shown occasionally to be functional **(Wagner *et al.* 1981).**

The dual, but interrelated, goals of this research were to: (1) Construct transgenic Japanese quail's population by introducing foreign DNA and genes represented as part of growth hormone gene from pituitary gland of broiler breeder chickens and/or Muscovy duck individually into the embryonic cell of two strains of Japanese quails (Brown &Golden), to be a nucleus population; and (2) Detect the stability of DNA insertion into Japanese quail over generations.

MATERIALS AND METHODS

The experimental work was carried out in the Biotechnology Research Center (BRC), and Poultry Biotechnology Research Laboratory, Animal Production Department, Faculty of Agriculture, Suez Canal University, and Poultry Research Laboratory, Faculty of Agriculture (Saba Bacha), Alexandria University. The present study was conducted as an attempt to introduce functional growth hormone (GH) gene isolated from pituitary gland genes through its fragmented purified DNA and genes either from Hubbard broiler breeder's chickens (HB) or Muscovy ducks (MD), into embryo cell of Japanese quails strains (Brown and Golden). The effects of these treatments on the productive and reproductive performance, carcass, egg quality, and quality traits of hatched chicks at their suitable ages. In addition, hematological and biochemical genetic changes will be evaluated and studied in proliferate researches for two subsequent generations.

Preparation of Genomic DNA and genes of Hubbard Broiler Breeder's chickens and Muscovy ducks:

Selection of the Open Reading Frame (ORF):

A chicken Open Reading Frame (ORF) is a portion of a chicken genome which contains a sequence of bases that could potentially encode a protein. In a gene, ORFs are located between the start-code sequence (initiation codon) and the stop-code sequence (termination codon). In the current study, Open Reading Frame Finder Program at http://www.ncbi.nlm.nih.gov/ has been used to find the ORF within the complete GH cds, and resulted in six open reading frames. An open reading frame was selected according to the number of translated amino acid within this frame. To confirm the location of ORF within the exon of the GH gene, the BLAST analysis was performed by using the selected ORF against complete chicken GH sequences located in the chicken whole genome sequence (WGS) by using BLAST (BLAST v2.2.18 software;

http://www.ncbi.nlm.nih.gov/blast) resulted in homologous with 100%. Homologous sequences were subsequently used to design the new markers.

Selection of markers and genes.

Potential chicken ORFs were first identified by using ORF finder. Primer pairs were designed preferably within both complete GH sequence, and selected ORFs. The gene promoter region has been included preferably within the primers which were designed by using the complete GH sequence. Primers were designed with the PRIMER3 program (Table 1). Amplification conditions for each marker were optimized by varying the annealing temperature to produce a single amplicon of the predicted length with chicken genomic DNA. Only primer pairs that gave a clear amplification product with the chicken genomic DNA were used for further analysis.

Table 1: The new designed Marker; the markers with asterisk were used for this study.

Marker	Forward primer sequence	Reverse primer sequence
ORF1	ACCATCTCCATCCCTTCCAC	CGGCACTTCATCACCTTCAG
ORF2*	GCACTCACAGGTGGACACAA	AAGGGTTGAGGTGAGCACAG
GH1	ATCCCCAGGCAAACATCCTC	CCTCGACATCCAGCTCACAT
GH2*	CTAAAGGACCTGGAAGAAGGG	AACTTGTCGTAGGTGGGTCTG

Extraction DNA from Tissue.

The DNA was Isolated from Both of Broiler Breeder's chickens and Muscovy ducks tissues using the extraction DNA method described by **Rabie et al. (2009)**

PCR conditions.

Polymerase Chain Reactions (PCR) were performed in PCR system eppendorf® using PCR eppendorf tubes, and was amplified in a 50 µl final volume containing 12 µl template DNA(<0.1µg/µl, and mixture of 10µl GoTaq® Flexi buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1.25 U GoTaq® polymerase (GoTaq® Flexi DNA Polymerase, Promega, USA) and 1.2 µmol of each primer. Each marker was typed in duplicate independently to be ready of working with. Amplification conditions for each locus was optimized to produce a single amplicon of the predicted length with chicken genomic DNA and the amplification with Internal marker (DNA Ladder, Takara®) to verify that primers targeted the appropriate gene. The PCR reactions were started with 5 min. at 95°C followed by 35 cycles for 30 sec. at 95°C, 45 sec. at 50°C or 55°C annealing temperature, and 60 sec. at 72°C, followed by a final elongation step at 72°C for 10 min. The PCR products

were tested with electrophoresis on 1.5% ethidium bromide stained agarose gels in 0.5x TAE buffer (Promega, USA).

Extraction of specific PCR product from the agarose gel.

The PCR products from all markers were isolated from agarose gel by using the following steps according to **Islam *et al.* (2002)**, which is followed the basic methods as described by **Thuring *et al.* (1975)**, and **Tautz and Renz (1983)**. Except that, the isolated DNA was resuspended at STE buffer. That is because the DNA soluble in STE buffer is more stable compared to that soluble in the water **(Rabie *et al.*, 2009)**.

Experimental Designe

Egg treatments.

Five-hundred and eighty-three eggs in one hatch were obtained from laying Japanese quail (Brown and Golden) reared under normal conditions at the Poultry Research laboratory, Faculty of Agriculture (Saba Bacha), Fac. of Agric. Alexandria University. The incubator was cleaned, fumigated and washed with a sterilizing solution (Hydrocyle 200 ml/litre water). Clean eggs were sterilized with a sterilizing solution (Hysine with concentration 100 ml /litre water). Eggs were fumigated before setting the forced draft-type incubator. At the third day of incubation, the fertile eggs were randomly assigned into eight experimental groups as shown in Table 2.

Inoculation of the eggs.

Inoculation of genomic DNA and gene into the quail's embryos (Eggs) was carried out as described by **Salter *et al.* (1986)**. During the first 72 hours of incubation, the blastoderm became positioned beneath the topmost area of the shell. The surface of the egg (shell) was wiped with 70 % ethanol, then a hole of 2 mm. was drilled over the air cell of the egg and certain DNA and gene were injected. The surface of egg was wiped again, sealed with liquid paraffin and returned to the incubator. On the 15th day of the incubation, all treatment eggs were transferred into the hatchery until the 18th day of incubation. On hatching day, all eggs that failed to hatch were broken out and examined for assign to early dead and late dead, and examine any abnormalities in the embryos. This part of experiment was done under strict hygienic measures as summarized at (Table 2).

Management of hatched chicks.

Hatched quail chicks from different families per treatment were wing-banded on the day of hatch and brooded in floor pens. The house temperature was kept at about 35°C during the first 3 days, 32°C during next 4

days and gradually decreased by 2°C weekly until the end of the third maintained at 24°C. Chicks were fed a growing diet of 24.1% protein and 2860 kcal/kg, till 5 wk of age. Pullets were fed a layer diet of 20.06% protein and 2820 kcal/kg, from 6-14 weeks of age. Feed and water were provided *ad-libitum* to all chicks. At sexual maturity the birds had been moved to other layer batteries.

Hatch characteristics

Fertility percentage (%).

Eggs that failed to hatch and having full opportunity to hatch were broken out and then examined macroscopically to estimate the embryonic development. Fertility percentages were calculated for the first (G_1) and Second (G_2) generations for all treatments.

Hatchability percentage (%).

All percentage data of Hatchability was subjected to arcsine square root percentage transformation prior to analyses. Hatchability percentages were calculated for the first (G_1) and second (G_2) generations for all treatments.

Mortality percentage (%).

It was recorded as a mortality percentage of the initial number of chicks in the sub-group (three replicates for each sub-group approximately) from 1 to 6 weeks of age for first and second generations.

Table (2): Classification of the different treatments of the experiment.

Breeds	Source	Type	Level	Number of eggs	No. of chicks (G_1)	No. of chicks (G_2)
Brown	HB	DNA	20	47	16	30
			40	26	8	21
		Gene	20	36	9	19
			40	19	6	16
	MD	DNA	20	48	17	31
			40	27	2	-
		Gene	20	-	-	-
			40	35	18	36
	C	C	C	68	21	33
Golden	HB	DNA	20	47	15	25
			40	25	3	15
		Gene	20	35	8	24
			40	20	8	18
	MD	DNA	20	47	15	29
			40	-	-	-
		Gene	20	-	-	-
			40	35	21	36
	C	C	C	68	24	37

HB =Hubbard Broiler Breeders MD= Muscovy Ducks & C= control

Statistical Analysis.

Data were analyzed using to SAS program (**SAS, Institute, Inc, 1997**), by the application of the General Linear Method Procedure (GLM). All percentage data was subjected to arcsine square root percentage transformation prior to analyses. Test of significance for the differences between treatments or levels were done according to **Duncan (1955)**.The statistical model used was as follows:

$$Y_{ijklm} = \mu + +BR_i(SO)_j + DG_k(BR*SO)_{ij} + LEV_l(BR*SO*DG)_{ijk} + e_{ijklm}$$

Where Y_{ijklm} = observations. μ = overall means, BR_i $(SO)_j$ =The interaction between breeds and source. DG_k $(BR*SO)_{ij}$ = The interaction between breeders, source and DG, LEV_l $(BR*SO*DG)_{ijk}$ = The interaction between breeders, source, DG and levels, and e_{ijklm} =Residual.

Protein Banding Patterns.

To confirm the inheritance of inoculated either DNA or the part of the GH gene from both Hubbard broiler breeder's chickens and Muscovy ducks into the quail's eggs from the ancestor to the following generations, the protein analysis for G_0, G_1, G_2, due to inoculated and control were performed individually and pooled samples. The protein changes were identified using Sodium Dodecylsulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) as described by **Laemmli (1970)**; and Its modified method (Suez Canal University, Personal Communication) in five progressively steps where 500µl of blood sample was homogenized with the extraction buffer, and then samples were centrifuged at 6000 rpm for 20 minutes. The supernatant was then transferred to a different eppindorf tube. Thirty µl marker dye were added to 10µl of the extract samples and well mixed. Samples were finally heated on a water bath for 3 minutes immediately before loading in the gel. Thirty µl of each sample was loaded in a different lane in the pre-prepared SDS-PAGE gel. Thirty µl of the standard protein molecular markers (BioRAD®, USA) ranging between 214-6.8 kDa was also loaded in a separate lane. Subsequently, the gel electrophoresis was carried out at 280 Volt and 100 Am for 6 hours. The separation gel was then lifted off the electrophoresis glass sheets and stained with "Coomassie Stain" for 12 hours then distained for more 12 hours. The separated gel was pictured, and analyzed on a "Gel Documentation System" (GDS).

Analysis of the separated protein bands.

In the current study, the SDS-PAGE images were analyzed by using the GDS followed by AAB software, therefore The Simple band match similarity has calculated by Unweighted Pair Group Method with Arithmetic mean (UPGMA) **(Michener and Sokal; 1957)** which basically, the algorithm iteratively joins the two nearest clusters (or groups of species), until one cluster is left. Migrating mobility (Rf) in SDS-PAGE was calculated according to the following equation:

$$Rf = \frac{\text{The migration distance of the band}}{\text{The migration distance of the indication dye}}$$

Simple band match has been calculated using AAB software depending on number of bands shared between the samples. This method is fast but requires that bands be marked. Percent error may be set: this is defined as a percent of bands distance that another band must be in order for the two to be called the same band. 3.2% has been used as a percent error where. Higher Percent Match = Higher Similarity. Percent match ranges from 100% to 0%.

RESULTS AND DISCUSSION

Fertility percentage

No significant differences were shown in fertility percentages (%) of transgenic Brown and Golden quails due to DNA source and genes (HB and MD) within the studied levels (20 or 40ng). The fertility percentage reported her is for Japanese quails (Brown and Golden) control group (75.93 and 86.46 %) was closer to the corresponding values recorded by **El-Tahawy (2005)** (84.05-87.4%) but higher than those found by **Mandour (1996)** (59.4-65.6%). Moreover, it was worth noted that introducing HB or MD DNA and gene increased fertility percentages compared to those indicated by **Mandour (1996)** while using Japanese quail bursal DNA (78.1%) for Gimmizah and (57.3%) for Golden Montazah strains, **Ali *et al.* (1999)** while using broiler (63.6 and 43.52% and turkey (64.83%) DNA, as well as **Ali (2001)** while using duck (48.6%) and Fayoumi (63.3%) DNA origin. However, these values were less than those recorded by **El-Tahawy (2005)** while using Japanese quail (91.63%) or broiler breeders (90.51%) DNA, but these values were within range found by **Doaa (2006)** while working on Bandarah and found that, the overall mean value of fertility percentage were not significantly differed due to microinjection of Bursa and Thymus DNA

(87.04 and 87.22%) and cells (87.86 and 86.07%), compared to control group (86.46%).

Hatchability percentages

In the first generation, the whole average of hatchability percentage for Brown quails treated by HB or MD source and control group were 30.35, 31.42, 30.88% respectively, and corresponding values for Golden quail were 26.69, 45.96, 35.29 %. It could be observed from Table 3 that the whole average value of hatchability percentage was improved due to microinjection of MD DNA source in Brown quail and Golden quail compared to control. However, MD DNA source induced higher increase (30.2 %) in hatchability percentage in Golden quail compared response in Brown quail (1.7 %). On the other hand, there no effect of HB DNA source on hatchability percentage in Brown quails; however, it was decreased in Golden quail by 24.4 %.

In the second generation, Brown and Golden quail showed no significant response to foreign DNA or gene of either source at different levels used ($P< 0.05$) for the whole average commercial hatchability percentage compared to the corresponding control group. Although MD gene (40 ng) inoculation induced high hatchability % in Brown quail fertile eggs (51.43 %) & Golden quail ones (60.00 %) during first generation, hatchability % was increased to (85.24± 5.85 %) during second generation (Tables 4 and 5). In addition, DNA inoculation of either source MD (Brown quail) or HB (Golden quail) decreased hatchability % during first generation to the least of all treatments (7.41 & 12 %). The average of commercial hatchability percentage of control group in (Brown and Golden) quail (62.91 and 73.90 %) were lower compared to those recorded by **El-Turky (1981)**, **El-Hossari *et al.* (1992)** (84.6%), and **Abd El-Galil (1993)** (75.5%), but higher than those reported by **Mandour (1996)** (59.4%), would be due to stress of egg drilling and injection with buffer solution of DNA and buffer solution of cells. Results reported herein for the effect of introducing DNA on hatchability were less than those found by **El-Tahawy (2005)** (86.5%) for Japanese quail-DNA, and 84.05% for (Broiler Breeders-DNA), but higher than those recorded by **Ahmed *et al.* (1994)** (24.1-1.01%) while injecting turkey DNA, and **Doaa (2006)** while working on Bandarah the effect of different foreign DNA sources and levels on hatchability percentages was significantly realized (maximum) for eggs injected by bursa DNA 4μg/egg (60.55%). Also **Doaa (2006)**. postulated that the reduction in hatchability percentages as a result of introducing foreign DNA or cells would be due to genetically unbalanced chromosome arrangement

in embryos, chromosomal aberration or disturbance, altering through physiological feed back mechanisms, replacing particular genes with mutant forms, or deleting genes which will be back bone to genetic disease model builders.

Mortality percentages:

There were no significant differences shown in mortality percentage of transgenic Brown and Golden quails due to (HB and MD) DNA source and genes within the studied levels (20 or 40 ng) (Tables 5 and 6). The least

Table (3): Means for the effect of Japanese quail breed, nucleic acid source (Broiler vs. Duck) and type (DNA vs. Gene) on hatchability, and mortality percentages during the 1st generation.

Traits		Hatchability %			Mortality %		
Breeds	Source / Type	HB	MD	C	HB	MD	C
B	DNA	32.41	21.42	30.88	9.38	2.94	23.81
	Gene	28.29	51.43	30.88	5.55	11.11	23.81
	Average	30.35	31.42	30.88	7.47	5.66	23.81
G	DNA	21.96	31.91	35.29	3.34	0.00	8.33
	Gene	31.43	60.00	35.29	18.75	0.00	8.33
	Average	26.69	45.96	35.29	11.04	0.00	8.33

HB = Hubbard Broilers. MD= Muscovy Ducks. C= control . B =Brown. and G= Golden

Table (4): Means for the effect of Japanese quail breed, nucleic acid source (Broiler vs. Duck), type (DNA vs. Gene) and level (20 vs. 40) on hatchability, and mortality percentages during the 1st generation.

Traits		Hatchability %				Mortality %			
Breeds	Type	DNA		Gene		DNA		Gene	
	Source	20 ng/µl	40 ng/µl	M4 20 ng/µl	M2 40 ng/µl	20 ng/µl	40 ng/µl	M4 20 ng/µl	M2 40 ng/µl
B	HB	34.04	30.77	25.00	31.58	6.25	12.50	11.11	0.00
	MD	35.42	7.41	-	51.43	5.88	0.00	-	11.11
	C	30.88	30.88	30.88	30.88	23.81	23.81	23.81	23.81
G	HB	31.91	12.00	40.00	22.86	6.67	0.00	25.00	12.50
	MD	31.91	-	-	60.00	0.00	-	-	0.00
	C	35.29	35.29	35.29	35.29	8.33	8.33	8.33	8.33

HB = Hubbard Broilers, MD= Muscovy Ducks, C= control , B =Brown, and G= Golden
M2= Open Reading Frame (ORF) sequence & M4= complete GH sequence. (20&40ng/µl /egg)

Table (5): Least square means ± standard errors for the effect of Japanese quail breed, nucleic acid source (Broiler vs. Duck) and type (DNA vs. Gene) on fertility and hatchability percentages during the 2nd generation.

Traits		Fertility %			Hatchability % *		
Breeds	Source / Type	HB	MD	C	HB	MD	C
B	DNA	77.63 ± 8.43 ^{a}	82.10 ± 15.02 ^{ax}	75.93 ± 4.43 ^{ax}	60.70 ± 9.48 ^{ax}	75.41 ± 17.06 ^{ax}	62.91 ± 5.00 ^{ax}
	Gene	85.67 ± 5.83 ^{a}	95.32 ± 2.02 ^{ax}	75.93 ± 4.43 ^{ax}	74.74 ± 4.94 ^{ax}	85.24 ± 5.85 ^{axw}	62.91 ± 5.00 ^{ax}
	Average	81.65 ± 5.58 ^{a}	88.71 ± 6.64 ^{aZ}	75.93 ± 4.43 ^{aZ}	67.72 ± 6.04 ^{aZ}	80.32 ± 7.81 ^{aZ}	62.91 ± 5.00 ^{aZ}
G	DNA	87.26 ± 5.89 ^{a}	83.89 ± 9.73 ^{ax}	86.86 ± 5.35 ^{ax}	73.91 ± 7.42 ^{ax}	78.89 ± 9.97 ^{ax}	73.90 ± 6.27 ^{ax}
	Gene	74.87 ± 10.55 ^{a}	80.67 ± 4.20 ^{ax}	86.86 ± 5.35 ^{ax}	62.54 ± 13.41 ^{ax}	58.64 ± 4.20 ^{axz}	73.90 ± 6.27 ^{ax}
	Average	81.06 ± 6.00 ^{a}	82.28 ± 4.34 ^{aZ}	86.68 ± 5.35 ^{aZ}	68.23 ± 7.42 ^{aZ}	68.77 ± 5.48 ^{aZ}	73.90 ± 6.27 ^{aZ}

$^{a-c}$ Means within the same row having different letters are significant different ($p \leq 0.05$).

$^{x-y}$ Means within the same column (Breed) having different letters are significant different ($p \leq 0.05$).

$^{Z-W}$ Means within the same column, between Average, having different letters are significant different ($p \leq 0.05$).

HB = Hubbard Broilers, MD= Muscovy Ducks, C= control , B =Brown, and G= Golden

* Hatchability % = as a percentage from the total number of setted eggs.

Table (5): Least square means ± standard errors for the effect of Japanese quail breed. nucleic acid source (Broiler vs. Duck) and type (DNA vs. Gene) on hatchability and mortality percentages during the 2nd generation.

Traits		Hatchability %**			Mortality %		
Breeds	Source / Type	HB	MD	C	HB	MD	C
B	DNA	75.98 ± 6.34 ^{ax}	88.10 ± 7.90 ^{ax}	82.14 ± 3.32 ^{ax}	16.46 ± 7.72 ^{ax}	22.78 ±13.62 ^{a}	19.37 ± 5.26 ^{a}
	Gene	87.67 ± 4.92 ^{ax}	89.14 ± 4.61 ^{ax}	82.14 ± 3.32 ^{a}	20.28 ± 3.55 ^{ax}	15.29 ± 5.52 ^{a}	19.37 ± 5.26
	Average	81.78 ± 4.20 ^{aZ}	88.26 ± 4.04 ^{aZ}	82.14 ± 3.32 ^{aZ}	18.37 ± 4.31 ^{aZ}	19.04 ± 6.78 ^{aZ}	19.37 ± 5.26 ^{aZ}
G	DNA	83.84 ± 3.96 ^{ax}	94.45 ± 5.56 ^{ax}	84.84 ± 4.31 ^{ax}	23.20 ± 5.82 ^{ax}	12.70 ± 1.59 ^{a}	24.36 ± 8.68 ^{a}
	Gene	79.75 ± 9.91 ^{ax}	73.32 ± 5.74 ^{ay}	84.84 ± 4.31 ^{a}	32.43 ± 14.26 ^{ax}	11.11 ± 0.00	24.36 ± 8.68
	Average	81.79 ± 5.10 ^{aZ}	83.88 ± 5.19 ^{aZ}	84.84 ± 4.31 ^{aZ}	27.82 ± 8.66 ^{aZ}	11.90 ± 1.06 ^{aZ}	24.36 ± 8.68 ^{aZ}

a-c Means within the same row having different letters are significant different ($p \leq 0.05$).

x-y Means within the same column (Breed) having different letters are significant different ($p \leq 0.05$).

Z-W Means within the same column, between Average, having different letters are significant different ($p \leq 0.05$).

HB = Hubbard Broilers. MD= Muscovy Ducks, C= control , B =Brown, and G= Golden

** Hatchability % = as a percentage from the total number of fertile eggs

Table (6): Least square means ± standard errors for the effect of Japanese quail breed, nucleic acid source (Broiler vs. Duck), type (DNA vs. Gene) and levels (20 vs. 40) on fertility and hatchability percentages during the 2nd generation.

Traits		Fertility				Hatchability % *			
Breeds	Type	DNA		Gene		DNA		Gene	
	Source	20 ng/µl	40 ng/µl	M4 20 ng/µl	M2 40 ng/µl	20 ng/µl	40 ng/µl	M4 20 ng/µl	M2 40 ng/µl
B	HB	80.26 ± 9.47 ^{ax}	75.00 ± 25.00 ^{ax}	78.48 ± 7.88 ^{ax}	92.86 ± 7.14 ^{ax}	75.57 ± 9.73 ^{ax}	45.84 ± 20.84 ^{ax}	70.32 ± 11.50 ^{ax}	79.17 ± 4.65 ^{ax}
	MD	82.10 ± 15.02 ^{ax}	-	-	95.32 ± 2.02 ^{ax}	75.41 ± 17.06 ^{ax}	-	-	85.24 ± 5.85 ^{ax}
	C	75.93 ± 4.43 ^{x}	75.93 ± 4.43 ^{x}	75.93 ± 4.43 ^{x}	75.93 ± 4.43 ^{x}	62.91 ± 5.00 ^{x}	62.91 ± 5.00 ^{x}	62.91 ± 5.00 ^{x}	62.91 ± 5.00 ^{x}
G	HB	83.60 ± 8.18 ^{ax}	90.91 ± 9.09 ^{ax}	70.75 ± 21.19 ^{ax}	78.99 ± 9.51 ^{ax}	66.00 ± 9.80 ^{ax}	81.82 ± 9.09 ^{ax}	62.94 ± 21.17 ^{ax}	62.15 ± 21.22 ^{ax}
	MD	83.89 ± 9.73 ^{ax}	-	-	80.67 ± 4.20 ^{ax}	78.89 ± 9.97 ^{ax}	-	-	58.64 ± 4.20 ^{ax}
	C	86.86 ± 5.35 ^{x}	86.86 ± 5.35 ^{x}	86.86 ± 5.35 ^{x}	86.86 ± 5.35 ^{x}	73.90 ± 6.27 ^{x}	73.90 ± 6.27 ^{x}	73.90 ± 6.27 ^{x}	73.90 ± 6.27 ^{x}

$^{a-c}$ Means within the same row having different letters are significant different ($p \leq 0.05$).
$^{x-y}$ Means within the same column (Breed) having different letters are significant different ($p \leq 0.05$).
HB = Hubbard Broilers, MD= Muscovy Ducks, C= control , B =Brown, G= Golden, M2= Open Reading Frame (ORF) sequence & M4= complete GH sequence. (20&40 nano gram/ micro liter/egg).
* Hatchability % = as a percentage from the total number of setted eggs

Table (6): Least square means ± standard errors for the effect of Japanese quail breed, nucleic acid source (Broiler vs. Duck) and type (DNA vs. Gene) on hatchability and mortality percentages during the 2nd generation.

Traits		*Hatchability %***				*Mortality %*			
Breeds	Type / Source	DNA		Gene		DNA		Gene	
		20 ng/µl	40 ng/µl	M4 20 ng/µl	M2 40 ng/µl	20 ng/µl	40 ng/µl	M4 20 ng/µl	M2 40 ng/µl
B	HB	*93.45 ± 2.94 ^{ax}*	*58.34 ± 8.33 ^{b}*	*89.04 ± 5.71 ^{ax}*	*86.31 ± 8.27 ^{ax}*	*24.80 ± 10.84 ^{ax}*	*8.12 ± 1.89 ^{ax}*	*26.11 ± 3.89 ^{ax}*	*14.46 ± 3.15 ^{ax}*
	MD	*88.10 ± 7.90 ^{ax}*	-	-	*89.14 ± 4.61 ^{ax}*	*22.78 ± 13.15 ^{ax}*	-	-	*15.29 ± 5.52 ^{ax}*
	C	*82.14 ± 3.32 ^{x}*	*82.14 ± 3.32 ^{x}*	*82.14 ± 3.32 ^{x}*	*82.14 ± 3.32 ^{x}*	*19.37 ± 5.26 ^{x}*	*19.37 ± 5.26 ^{x}*	*19.37 ± 5.26 ^{x}*	*19.37 ± 5.26 ^{x}*
G	HB	*77.79 ± 4.77 ^{ax}*	*89.90 ± 1.01 ^{ax}*	*85.64 ± 5.87 ^{ax}*	*73.86 ± 20.55 ^{ax}*	*21.41 ± 8.13 ^{ax}*	*25.00 ± 0.00 ^{ax}*	*15.34 ± 9.03 ^{abx}*	*29.17 ±14.11 ^{acx}*
	MD	*94.45 ± 5.56 ^{ax}*	-	-	*73.32 ± 5.74 ^{bx}*	*12.70 ± 1.59 ^{ax}*	-	-	*11.11 ± 0.00 ^{ax}*
	C	*84.84 ± 4.31 ^{x}*	*84.84 ± 4.31 ^{x}*	*84.84 ± 4.31 ^{x}*	*84.84 ± 4.31 ^{x}*	*24.36 ± 8.68 ^{x}*	*24.36 ± 8.68 ^{x}*	*24.36 ± 8.68 ^{x}*	*24.36 ± 8.68 ^{x}*

$^{a-c}$ Means within the same row having different letters are significant different ($p \leq 0.05$). $^{x-y}$ Means within the same column (Breed) having different letters are significant different ($p \leq 0.05$). HB = Hubbard Broilers MD= Muscovy Ducks C= control & B =Brown G= Golden. M2= Open Reading Frame (ORF) sequence & M4= complete GH sequence. ** Hatchability % = as a percentage from the total number of fertile eggs.

mortality % (0.00 %) was noticed due to inoculation of HB gene (40 ng) & MD DNA (40 ng) to Brown quail and HB DNA (40 ng), MD DNA (20 ng) & MD gene (40 ng) to Golden quail during the first generation, while the highest mortality % was recorded in control group during the first generation (23.80%) and HB gene (40ng) Golden quail group (29.52 ± 14.11 %) during the second generation.

The mortality percentage of control group of Brown quail (19.37 %) was within the range found by **Ali (2001)** (13.3-37.9%), but higher than those recorded by **El-Naggar (2002)** (6.66 %) on Japanese quail. Meanwhile, the percentages mortality detected in transgenic Japanese quail were lower than that found in transgenic White Leghorn but less than their corresponding treatments in Norfa strain **(Ali, 2001)**, but higher than those obtained by **El-Naggar (2002)** (3.33-6.66 %) on transgenic quail.

The separated protein bands

The SDS-PAGE analysis have used in this study to ensure that the transgenic materials are presence in consequence generations. The results revealed the presence of 33 fractions of protein bands which were discovered in the quail samples. Approximately one-third of the electrophoretic spectrum of the protein bands was composed of strong fractions, forming brightly stained bands in the gel. The remaining fractions stained much less strongly, and they included. One-third of the separated protein bands contained fractions of high-molecular-weight with mol. wt. of over 95 kD (Table 7). The SDS-PAGE method was also used by **Ghislaine *et al.*, (1999)** who found the existing of S3 gene ORF for Muscovy duck was similar to that of the chicken reovirus S3 gene product. The RF values for all transected genes were presented in Table 8 calculated by using gel documentation system (GDS).

Analyzing the pictured stained SDS-PAGE by gel documentation system (GDS) indicated the presence of 4 bands in the control sample, and 7-9 bands in the MD DNA (20ng), HB DNA (20 and 40ng). The UPGMA clustering analysis and simple band match has been used. The effect of all treatments summarized as follow.

HB DNA (20 ng)

As shown in Figure (1), although there were slightly differences between G_1 and G_2 samples (7.69 and 18.68) according to the bands match similarity between them, the results showed that the control differed than the treatments enormously (30.91).

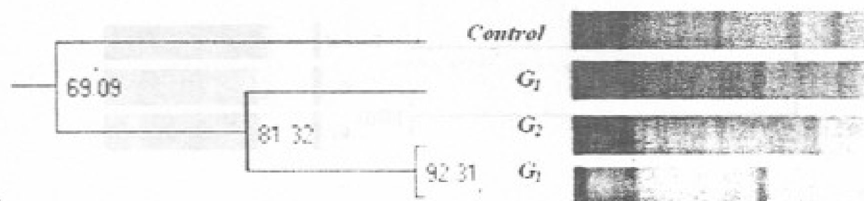

Figure (1): Separated protein bands dendogram calculated as simple band match for HB DNA (20ng) by AAB software using UPGMA cluster analysis.

HB DNA (40 ng)

The same trend resulted in HB DNA (40ng) where the bands match similarity between the control and the treated G_1 and G_2 was 66.36 compared by 92.31 between the G_1 and G_2 samples as shown in Figure (2).

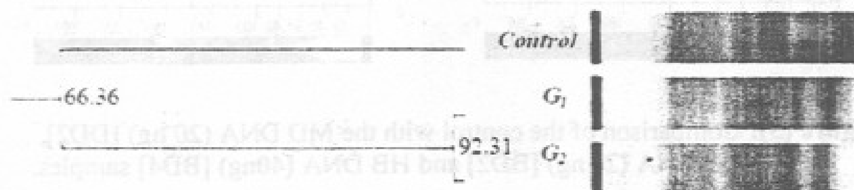

Figure (2): Similarity dendogram for the separated protein bands calculated as simple band match for treated quails by HB DNA (40ng) using AAB software.

MD DNA (20ng).

Compared the control against the treatment resulted in 27.27% differences, and there were no differences between G_1 and G_2. This results confirmed that the transfer genomic MD DNA (20ng) is not efficient.

HB gene (40ng):

Chicks that had been transected with HB gene (40ng) presented higher levels of expression then those not treated (Figure 4) whereas the there were 15% from the separated proteins were differed between G_1 and G_2, and those were differed dramatically from the control (58.82%), though the efficiency of the transfection was high.

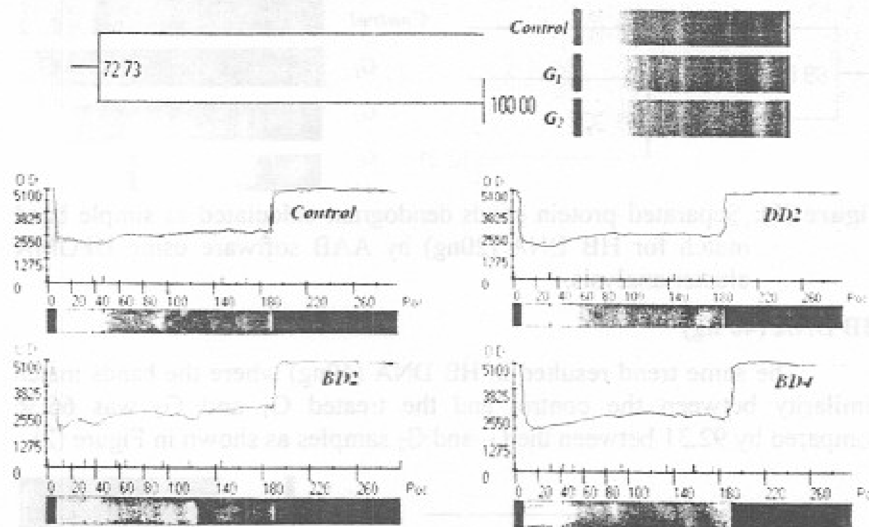

Figure (3): Comparison of the control with the MD DNA (20 ng) [DD2], HB DNA (20 ng) [BD2] and HB DNA (40ng) [BD4] samples.

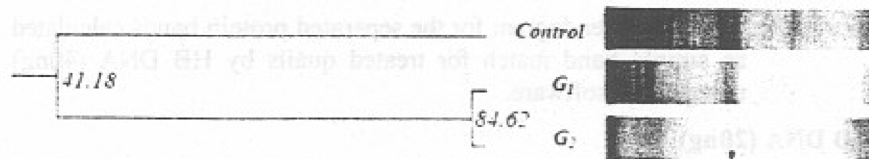

Figure (4): Protein dendogram analysis between treated quails by Hubbard broiler breeder's gene at level 40ng ($G_1$and G_2) compared to control.

HB gene (20ng).

In HB gene (20ng) treatment, the G_1 had different picture ranged the similarity from 77 to 81% approximately, the control showed 50% differences than the treated quail over two generations as shown in Figure (5).

Table (7): Molecular weight Comparison for separated protein bands for the treated quails by MD gene (40ng) [GD4], HB gene (40ng) [GBD24], and HB gene (20ng) [GBD42].

Band	Mol.Wt.			GD4		GBD24		GBD42	
		Marker	Control	G_1	G_2	G_1	G_2	G_1	G_2
		Molecular weight (Mol. Wt.)							
1	328.25								328.25
2	214	214							
3	184.08							184.08	
4	171.83					171.83			
5	160.88						160.88		
6	150.97			150.97	150.97				
7	133.59						133.59		133.59
8	118.71				118.71			118.71	
9	111.25	112							
10	103.06					104.15	103.05		
11	98.79			99.1	97.33			100.03	100.03
12	90.91	92				90.36			
13	87.83				87.21		87.21		
14	85.1							85.69	84.22
15	72.94					73.55	72.34		
16	69.18		69.99	68.85				68.85	
17	67				67.73				66.63
18	64.76							64.5	
19	54.47					54.82	54.82	53.93	
20	52.59	52.2	53.06	53.06	52.2				53.06
21	47.25						47.8		47.06
22	44.62			44.62		44.29			
23	38.97							39.13	38.57
24	37.18				36.71	37.76		36.97	
25	35.84	35.7		36.46		35.95			35.6
26	32.42					32.12		32.19	
27	30.85		30.86	30.99	30.92				30.27
28	28.88	28.9				28.96	29.13	29.07	
29	27.14								27.19
30	25.99					25.9			
31	14.66		14.85	14.58	14.72	14.72	14.72	14.85	14.58
32	9.75			9.66	9.78				
33	9.35						9.29	9.29	
Total Band		**6**	**4**	**9**	**10**	**12**	**10**	**13**	**12**

Table (8): Relative mobility (Rf) comparison for separated protein bands for the treated quails by MD gene (40ng) [GD4], HB gene (40ng) [GBD24] and HB gene (20ng) [GBD42].

Band	Mol.Wt.			GD4		GBD24		GBD42	
		Marker	Control	G_1	G_2	G_1	G_2	G_1	G_2
		Rf							
1	328.25								0.0
2	214	0.01							
3	184.08							0.02	
4	171.83					0.02			
5	160.88						0.02		
6	150.97			0.03	0.03				
7	133.59						0.03		0.03
8	118.71				0.03			0.03	
9	111.25	0.04							
10	103.06					0.05	0.05		
11	98.79			0.06	0.07			0.06	0.06
12	90.91	0.08				0.09			
13	87.83				0.09		0.09		
14	85.1							0.09	0.1
15	72.94					0.11	0.12		
16	69.18		0.12	0.12				0.12	
17	67				0.13				0.13
18	64.76							0.13	
19	54.47					0.16	0.16	0.16	
20	52.59	0.16	0.16	0.16	0.16				0.16
21	47.25						0.19		0.19
22	44.62			0.21		0.21			
23	38.97							0.25	0.26
24	37.18				0.27	0.26		0.27	
25	35.84	0.28		0.28		0.28			0.28
26	32.42					0.38		0.38	
27	30.85		0.42	0.42	0.42				0.44
28	28.88	0.5				0.49	0.49	0.49	
29	27.14								0.52
30	25.99					0.53			
31	14.66		0.7	0.7	0.7	0.7	0.7	0.7	0.7
32	9.75			0.79	0.79				

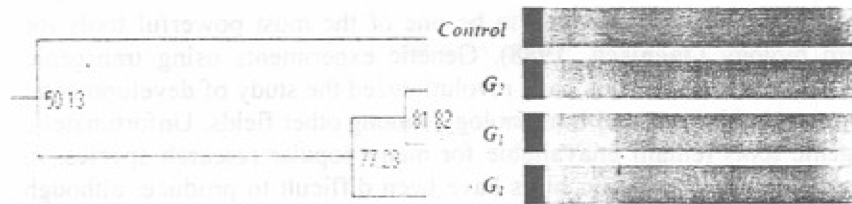

Figure (5): Protein dendogram shows the similarity between treated quails by Hubbard broiler breeder's gene at level 20ng ($G_1$and G_2) compared to control.

MD gene (40ng).

With the following figure, the MD gene (40ng) treatment shows higher percentage of band similarities between G_1 and G_2 (90%), thus differ than the control by 38% approximately (Figure 6).

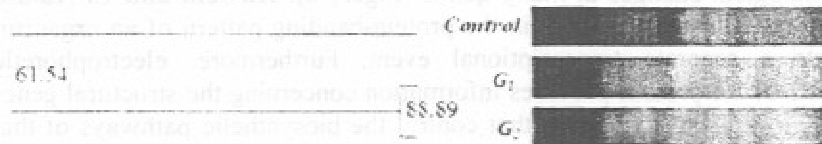

Figure (6): Protein dendogram analysis for treated quails by Muscovy duck gene at level 40ng ($G_1$and G_2) compared to control.

Comparing all treatments

The results confirmed that all the treatments had superior differences than the control as shown at Figure 7.

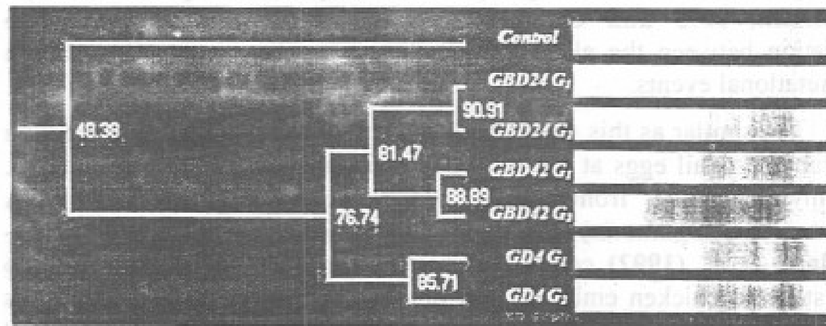

Figure (7) The multiple comparisons between the control and all transected quail by using different sources and levels of growth hormone gene.

Transgenesis has proven to be one of the most powerful tools for modern biology **(Jaenisch, 1988)**. Genetic experiments using transgenic mice, fish, worms, and flies have revolutionized the study of developmental biology, neurobiology, and immunology, among other fields. Unfortunately, transgenic tools remain unavailable for many popular research species. In particular, useful transgenic birds have been difficult to produce, although numerous attempts have been made for >25 years (**Sang, 2004**). The high degree of homology between chicken and quail genomes allows researchers to design highly specific DNA constructs for the production of transgenic birds. In addition, transgenic quail offer all of the advantages of the classic avian developmental model system, such as the ability to readily produce quail:chick transplant chimeras (**Poynter *et al.*, 2009**). In current study, the transgenic quail's population has been constructed successfully by inoculated either broiler or duck growth hormone gene, and confirmed not only by the protein-banding pattern of each treated group but as a morphological changes at many quails (Figure 8). **Hussein and El-Abidin (1985)** stated that each band in the protein-banding pattern of an organism reflects a separate transcriptional event. Furthermore, electrophoretic analysis of the protein provides information concerning the structural genes and their regulatory systems that control the biosynthetic pathways of that protein. An important proportion of the protein-coding genes are polymorphic, i.e. they exist in the form of one or more alleles. Changes events such as transgenic effect, therefore, have to be considered as the reasonable interpretation for the observed banding pattern changes. In this study, the protein band changes have been discovered for two subsequent generations. Therefore, the results confirmed that all transgenic birds were differing than the control group which emphasized by **Muller and Gottschalk, 1973 and Gamal El-Din *et al.*, 1988 who found the** correlation between the alterations in the electrophoretic banding pattern and mutational events.

The similar as this study, **El-Naggar (2002)** found that the injection of incubated quail eggs at the third day of incubation by DNA of adrenal, and thyroid gland from naked neck chicken and bacterial DNA (*Streptococcus agalactia*) produced transgenic quails successfully. **El-Wardany *et al.* (1992)** concluded that insertion of foreign material into early stage of chicken embryos may permit the transfer of favorable traits and may affect the long-rang improvement of poultry. Although all transgenic birds in this study completely differ than the control group by using inoculated method, Direct DNA injection to produce transgenic chickens through the injection of DNA into the vicinity of the pronuclei of

the newly fertilized egg **(Love *et al.*, 1994),** resulted in the efficiency of this process was low, and only a

few transgenic birds have been produced through DNA injection. **Gordon and Ruddle (1982)** reported that genetic manipulation of the avian genome to produce transgenic embryos by DNA micro-injection has lagged behind the technology available in mammalian systems. Moreover, the gene transfer has several advantages not only increasing the genetic variation by 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 discover desirable gene that may lead to improve and solve breeding problems **(Shuman and Shoffner, 1986).**

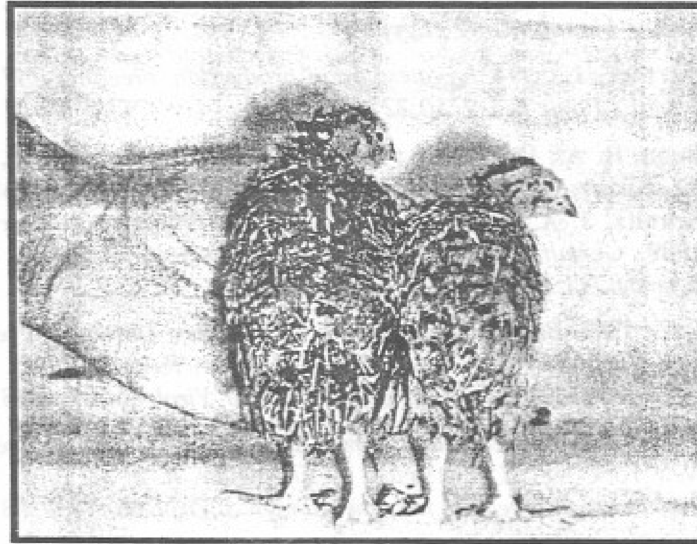

Figure 8: Effect of the treatment by GD4 on morphological changes compared to control.

In conclusion, transgenic quail's population has been produced successfully expressing either broiler or duck growth hormone gene which was superior to the control group. The quail as a model should provide a valuable tool for the study of the functions of gene in metabolism, production, and reproduction performance. More studies are needed to confirm the presence of the transected genes by using advanced techniques than that used in this study.

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الملخص العربي

بناء قطيع السمان النقلجيني بواسطة طريقة الحقن.

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[1]جامعة قناة السويس، كلية الزراعة، قسم الإنتاج الحيواني و الدواجن، ٤١٥٢٢ الإسماعيلية، جمهورية مصر العربية.

[2]جامعة الأسكندرية، كلية الزراعة (سابا باشا)، قسم الانتاج الحيواني و الأسماك، جمهورية مصر العربية.

للطيور أهمية خاصة لأغراض البحث العلمي المتعددة، و عليه فإن الطرق الفعالة للتعامل مع جينوم الطيورتقدمت بسرعة كبيرة في المجالات التي تعتبر الطيور كنظام نموذجي للأبحاث البيولوجية مثل التطور البيولوجي و سلوكيات البيولوجية العصبية . استخدمت طريقة الحقن بكفائة لإنتاج السمان النقلجيني الذى يعبر عن الدنا الجينومي genomic DNA و/أو اطار القراءة المفتوح Open Reading Frame لجين هورمون النموالمأخوذ من امهات دجاج اللحم هابارد أو البط المسكوفي.

تم انتاج٢جيلين من طيور السمان النقلجيني والذى أكد أن طريقة الحقن يمكن استخدامها على مستوى انتاجي واسع لإنتاج قطعان نقلجينية. أوضحت النتائج أن حقن جين البط المسكوفي عند مستوى ٤٠ نانوجرام أدت إلى زيادة نسبة الفقس في الجيل الثاني بنسبة ٢٠% تقريبا.

لايوجد اختلافات معنوية راجعة إلى المعاملات على نسبتي الخصوبة و النفوق. كان التعبير الجيني كافيا لإظهار إختلافات مظهرية في العديد من طيور السمان المعاملة. قدم التعبير النقلجيني المحدد عند المستويات المرتفعة أداه قوية للأبحاث البيولوجية و تفتح افاق جديدة لتداول المادة الوراثية في طيور السمان.