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IMPACT OF GROWTH HORMONE GENE TRANSFER BY SPERM-MEDIATED GENE TRANSFER ON EGG PRODUCTIVE AND REPRODUCTIVE TRAITS OF BANDARAH LOCAL CHICKEN

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ABSTRACT: The experimental was aimed to improve genetic of Bandarah Local chicken strain to insert Growth hormone gene (cGH) from (Cobb 500) broiler strain by method sperm-mediated gene transfer technique (SMGT). This study investigated for two generations. Total RNA was extracted from chicken liver tissue and the cDNA was successfully prepared. PCR Products GH, mRNA normal Length 810bp for transgenic Bandarah chickens which treated by SMGT method, the same result was found in the first and second generation. The averages of fertility percentage were 88.97% and 92.14 % for SMGT and control, respectively. The overall mean of the second generation was nearly value 90.76% of the first one 90.38%.The responses of the transgenic techniques SMGT for fertility percentage were 5.64%. The responses of hatchability for SMGT were 18.35% and 21.82% for fertile and total eggs, respectively The SMGT of transferring was decreased the age at sexual maturity ASM at the second generation by 35.01 d. Egg number which produced during the first 90 days from SMGT method significantly increased compared by the control. There was highly significant difference between treated found, the SMGT method had the heaviest egg weight 49.20 g followed by control one 48.22 g. After two generations, the SMGT technique was improved egg number by 10.62 egg, decreased egg weight by 0.9g and improved egg mass during the first 90 days of laying by 512.62 g. SMGT is an efficient method that will hopefully facilitate the implementation of strategies for securing the benefits that can be expected to arise from the introduction of transgenic chicken. Chicken cGH gene was affected in egg productive traits and reproductive traits and moved from the first generation to the second generation with the same shape and increased the effect. Growth hormone gene transfer by SMGT will save time to improve egg productive traits and reproductive traits.

Key words: Egg traits, reproductive traits, GH gen, gene transfer, SMGT

INTRODUCTION

Poultry production is an important and diverse component of agriculture all over the world. Today, more attention has been given to indigenous animals in general, and poultry in particular; due to their quality of meat and egg production (Kaya and Yıldız, 2008).The chicken growth hormone (GH) gene has an important function in chicken growth and reproduction (Samaneh Gorji Makhsous *et al.,* 2013) who found that (GH) gene could be a genetic locus or linked to a major gene significantly affecting egg number and rate of lay traits in chickens, and the relationship between these traits may be useful for molecular marker-assisted selection (MAS) to improve the chickens breeding programs.

Polymorphisms are the most frequently found DNA sequence variations in the animal genome and can be used as genetic markers for association analysis with economic traits. The Growth hormone gene (GH) is polypeptide that regulates the growth of many types of mammalian cells (Malak *et al.,* 2008). Most of the functions of the growth hormone in chickens are mediated by insulin-like growth factors (IGF) which stimulate amino acid uptake, glucose metabolism and DNA synthesis (McMurtry, 1998). Furthermore, RT-qPCR demonstrated that the GH gene could be a candidate gene for reversing nesting behavior of the Muscovy duck. (Wu. *et al.,* 2014). Transgenic animals represent one of the most potent and exciting research tools in the biological sciences. A transgenic animal is an animal whose genome foreign Deoxyribose Nucleic Acid (DNA) has been transferred for the purpose of studying and manipulating that DNA. The establishment of stable transgenic animals implies that the foreign DNA is present in gametes or one cell embryos to allow its transmission to progeny. To reach this goal, the foreign gene can be transferred using different methods according to animal species (Tekalign Tadesse and Deme Koricho., 2017). Specific developmental characteristics of the chicken make it an attractive model for generation of transgenic organisms (Bahrami *et al.*,2020) Sperm Mediated Gene Transfer (SMGT) is based on the ability of sperm cells to bind and internalize exogenous DNA and to transfer it into eggs at fertilization. The first report on production of transgenic animal by stable integration of foreign DNA by SMGT and its transmission to the progenies by Mendelian inheritance was provided by (Lavitrano *et al.*1989). Sperm mediated gene transfer involves the use of sperm as vectors for gene transfer into chickens (Naito *et al.,* 1994; Jahav and Siddiqui, 2007).

The objective of this study was investigating the effect of insert the growth hormone gene from Cobb 500 by using (SMGT) technique Sperm Mediated Gene Transfer on some egg productive and reproductive traits of Bandarah local chicken strain through two generations .

MATERIALS AND METHODS

This study was carried out at Faculty of, Agriculture Damanhour University Animal and Poultry Production Department, El-Sabahia Poultry Research Station in Alexandria, Animal Production Research Institute, Agricultural Research Center. with the cooperation of Genetic Engineering and Biotechnology Research Institute, University of Sadat City, Egypt . This experiment was aimed to improve Bandrah Local chicken by insert Growth hormone gene (cGH) which transfer from Cobb 500 broilers as a high producing exotic broiler strain, then transfer chicken (cGH) by using sperm-mediated gene transfer (SMGT) in develop chicken strains Bandarah.It found from White Cornish and the Gimmizah were utilized as base population when developing Bandarah chicken for four generations. (Mahmoud *et al.,* 1989).

Experimental birds and treatments:

A total 75 hens and 15 cocks at eight months of age from Bandarah. a local chicken strain was used to start this study to produce generation one. The birds were assigned in individual cages; feed and water provided and randomly divided into two groups. Group A used method cGH SMGT technique contain 50 hens and 10 cocks, group B contain 25 hens and 5 cocks used classic artificial insemination. Second generation has done by classic artificial insemination between cocks and chicken from each group.

Molecular isolation and cloning Growth hormone gene:

Chicken fresh liver samples were immediately excised from chicken fast growing (Cobb500 broilers) as a high producing exotic broiler strain at three weeks age. The collected liver tissue was rapidly dissected into small pieces using sterile scalpel, immediately stored at -80ºC until RNA extraction. Total RNA was extracted from the liver sample using RNA-spin^{TM} Total RNA Extraction Kits (iNtRON Biotechnology, Inc) following manufacturer's recommendations. RNA was quantified using nano Drop technology with the Epoch Multi-Volume Spectrophotometer System (Biotech, Winooski, VT, USA).

Amplification of chicken growth hormone (cGH) cDNA by reverse transcriptase polymerase chain reaction (RT-PCR):

One microgram of each total RNA was reverse transcribed with Superscript II reverse transcriptase kits according to the manufacturer's directions (Life Technologies, Inc., Grand Island, NY). A 0.1-ml aliquot of the reaction was used in each PCR, using specific primers for GH. The forward primers located in exons one (PE1F) and four (PE4F) of the chicken GH (cGH) gene were 5\ TCAAGCAACACCTGAGCAACTC 3\ and 5\ TTTTGGCACCTCAGACAGAGTG 3\, respectively, and the reverse GH primer located in exon 5 (PE5R) was CTGTGGGTTTATTCCTCGTGT. PCR was carried out using TAKARA Taq DNA polymerase (TAKARA, Otsu, Japan) and a thermal cycler (Gene Amp PCR System 9700, PE Applied Biosystems, Foster City, CA). The PCR condition were 30s at 95 °C followed by 35 cycles for 1 min at 60°C and 10 min at 60°C. A 10 µL aliquot of each resulting reaction was electrophoresed on a 1.5% agarose gel with 50 ml TAE buffer, stained with ethidium bromide, and photographed under UV illumination. The amplified cDNA fragments were then subcloned into a pGEM3Zf+ plasmid and subjected to sequencing. DNA sequencing was performed using fluorescent primers and an automated DNA sequencer (PE Applied Biosystems 373A).

cGH gene transfer by using sperm-mediated gene transfer technique:

The foreign GH gene was transferred using SMGT. Semen was collected from 15 Bandarah adult cocks (eight months old); following the dorsal-abdominal massage method according to (Lake, 1957). A total of 20 µL gene doses (10 µg) of cGH gene were mixed with 80 µL from two skim milk media dilution in separate Eppendorf tube. On the same time 20 µL Lipofectin reagents was mixed 80 µL in each media dilution and stay in room temperature for 30-45 minutes then the two parts were mixed in one tube after adding 800 µL in each media. After that adding1000 µL of semen in each strain and incubated in water bath at 37 ° c for 30 min according to (Lavitrano *et al.*, 2000). Lipofectin reagent product by (KOMA BIOTECH INC. Company, Korea) that is considered to be suitable for transfection process of the foreign DNA into nuclear sperm cells according to (Bioconjugate *et al.* 2004).

Artificial insemination to produce generation one:

First group SMGT taking 2 mL of mixed solution contains 1 mL of semen 10µg dose of cGH gene, Lipofectin reagent and salin,control group taking 2 mL of mixed solution contains 1 mL of semen without any gene treatment, Lipofectin reagent and saline used in dilution.

Artificial insemination to produce generation two:

Semen was collected from adult cocks in each group (eight months old); following the dorsal-abdominal massage method according to **(Lake, 1957).** Hen for each group artificial insemination by cocks from the same group and applied by assigning five females to each male.

Incubation technique:

A total of 350 eggs obtained from all experimental groups were incubated in forced draft incubator at incubating laboratory belonging to the El-Sabahia Poultry Research Station in Alexandria, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture. Before incubation, the collected eggs were cleaned by dried piece of wool cloth. Fumigation and temperature of incubator were adjust med and applied one day before incubation. Eggs were incubated at 99.7°F (37.6°C) with relative humidity of 55% during the first 18 days of incubation period. Eggs were automatically turned every one-hour interval. Ventilation channels were automatically opened and closed according to temperature fluctuation. Ventilator limits were between 0.5 to 3.0 ventilation units (V.U.) until the 18th day of incubation period and 1.5 to 4.0 (V.U.) thereafter. Incubated eggs were transferred at the 18th day to a separate hatcher of 98.37°F (37.09°C) with relative humidity of 70%.

Birds and their management:

Chicks hatched from different experimental groups of artificial insemination experiments were kept under similar hygienic and environmental conditions. Hatched chicks Vaccination and medication were done according to the used program in the station.

At hatching day, the chicks were wing-banded, weighed and brooded on floor brooders with electric heaters for brooding chicks, at 32°c during the 1st week and 3°c was decreased each week thereafter till it reached 22-24°c. Wheat straw of 10 cm depth was used in brooding house. The wet litter was changed with dry one.

The chicks were subjected to 24 hours lighting on intensity of 3 watt / m^2 along till the four weeks of age then reduced to 10:11 hours of light during the rearing period. Experimental groups were reared under similar managerial and hygienic conditions. Fresh water was automatically available at all time by stainless steel nipples for each cage. The experimental diets were offered to the chicks *ad libitum* in mash form.

At 18 weeks of age the light period gradually increased to 14 hours daily. During laying period, the light was 16 hours daily (Chao and Lee 2001).

Chicks were fed a basal starter diet from 0-8 weeks of age23.3%protein and 2900Kcal and a basal grower diet from 8-20 weeks of age18.3%protein and 2850Kcal and16.5%protein and 2750 Kcal and 4% calcium and0.4 Phosphorus available at egg production period.

Studied traits in each generation:

The reproductive traits of experimental groups were evaluated by estimating the following parameters in different groups of transgenic chicks and control group.

Fertility:

$$\text{Fertility (\%)} = \frac{\text{Fertile eggs}}{\text{Total incubated eggs}} \times 100$$

Hatchability for fertile eggs (%):

$$\text{Hatchability (\%)} = \frac{\text{Hatched chicks}}{\text{Fertile eggs}} \times 100$$

Hatchability for total eggs (%):

$$\text{Hatchability (\%)} = \frac{\text{Hatched chicks}}{\text{Total eggs}} \times 100$$

Egg production traits:

Individual age and body weight at sexual maturity were calculated, in days and grams, at the day of laying the first egg. Egg number during the first 90 days of production, egg weight during the first 90 days of production and egg mass during the first 90 days of production were recorded.

Genetic analyses:

At the age eight week of the experiment, nine birds from each group in every generation were randomly chosen for collection of blood samples to genetic analyses.

Random amplified cDNA ends (RACE) analysis of GH mRNA:

The 5′RACE analysis was performed using a 5′/3′RACE kit (Roche, Sandhofer Strasse, Mannheim, Germany) according to the manufacturer’s directions. Total RNA was prepared from blood using a GLASS MAX RNA Micro-isolation Spin Cartridge System (Life Technologies, Inc.), and 2 µg of each total RNA were reverse transcribed using PE5R as reverse gene-specific primer and two forward gene-specific primers were used in subsequent amplification of cGH cDNA.

PCR amplification was conducted under the following conditions: 95ºC for five minutes, followed by 30 to 35 cycles at 95ºC for 45 s, 58ºC to 68ºC for 30 to 45 s, and 72ºC for 30 to 45 s; followed by a final extension at 72ºC for five minutes.

A l0 ul aliquot of each PCR reaction was electrophoresed on a 2.0% agarose gel, stained with ethidium bromide, and photographed under UV illumination. The amplified cDNA fragments were subcloned into a pGEM3Zf$^+$ plasmid and subjected to sequencing. Sequence analysis was carried out using GENETYX software.

Gene Transferring response (R):

Gene Transferring responses due to gene transfer in the second generation compared to the first generation

$R_t = (S_t - S_{t-1}) - (C_t - C_{t-1})$.

Where: R_t: realized gain due to transferring methods in t^{th} generation.

S and C: average performance of the transferring methods and control populations (Becker, 1985).

Statistical analysis:

Data were analyzed using SAS (2004) software (SAS, 2004) by using two ways ANOVA to (method of transferring and generation).The difference among treatment were tested using Duncan' s multiple range test at $p \leq 0.05$

Model:

$X_{ijk} = \mu + G_i + F_j + GF_{ij} + e_{ijk}$

X_{ijk} = the observation of ijk.

μ = overall mean.

G_i= effect of the i^{th} group methods of transferring cGH gene.

F_j = effect of the j^{th} generation.

GF_{ij}= the interaction between G^{th} groups and F^{th} generation

e_{ijk}= the experimental random error.

RESULTS AND DISCUSSIONS

Isolation, cloning and sequencing of (cGH) gene:

Total RNA was extracted from chicken liver tissue and the cDNA was successfully prepared. PCR amplification with cGH specific primers generated 429bp fragment.

The amplified cDNA fragments were then sub cloned into pGEM3Zf+ plasmid. Plasmid purification and perform standard PCR shows a fragment of about 429bp when using specific cGH primers and the same fragment size was generated by double digestion of recombinant plasmid.

Finally, the recombinant cDNA with the Gen Bank reference sequence accession number: LC441152.1 (Fig.1). The nucleotide and deduced amino acids were aligned and compared with reference sequence which showed about 99% matching due to heterozygous of the extracted cDNA.

The result of this study is suggested an easy method to isolate and cloning of targeted varieties of chicken genes which may be useful to improve the local breed. Molecular biologists exploit the replicative ability of cultured cells to clone genes. Gene cloning also enables scientists to manipulate and study genes in isolation from the organism they came from. This allows researchers to conduct many experiments that would be impossible without cloned genes.

From this result it could be concluded that a growth candidate gene in broiler chicken can take the advantages of this gene and make a transgenic chicken carrying this promising candidate by the molecular and transgenic tools in local Egyptian chicken strain.

Genetic analyses of GH mRNA

The amplified cDNA fragments from blood sample of first and second generation were sub cloned into a $pGEM3Zf^+$ plasmid and subjected to sequencing. Sequence analysis was carried out using GENETYX software.

PCR Products cGH, mRNA normal Length 798bp were found for Bandarah chicken control without any gene treatment, the same result was found in first and second generations.

The changed in gene GH sequence between traits approved that gene GH which isolated from Cobb 500 was successfully transferred by SMGT technique to Bandarah chicken strain. The result of gene transferred was pass from parents to progeny.

PCR Products GH, mRNA normal Length 810bp were found for transgenic chicken Bandarah chicken which treat by SMGT method, the same result was found in first and second generations.

Percentage of fertility and hatchability for different experimental groups:

Fertility percentage:

Means ± SE of fertility percentage as affected by group and generations are presented in table (1). After semen treat and incubation using methods of Sperm Mediated Gene Transfer (SMGT) and control without any gene treatment.

Results showed that there were decrease in fertility with insert cGH gene transfer but with no significant difference between treated or generations, the averages of fertility were 88.97% and 92.14 % for SMGT and control, respectively. The overall mean of the second generation was nearly value 90.76% of the first one 90.38%.

These results may be due to, the reduction in fertility of cock spermatozoa which used as a vector to transfer the foreign chicken growth hormone (cGH) gene because many factors such as lipofectin reagent was attributed to the depression in sperm viability. In addition, foreign cGH gene which reduced sperm fertilizes ability. Furthermore, time of incubation has negative effect on sperm motility and viability. All of these factors had direct effect on percentage of fertility %.

The responses of the transgenic techniques SMGT for fertility percentage were 5.64% Table (3). This result may be due to the positive effect of transferring techniques.

The obtained results go in agreement with (Niemann and Wrenzycki., 2003; Elokil 2015; Anzar and Buhr., 2006; El-Gendy *et al.* 2007 and Zhang *et al.*, 2012) who found that fertilization of egg involves penetration by a number of sperm, which helps to increase the entry of exogenous DNA into the egg and improves the efficiency of SMGT because many sperms were degraded after using SMGT. In addition, (Anzar and Buhr., 2006) found that although transgenic animals have been obtained using SMGT, its efficiency is still low, mainly due to the spermatozoon's low uptake of exogenous DNA, thereby reducing the number of fertilized oocytes with transfected spermatozoa and disagree with the findings of (Nakanishi *et al.*, 2002) stated that lipofectin added to chicken sperm did not affect fertility, although it slightly reduced mortality. (Khoo, 2000; Kuznetsov*et al.,* 2000 and Garcıa-Vazquez *et al.,* 2011) found that the negative relation between advantages of SMGT and semen traits were used individual motility, mass motility and fertility. (Elokil, 2015) found that after semen incubation using of (SMGT) techniques, IGF-1 gene doses had significant effect on the percentage of fertility. The zero doses were highest in average of fertility 79.76 and lowest average of fertility % (73.21 and 73.29) appeared with 10 and 15 μg doses of GF-1 gene, respectively.

Hatchability OF fertile and total eggs percentages:

Means ± SE of hatchability percentage are shown in table (1). After semen treat and incubation using (SMGT) and control. There were no significant differences between treated or generations for hatchability of fertile and total eggs percentages. The values were found in the control one in the first generation 95.64% and 88.49% for hatchability of fertile and total eggs percentage, respectively.

The responses of SMGT were 18.35% and 21.82% for hatchability of fertile and total eggs, respectively table (3). The obtained results in harmony with (El-Garhy, 2004) who found that the injection of White Leghorn (male) DNA into Fayoumi embryonic cells significantly decreased hatchability and increased embryonic mortality compared with control groups. Witter, (1993) found that transgenes in chickens may show undesirable side-effects including high death rate, short life span biological weakness and pathological syndromes. Natio *et al.*, (1994) showed that hatching rate mounted 11.8% when exogenous DNA was injected into the germinal disc of fertilized ova. El-Naggar, (2002) reported that hatchability was significantly affected by foreign DNA administration. Treated eggs showed lower hatchability as compared with intact eggs. The intact eggs showed significantly higher hatchability percentage than drilled eggs indicating that the negative effect obtained on hatchability may be mainly due to the technique applied for introducing foreign DNA into eggs. He stated that, early embryonic mortality records were indirectly related with the value of hatchability being significantly lower in the controls than in DNA treated groups without any significant difference due to doses within treatments. On the other hand, El-Tahawy, (2005) noticed improving hatchability percentage of Gimmizah eggs due to microinjection with Japanese quail DNA (86.5%) or broiler breeders (84.05%) comparing with control group (75.9%). El-Khalea, (2005) reported that the introduction of Hubbard (male) DNA into Fayouni embryos cells decreased hatchability percentage and increased embryonic mortality and percentages of abnormalities. He added that the rate of decrease was greater as the dose of injected foreign DNA increased.

Ahmed, (2006) concluded that introducing the foreign DNA of Japanese quail (*Coturnix coturnix*) purified from thymus and Bursa of Fabricious gland with different levels (1, 2, 4 µg/egg) into the embryonic cells of Bandarah chicken has significantly effect on hatchability percentage realizing it maximum value (60.55%) for eggs injected by 4µg/egg of Bursa of Fabricious–DNA. He added that either early or late embryonic mortality were significantly increased as compared to controls.

Egg production traits:

Age at sexual maturity

Means ± SE of age at sexual maturity (ASM) as affected by groups and generations are shown in table (2). Pullets of SMGT method matured significantly (P<0.01) early (168.19 d) than the pullets of control (182.32 d). On the other hand, there were highly significant different between generations for ASM, the overall means of ASM for the two generations were 180.65 and 166.02 d, respectively. The results in table (3) indicated that the method of transferring was decreased the ASM at the second generation by 35.01 d on SMGT method.

Body weight at sexual maturity

Table (2) displayed the effect of treated and generations on body weight at sexual maturity **(**BWSM). Pullets of SMGT method were significantly (P<0.01) heavier than the control.

The overall means were 1532.53 and 1487.04 g for the first and second generations. Analysis of variance showed that the differences between generations were highly significant "(P<0.01).

Results in table (3) indicated that the response of BWSM was negative (-81.47) as a result of SMGT method.

Egg number during the first 90 days of laying

Table (2) presented the means and SE of eggs number (EN) during the first 90 days of laying by treated and generations. Egg number which produced during the first 90 days from SMGT method significantly increased compared with the control.

Depending on the overall mean, the first-generation pullets produced significantly (P<0.01) more eggs than the second one (63.52 vs 60.57).

Table (3) indicated that the SMGT method was improved egg number by 10.62 egg.

Average egg weight of the first 90 days of laying

Means and SE of the egg weight (EW) of the first 90 days of laying as influenced by treated and generation shown in table (2). The average of eggs produced from pullets of the second generation was significantly (P<0.01) heavier (50.45g) than this produced by the first one (46.81g). For treated there were highly significant difference between treated found, the SMGT method had the heaviest egg weight 49.20 g followed by the lightest egg weight the control one 48.22g.

The responses of treated of transferring are shown in table (3). The SMGT method decreased egg weight by 0.9 g.

Egg mass during the first 90 days of laying

Table (2) displays means and SE of egg mass (EM) during the first 90 days of laying as influenced by treated and generations. There were non-significant different between generations for (EM) while, there were high significant different between treated for the same trait. The SMGT method produced an egg mass of 3202.43g while, the pullets of the control had a lower value of egg mass during the first 90 days of laying (2492.37 g.). SMGT improved egg mass during the first 90 days of laying by 512.62 g Table (3).

Obtained results agree with those of (Samaneh Gorji Makhsous *et al.*, 2013) who found that GH gene could be a genetic locus or linked to a major gene significantly affecting egg number and rate of lay traits in chickens, and the relationship between these traits may be useful for molecular marker-assisted selection (MAS) to improve the chickens breeding programs. (Vasilatos, Y. R. *et al.* 1997) The chicken growth hormone (cGH) gene is considered one of the most candidate genes that can influence chicken performance traits because of its crucial function in egg production reproduction and metabolism. . (Deeb and Lamont 2002) stated that insulin-like growth factors, as molecular markers for egg production enhancement in native breed. (Kuhnlein, *et al.,* 1997 and Feng, *et al.,* 1997) confirmed that polymorphism of the GH gene and its haplotypes is related to chicken egg production traits. RFLPs have been identified in the cGH gene showing that these polymorphisms are associated with egg production traits, resistance to Marek's disease and avian leucosis.

On the other hand, these results disagree with findings of (El-Garhy, 2011). Found that Insertion of foreign DNA significantly delayed sexual maturity with different degree depending on the level applied, pronounced decrease of egg production was observed and similar results were obtained between the mode of variation in average egg mass and each of average egg weight and average egg production rate.

CONCLUSION

In summary, total RNA was extracted from (Cobb 500) broiler and the cDNA was successfully prepared for cGH gene and transferred by using sperm-mediated gene transfer (SMGT) technique to Bandarah developed chicken strains. Chicken cGH gene was affected in egg production and reproductive traits and moved from the first generation to the second with the same shape and increased the effect. The responses of the transgenic technique SMGT for fertility percentage were 5.64%. Also, hatchability percentages of fertile and total eggs were 18.35% and 21.82% respectively. The treat of transferring was decreased the ASM at the second generation by 35.01 d on SMGT technique. After two generation, the SMGT technique was improved egg number by 10.62 egg, decreased egg weight by 0.9g and improved egg mass during the first 90 days of laying by 512.62 g.

Table (1): Means ± stander errors (SE) of Fertility and hatchability percentage by SMGT method of transferring technique and generations for Bandarah strain

Generation	Groups of gene transferring	Fertility %	Hatchability for fertile eggs %	Hatchability for total eggs %
One	SMGT	88.97±3.17	85.66±1.21	76.42±3.44
	Control	92.14±2.59	95.64±2.84	88.49±4.61
	Total	90.38±2.08	90.09±1.83	81.79±3.08
Two	SMGT	92.00±1.33	95.05±1.33	87.33±0.58
	Control	89.53±0.86	86. 68±3.72	77.58±3.37
	Total	90.76±0.87	90.86±2.42	82.45±2.43
over all mean	SMGT	89.84±2.29	88.34±1.49	79.53±2.78
	Control	91.27±1.75	92.65±2.51	84.85±3.53
Factors	**Significant**			
Generation (F)	NS		NS	NS
Groups (G)	NS		NS	NS
F X G	NS		**	*

SMGT: sperm mediated gene transfer, F: generation, G: group, NS: non-significant,* Significant at ≤0.05, ** Significant at ≤0.001, [a ,b,c] Means within the same column in the same trait with different superscripts are significantly different (P≤0.05).

Table (2): Means ± stander errors (SE) of egg production traits by using SMGT method of transferring techniques and generations for Bandarah strain

Generation	Groups of gene transferring	ASM	BWSM	EN 90	EW 90	EM 90
One	SMGT	181.62±1.79	1571.86±2281	65.84±0.8	47.14±0.29	3102.52±40.57
	Control	178.86±1.88	1434.20±3190	58.0±1.75	46.04±0.50	2671.58±87.91
	Total	180.65±1.04[A]	1532.53±1525[A]	63.52±0.87[A]	46.81±0.23[B]	2975.06±44.91
Two	SMGT	153.23±0.62	1499.42±213	64.55±0.89	50.50±0.31	3264.88±53.35
	Control	185.48±0.65	1443.23±36.69	46.09±1.89	50.30±0.75	2321.32±106.38
	Total	166.02±2.14[B]	1487.04±154[B]	60.57±1.10[B]	50.45±0.29[A]	3061.36±61.19
Overall	SMGT	168.19±1.93[c]	1524.93±16.18[a]	65.05±0.63[a]	49.20±0.25[a]	3202.43±36.89[a]
	Control	182. 32±1.07[a]	1439.30±24.71[b]	51.91±1.57[c]	48.22±0.56[b]	2492.37±73.64[c]
Factors		**significant**				
Generation (F)		**	**	**	**	NS
Groups (G)		**	**	**	**	**
F X G		**	**	**	**	**

SMGT: sperm mediated gene transfer, F: generation, G: groups, ASM: age at sexual maturity, BWSM: body weight at sexual maturity, EN 90: egg number during the first 90 days of laying, EW 90: egg weight during the first 90 days of laying, EM: egg mass during the first 90 days of laying, NS: non-significant,* Significant at P ≤0.05, ** Significant at P ≤0.001, a ,b,c Means within the same column in the same trait with different superscripts are significantly different (P≤0.05).

Table (3): Response of SMGT method of gene transferring for reproductive traits

Traits	Response
Fertility %	**5.64**
Hatchability for fertile eggs %	18.35
Hatchability for total eggs %	21.82
Age at sexual maturity (day)	-35.01
Body weight at sexual maturity (gm)	-81.47
Egg number during the first 90 day of laying	10.62
Egg weight during the first 90 day of laying(gm)	-0.90
Egg mass during the first 90 day of laying(gm)	512.62

SMGT: sperm mediated gene transfer

LC441152.1 Gallus gallus GH mRNA for somatotropin precursor,

ATCGACATCCCCTGAGCAACTCTCCCGGCAGGAATGGCTCCAGGCTCGTGGG
TTTCTCCTCTCATCATCGCTGTGGTCACGCTGGGACTGCCGCAGGAAGCTGCT
GCCACCTTCTCTGCCATGCCCCTCTCCAACCTGTTTGCCAACGCTGTGCTGAG
GGCTCAGCACCTCACCTCCTGGCTGCCGAGACATATAAAGAGTTCGGACGCA
TCTATATTCCGGAGGACCAGAGGTACACCAACAAAAACTCCCAGGCTGCGTT
TTGTTACTCAGAAACCATCCCAGCTCCCACGGGGAAGGATGACGCCCAGCAG
AAGTCAGACATGGAGCTGCTTCGGTTTTCACTGGTTCTCATCCAGTCTTGGCT
GACCCCCGTGCAATACCTAAGCAAGGTGTTCACGAACAACTTGGGTTTTGGC
ACCTGCGCA

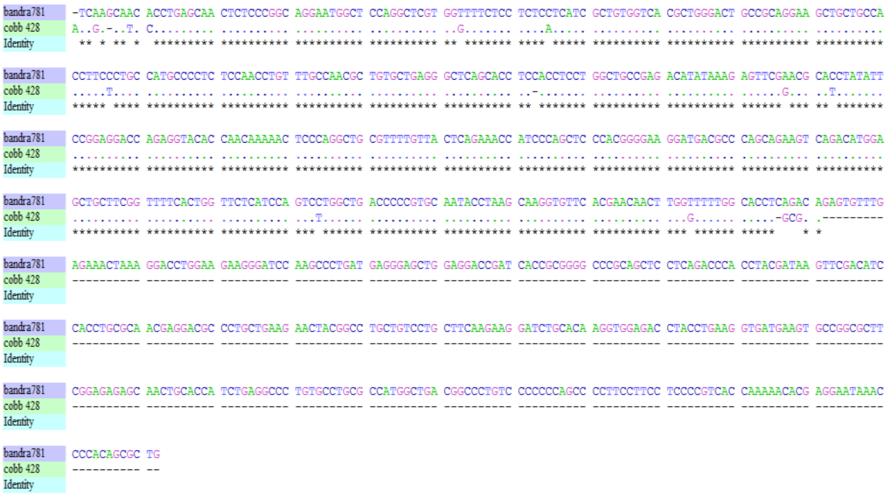

Fig (1): Sequencing alignment result of cGH recombinant gene by standard Sanger sequencing method using T7 forward primer.

Fig (2)Name: Bandarah control without any gene treatment
(Bandarah growth hormone (GH), mRNA)

Sequence:
1 ggtgttccctgtctacagtgtctgtgtaatgtagataaatgtgagaattttctctaaatc
61 ccccttctgtttgctaaatctcactgtcactgctaaaatcagagcagatagagcctgcgc
121 aatggaataaagtcctcaatattgaaatgtgacattgctctcaacatctcacatctctct
181 ggatttctttttctcatcattactgctaacaacttcatttccagactttgcacttttaa
241 gaagcaatggaacaaatcaacagtctttcaacacaattagttaagtgctgcttttgtgat
301 ttcttgaaggtgaagatgcacactgtgtcctacattcatttcttctaccttggcctgtgt
361 ttgcttaccttaaccagttctgctgctgccagaggcttctcactgtgtggtgctgagctg
421 gttgatgctcttgagttcgtatgtggagacacttcagtaaggcccagaaagcctacaggg
481 tttggatccagcagtagacgcttacaccacaagggaatagtggatgagtgctgcttccag
541 agttgtgacctgaggaggctgaagatgtactgtgctccaataaagccacctaaatctgca
601 cgctctgtacgtgctcagcgttccactgatatgccaaaagcacaaaaggaagtgcatttg
661 aagaatacaagtagagggaacacaggaaacagaaactacagaatgtaagatcatgccatc
721 cacaagaatgaagaatgaatgtgccatctgcagagtactttgctgtaaataaattatccg
781 ttacacattggacgactga c

Fig (3)Name: Bandarah with cGH of Cobb500 which transferring by SMGT method.
(Bandarah growth hormone (GH), mRNA)

Sequence:
1 ccaagcaacccctgagcaactctcccggcaggaatggctccaggctcgtggttttctcct
61 ctcctcatcgctgtggtcacgctgggactgccgcaggaagctgctgccaccttccctgcc
121 atgcccctctccaacctgtttgccaccgctgtgctgagggctcagcacctccacctcctg
181 gctgccgagacatataaagagttcgaacgcacctatattccgaaggaccagaggtacaac
241 aacaaaaactcccaggctgcgtttggttactcagaaaccatcccagctcccacggggaag
301 gatgacgcccagcaggagtcagacatggagctgcttcggttttcactggttctcatccag
361 tcctggctgaccttggtgcaatacctaagcaaggtgttcacgaacaacttggtttttggc
421 acctcagacagagtgtttgagaaactaaaggacctggaagaagggatccaagccctgatg
481 agggagctggaggaccgatcaccgcggggcccgcagctcctcagacccacctacgataag
541 ttcgacatccacctgcgcaacgaggacgccctgctgaagaactacggcctgctgtcctgc
601 ttcaagagggatctgcacaaggtggagacctacctgaaggtgatgaagtgccggcgcttc
661 ggagagaacaactgcaccatctgaggccctgtgcctgcgccatggctgacggccctgtcc
721 cccccagcttccccccttcctccccgtcaccaaaaacacgaggaataaaccccacagcgct
781 tcctggctgacccccgtgaaatacctaagc

REFERENCES

Ahmed, D. F. 2006. The use of modern biotechnology in the production of high immune response chickens. M.Sc. thesis, Fac. of Agric., Alex. Univ. Egypt.

Anzar, M. and Buhr, M. M. 2006. Spontaneous uptake of exogenous DNA by bullspermatozoa. Theriogenology, 65 683–690.

Bahrami S., Yekta A.,Daneshipour A., Jazayeri S., Mozdziak P.,Sanati M.and Gourabi H.(2020). Designing a transgenic chicken :Applying new approaches toward promising bioreactor.Cell journal V.22:133-139

Becker (1985). Manual al proceluyeo in qutantitatire geneticy Vash. State, berero, plama, Washington.

Bioconjugate C., Kim H. S., Moon J., Jang D. O., Park and Y. S. 2004. Gene transferring efficiencies of novel diamino cationic lipids with varied hydrocarbon chains, Bioconj. Chem., 15:1095-1101.

Chao, C. H. and Lee Y. P. 2001. Relationship between reproductive performance and immunity in Taiwan country chickens. Poul. Sci., 80:535-540.

Deeb, N. and Lamont, S. J. 2002. Genetic architecture of growth and body composition in unique chicken populations. J. Hered. 93:107–118.

El-Garhy, O. H. M. 2004. Effect of some biotechnological methodology on some productive and reproductive traits in fowl. M.Sc. Thesis, Fac. of Agric., Moshtohor, Zagazig Univ. Benha Branch,Egypt.

El-Garhy, O. H. M. 2011. Study of the effect of genetical factors using genetic engineering technology with relation to some metabolic and productive traits in fowl. Ph.D Thesis, Fac. of Agric., Benha Univ, Egypt.

El-Gendy, E. A.; Gad, A. Y. and Mostageer, A. 2007. Sperm-mediated gene transfer in poultry. 1. The relationship with cock sperm viability. Arab J. Biotech. 10:(1): 1-12.

El-Khalea, M. E. 2005. Factors affecting embryonic mortality, growth rate and production of new hatched chicks. M.Sc. Thesis, Fac. of Agric., Moshtohor, Zagazig Univ. Benha Branch,Egypt.

El-Naggar, R. A. 2002. Genetical and physiological studies on Japanese quail. Ph.D. Thesis, Fac. of Agric. Kafr El-Sheikh, Tanat Univ.

Elokil A. A. 2015. Applicability of the modern biological technology for improving the productive performance of some local poultry breeds. Ph.D Thesis,Fac. of Agric. Benha Univ. Egypt.

El-Tahawy, W. S. 2005. Production of transgenic local chicken by injection of DNA from Japanese quails or broiler breeds. Ph.D Thesis, Fac. of Agric. Damanhour Branch, Alex. Univ. Egypt.

Feng XP, Kuhnlein U, Aggrey SE, Gavora JS & Zadworny D. 1997. Trait association of genetic markers in the growth hormone and the growth hormone receptor gene in a White Leghorn strain. Poultry Science, 76: 1770-1775.

Garcıa-Vazquez, F. A.; Ruiz, S.; Grullo´n, L. A.; Ondiz, A. D. and Gutierrez-Adan, J. 2011. Factors affecting porcine sperm mediated gene transfer. Res. Vet. Sci. 91: 446-453.

Jahav, N, V. and Siddiqui, M. F., 2007. Hand Book of Poultry and Management. 2nd Edition, Jay Pee Brothers Medical Publishers (P) Limited. New Delhi.

Kaya, M. and M. A. Yıldız. 2008. Genetic diversity among Turkish native chickens, Denizli and Gerze, estimated by microsatellite markers. Biochem. Genet. 46:480-491.

Khoo, H. W. 2000. Sperm-mediated gene transfer studies on zebrafish in Singapore. 56:278–280.

Kuhnlein U, Ni L, Zadworny D, Weigend S, Gavora JS & Fairfull W. 1997.DNA polymorphisms in the chicken growth hormone gene: response to selection for disease resistance and association with egg production. Animal Genetics, 28: 116-123.

Kuznetsov, A. V.; Kuznetsova, I. V. and Schit, I. Y. 2000. DNA interaction with rabbit sperm cells and its transfer to ova in vitro and in vivo. Mol Reprod. 56:292-297.

Lake, P. E. 1957. Fowl semen as collected by the massage method. J. of Agri. Sci. 49: 120-126.

Lavitrano, M., Camaioni, A., Fazio, V.M., Dolci, S., Farace, M.G. and Spadafora, C. 1989. Sperm cells as vectors for introducing foreign DNA into eggs: genetic transformation of mice. Cell, 57: 717–723.

Lavitrano, M.; Forni, M.; Varzi, V.; Pucci, L.; Bacci, M. L.; Di Stefano, C.; Fioretti, D.; Zoraqi, G.; Moioli, B. and Rossi, M. 2000. Sperm-mediated gene transfer: production of pigs transgenic for a human regulator of complement activation. Transplantation Proceedings. 29: 3508–3509.

Mahmoud, T.H.; J.E. Abd El-Hameid and A.I. El-Turkey, 1989. “Bandara” A new breed of chickens. Agric. Res. Rev., 67:229.

Malak, M, Zahran, J. and Aboul-Soud, A. M. 2008. Isolation and sequencing of insulin-like growth factor 1 (IGF-1) from Egyptian buffalo via RT-PCR Arab J. Biotech., 11 (1): 19-28.

McMurtry, J. P. 1998. Nutritional and developmental roles of insulin-like growth factors in poultry. J. Nutrition 1(28):302-310.

Naito, M., Sasaki, E., Ohtaki, M. and Sakurai 1994. Introduction of exogenous DNA into somatic and germ cells of chickens by microinjection into germinal disc of fertilized ova. Molecular Reproduction Development 37: 167-171.

Nakanishi, A.; Sakabe, A. and Iritani, A. 2002. Gene transfer in the chicken by sperm-mediated methods. Mol. Reprod. Dev. 36 258–261 .

Niemann, H, Rath, D. and Wrenzycki, C.2003. Advances in biotechnology: new tools in future pig production for agriculture and biomedicine. Reprod. in Domes. Anim. 38: 82–89.

SAS. 2004. SAS procedure guide version 6.12th Ed., SAS institute Ine., cary, NC, USA.

Samaneh Gorji Makhsous1, , Seyed Ziaeddin Mirhoseini, Mohammad Javad Zamiri, and Ali Niazi 2013. Polymorphisms of growth hormone gene in a native chicken population: association with egg production. Bull Vet Inst Pulawy 57, 73-77.

Tekalign Tadesse and Deme Koricho 2017. Biomedical Application and Future Prospects of Transgenic Animal: Review. Journal of Natural Sciences Research ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online) www.iiste.org. Vol.7 No.23 2017.

Vasilatos Y R, Dunnington E A, Siegel P B and McMurtry J P. 1997. Tissue-specific alterations in insulin- like growth factor-1 concentrations in response to 3, 3, 5-triiod L-thyronine supplementation in the growth hormone receptor-deficient sexlinked dwarf chicken. General Comparative Endocrinology 6: 105

Witter, R. L. 1993. "Biotechnology: where are and what lies ahead?" Poultry Digest. Feb: 34-39.

Wu X., Yan M. J., Lian S. Y., Liu X. T. And Li A. 2014. GH gene polymorphisms and expression associated with egg laying in muscovy ducks (Cairina moschata). Hereditas 151: 14 – 19. Lund, Sweden. eISSN 1601-5223.

Zhang, X.; Xue, R. Y.; Cao, G. L.; Hu, X. L.; Wang, X. J.; Pan, Z. H.; Xie, M.; Yu, X. H. and Gong, C. L. 2012. Effects of egt gene transfer on the development of Bombyxmori. Gene. 491:272–277.

الملخص العربي

تاثير نقل جين هرمون النمو باستخدام تكنيك نقل الجينات بواسطه الحيوانات المنوية علي صفات انتاج البيض والتناسل لسلاله دجاج البندره المحلي

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هدفت هذه التجربة إلى دراسه مدي التحسين الوراثي لسلالة دجاج البندره المحليةالمحسنه عن طريق نقل جين هرمون النمو من دجاج التسمين (Cobb500) باستخدام تكنيك نقل الجينات بواسطه الحيوانات المنوية. تم اجراء هذه التجربه لمده جيليين وتمت التجربه في كليه الزراعه بدمنهور ومركز البحوث الزراعيه بمحطه الصباحيه. تم استخلاص الحمض النووي RNA من أنسجة كبد دجاج التسمين (cobb 500) وتم تحضير (DNA)المعاد الالتحام بنجاح. كان حجم الحمض النوويmRNA لهرمون النمو ٨١٠ قاعده نيتروجينيه في سلاله دجاج البندره المحور وراثيا وكان طول جين هرمون النمو ثابت في الجيل الاول والجيل التاني في التجربه وكذلك تتابع القواعد النيتروجينيه ظل ثابت من الجيل الاول حتي الجيل الثاني وانتقل الجين من الاباء الي الامهات بنفس الترتيب والطول وذلك من خلال استخدام تكنيك نقل الجينات بواسطه الحيوانات المنويهSMGT لدجاج البندره المحور وراثيا. كان متوسط صفه نسبة الخصوبة لكل من المجوعه المعامله والمجموعه الكنترول هي ٨٩.٨٤ و ٩١.٢٧٪ علي التوالي.كانت نسبه الاستجابه لصفه نسبه الخصوبه في في المجموعه التي تم نقل جين هرمون النمو اليها باستخدام تكنيك SMGT هي ٥.٦٤%. لم يكن هناك اختلافات معنويه في نسبه التفريخ بين المعاملات وكانت الاستجابه لنسبه التفريخ للبيض المخصب والبيض الكلي هي ١٨.٣٥% ٢١.٨٢% علي التوالي. حدث انخفاض في عمر البلوغ الجنسي في الجيل الثاني بمقدار ٣٥.٠١ يوم في المجموعه التي تم نقل جين هرمون النمو اليها باستخدام تكنيك SMGT. كذلك حدث زياده في عدد البيض الذي تم إنتاجه خلال اول تسعين يومً من الانتاج في المجموعه التي تم نقل جين هرمون النمو اليها باستخدام تكنيك SMGT عن الكنترول وكانت زياده معنويه. كان هناك فروق معنويه بين المجموعه التي تم نقل جين هرمون النمو اليها باستخدام تكنيك SMGT المعامله والكنترول في صفه متوسط وزن البيضه حيث كانت في المجموعه المعامله هي ٤٩.٢٠ جم تليها الكنترول ب ٤٨.٢٢ جم. بعد جيلين حدث تحسن في صفه عدد البيض بمعدل ١٠.٦٢ بيضه وكذلك حدث انخفاض في متوسط وزن البيضه بمعدل ٠.٩ جم وكذلك تم زياده كتله البيض بقيمه ٥١٢.٦٢ جم وذلك للبيض المنتج خلال اول ٩٠ يوم من انتاج البيض في المجموعه التي تم نقل جين هرمون النمو اليها باستخدام تكنيك SMGT .استخدام تكنيك SMGT في نقل الجينات في الدجاج هي طريقة فعالة وامنه وتحقق نتائج جيده في عمليه نقل الجينات وانتاج دجاج معدل وراثيا. جين cGH هرمون النمو عمل علي التحسين الوراثي لسلاله البندره وقد ادي الي تحسين صفات انتاج البيض وصفات التكاثر سواء نسبه الخصوبه والتفريخ.وقد تم توريث جين هرمون النمو من الاباء الي الابناء وتم انتقال الجين من الجيل الاول الي الجيل الثاني بنفس التتابع ونفس التاثير علي صفات انتاج البيض . كانت تاثيرات الجين علي صفات انتاج البيض في الجيل الثاني اعلي من الجيل الاول. وبشكل عام يمكن القول بان استخدام تكنيك نقل الجينات بواسطه الحيوانات المنويه سوف يوفر الوقت ويعمل علي التحسين الوراثي لصفات انتاج البيض وصفات التكاثر .

الكلمات الداله: صفات انتاج البيض ، صفات التكاثر ، جين GH ، نقل الجينات ،تكنيك SMGT