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

W. S. EL-Tahawy;1 A.E.Abd Al Hamid ;1Ghanem H.H.;2 A.N.Nawar2 and O.M.Aly2

1Anim. and Poult. Prod. Dep., Fac. of Agric., Damanhour Uni., Egypt

Corresponding author: Waleed salah EL-Tahawy ;Email:waleed.eltahawy@agr.dmu.edu.eg

ABSTRACT: The experimental was aimed to improve genetic of Bandara 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 gametos 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
Egg traits, reproductive traits, GH gen, gene transfer, SMGT

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 -80°C until RNA extraction. Total RNA was extracted from the liver sample using RNA-spin™ 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’ TTTTGGCACCTCAGACAGATG 3’, respectively, and the reverse GH primer located in exon 5 (PESR) was CTGTGGGTATTCTCCTGTG. 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 adding 1000 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 SM GT taking 2 mL of mixed solution contains 1 mL of semen 10 µg dose of cGH gene, Lipofectin reagent and saline, 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 / m² 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
Egg traits, reproductive traits, GH gen, gene transfer, SMGT

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 and 16.5%protein and 2750 Kcal and 4% calcium and 0.4 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:**
Fertility (%) = \( \frac{\text{Fertile eggs}}{\text{Total incubated eggs}} \times 100 \)

**Hatchability for fertile eggs (%):**
Hatchability (%) = \( \frac{\text{Hatched chicks}}{\text{Fertile eggs}} \times 100 \)

**Hatchability for total eggs (%):**
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: 95°C for five minutes, followed by 30 to 35 cycles at 95°C for 45 s, 58°C to 68°C for 30 to 45 s, and 72°C for 30 to 45 s; followed by a final extension at 72°C for five minutes. A 10 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.

$\mu$ = 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; Kuznetsovet al., 2000 and Garcia-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.
Egg traits, reproductive traits, GH gen, gene transfer, SMGT

### 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 (50.45 g) than this produced by the first one (46.81 g). 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 48.22 g. 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 highly significant different between treated for the same trait. The SMGT method produced an egg mass of 3202.43 g 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 0.9 g 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

<table>
<thead>
<tr>
<th>Generation</th>
<th>Groups of gene transferring</th>
<th>Fertility %</th>
<th>Hatchability for fertile eggs %</th>
<th>Hatchability for total eggs %</th>
</tr>
</thead>
<tbody>
<tr>
<td>One</td>
<td>SMGT</td>
<td>88.97±3.17</td>
<td>85.66±1.21</td>
<td>76.42±3.44</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>92.14±2.59</td>
<td>95.64±2.84</td>
<td>88.49±4.61</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>90.38±2.08</td>
<td>90.09±1.83</td>
<td>81.79±3.08</td>
</tr>
<tr>
<td>Two</td>
<td>SMGT</td>
<td>92.00±1.33</td>
<td>95.05±1.33</td>
<td>87.33±0.58</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>89.53±0.86</td>
<td>86.68±3.72</td>
<td>77.58±3.37</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>90.76±0.87</td>
<td>90.86±2.42</td>
<td>82.45±2.43</td>
</tr>
<tr>
<td>over all mean</td>
<td>SMGT</td>
<td>89.84±2.29</td>
<td>88.34±1.49</td>
<td>79.53±2.78</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>91.27±1.75</td>
<td>92.65±2.51</td>
<td>84.85±3.53</td>
</tr>
</tbody>
</table>

Factors

<table>
<thead>
<tr>
<th>Generation (F)</th>
<th>Significant</th>
<th>Generation (G)</th>
<th>Significant</th>
<th>F X G</th>
<th>Significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td></td>
<td>NS</td>
<td></td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

SMGT: sperm mediated gene transfer, F: generation, G: group, NS: non-significant,* Significant at ≤0.05, ** Significant at ≤0.001, Mean within the same column in the same trait with different superscripts are significantly different (P≤0.05).
Table (2): Means ± standard errors (SE) of egg production traits by using SMGT method of transferring techniques and generations for Bandarah strain

<table>
<thead>
<tr>
<th>Generation</th>
<th>Groups of gene transferring</th>
<th>ASM</th>
<th>BWSM</th>
<th>EN 90</th>
<th>EW 90</th>
<th>EM 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>One</td>
<td>SMGT</td>
<td>181.62±1.79</td>
<td>1571.86±2281</td>
<td>65.84±0.8</td>
<td>47.14±0.29</td>
<td>3102.52±40.57</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>178.86±1.88</td>
<td>1434.20±3190</td>
<td>58.0±1.75</td>
<td>46.04±0.50</td>
<td>2671.58±87.91</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>180.65±1.04</td>
<td>1532.53±1525</td>
<td>63.52±0.87</td>
<td>46.81±0.23</td>
<td>2975.06±44.91</td>
</tr>
<tr>
<td>Two</td>
<td>SMGT</td>
<td>153.23±0.62</td>
<td>1499.42±213</td>
<td>64.55±0.89</td>
<td>50.50±0.31</td>
<td>3264.88±53.35</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>185.48±0.65</td>
<td>1443.23±36.9</td>
<td>46.09±1.89</td>
<td>50.30±0.75</td>
<td>2321.32±106.38</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>166.02±2.14B</td>
<td>1487.04±154B</td>
<td>60.57±1.10B</td>
<td>50.45±0.29A</td>
<td>3061.36±61.19</td>
</tr>
<tr>
<td>Overall</td>
<td>SMGT</td>
<td>168.19±1.93c</td>
<td>1524.93±16.18</td>
<td>65.05±0.63a</td>
<td>49.20±0.25a</td>
<td>3202.43±36.89a</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>182.32±1.07a</td>
<td>1439.30±24.71b</td>
<td>51.91±1.57c</td>
<td>48.22±0.56b</td>
<td>2492.37±73.64c</td>
</tr>
</tbody>
</table>

Factors significant

<table>
<thead>
<tr>
<th>Generation (F)</th>
<th>**</th>
<th>**</th>
<th>**</th>
<th>**</th>
<th>NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups (G)</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>F X G</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Traits</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hatchability for fertile eggs %</td>
<td>18.35</td>
</tr>
<tr>
<td>Hatchability for total eggs %</td>
<td>21.82</td>
</tr>
<tr>
<td>Age at sexual maturity (day)</td>
<td>-35.01</td>
</tr>
<tr>
<td>Body weight at sexual maturity (gm)</td>
<td>-81.47</td>
</tr>
<tr>
<td>Egg number during the first 90 day of laying</td>
<td>10.62</td>
</tr>
<tr>
<td>Egg weight during the first 90 day of laying (gm)</td>
<td>-0.90</td>
</tr>
<tr>
<td>Egg mass during the first 90 day of laying(gm)</td>
<td>512.62</td>
</tr>
</tbody>
</table>

SMGT: sperm mediated gene transfer

LC441152.1 Gallus gallus GH mRNA for somatotropin precursor,

ATCGACATCCCCCTGAGCAACTCTCCCGCCAGGAGATGGCTCAGGCAGGCAGTGGG
TTTCTCTCTCCTCATCTACGCTGTGGTCAGCTCAGCAGCTGAGCTGAGCCTGCC
GGCACTTTCTCTGCCATGCCCTCTCTCCACCTGTTTGGCAACGGCTGAGCTG
GGCTCAGCACCCTCCTTGGCTGCGGAGACATATAAAGAGTTTCCGAGAC
TCTATATTCCGGGAGACCAGAGGTACACCAACAAAAACTCCAGAGCTGCCTT
TTGTTACCTCAGAAACCATCCAGCTCCACCGGAGAGTGGACCGCAGCAG
AACAGAGACATGGAGCTGCTTCGGTTTCACTGGTTCTCATCAGTCTTGGCT
GACCCCGTGCACAATACCTAAGCAAGGTTTTGTCAGCAAAACTTGGGTTTTGGC
ACCTGGCC

Fig (1): Sequencing alignment result of cGH recombinant gene by standard Sanger sequencing method using T7 forward primer.
Egg traits, reproductive traits, GH gen, gene transfer, SMGT

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

Sequence:
1 ggtgcctcgttctcatacgctgttgtgtgaatgtagataaattgtgagaattttctctaaatc
61 cccctctgttgtctaaactcactgcttgtaaactatccagagcagatagctgtgccgc
121 aatggagaaatgtcctcaatattgaagaatgtgacatgtctctcaaatcatactcactctctct
181 gatgtcttttttttcataattaattgctaataaactacttctttcagacttattttcattaaaa
241 gaagcaatggagaattctttctcaacatattgaattaagttgtgatgtttttgtgat
301 ttctgaaagttgaagctcactgtctgctctctacttttttttcattatcattgctgtgtgtgt
361 ttgaattcttctacagatgctgtgctgagttctcactctggtgtgctgtgctgtgctgctgagttctg
421 ttagtactctcctgtttttgtctttttttttgagggctttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttt
REFERENCES


Egg traits, reproductive traits, GH gen, gene transfer, SMGT

egg production. Animal Genetics, 28: 116-123.


الفصل العربي
تأثير نقل جين هرمون النمو باستخدام تكنيك نقل الجينات بواسطة الحوادث التنوية على صفات
التثدي والتناسل لسلالة دجاج البيدر المحلي
وليد صلاح الطحاوي 1، عبد الحميد السيد عبد الحميد 2، حنان حسن غالي 2، أحمد نبيل نوار 3
1-اسماء محبوب
2-قسم التثدي الحيواني، كلية الزراعة، جامعة المنوفية، مصر
3-معهد بحوث التثدي الحيواني، مركز البحوث الزراعية، الجيزة، مصر

هدفت هذه التجربة إلى دراسة مدى التحسين الوراثي لسلالة دجاج البيدر المحلية المحصنة عن طريق نقل جين هرمون النمو من دجاج التسمين (500) باستخدام تكنيك نقل الجينات بواسطة الحوادث التنوية. تم إجراء هذه التجربة لمدتين وتتم التجربة في كلية الزراعة بجامعة منصوره ومركز البحوث الزراعية بمحافظة الصعيد. تم استخلاص الحمض النووي (DNA) من نسخة كبد دجاج التسمين (500) و تحضير (cobb 500) رإعة يتيروجينيه في سلالة دجاج البيدر SMGT وثامرا. كان طول جين هرمون النمو ثابت في الجيل الأول والجيل الثاني في التجربة وكذلك تتابع القواعد التكروجينيه طول ثابت في الجيل الأول حتى الجيل الثاني والانقل الجين من الآباء إلى الأحفاد بنفس الترتيب لدجاج البيدر المحور SMGT وطول وذلك من خلال استخدام تكنيك نقل الجينات بواسطة الحوادث التنوية وثامرا. كان متوسط صفر نسبة الخصوبة لكل من المجموع المعاملة والمجموع الكنترول هي 98.27 و 91.27% على التوالي. كانت نسبة الاستجابا فصbee الخصوبة في المجموعة التي تم نقل جين هرمون النمو القي في استخدام تكنيك SMGT هي 56.14%. لم يكن هناك اختلافات معنوية في نسبة التكرار بين المعاملات وكانت استخدام تكنيك SMGT لدجاج البيدر المحور SMGT والطائرات وذلك من خلال استخدام تكنيك SMGT في المجموع معنوية بين المجموعات التي تم نقل جين هرمون النمو القي في استخدام تكنيك SMGT وال净土ول وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا.

عمر البلوغ الجنسي في الجيل الثاني بمقدار 35.1 يوم في المجموعة التي تم نقل جين هرمون النمو القي في استخدام تكنيك SMGT وثامرا. كان هناك حدث زياده في عدد البيض الذي تم إنتاجه خلال أول تسعة يوم من الخلق في المجموع معنوية بين كنترول عن الكنترول وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. في صفة وزن وجبة كانت في المجموع المعاملة هي 48.12 جم. بعد ثلاثين، حدث تحسن في صفة عدد البيض بمعدل 1.25 بيضه وذلك حدث انخفاض في متوسط وزن البيض بمعدل 1.26% وثامرا. كذلك حدث انخفاض في متوسط وزن البيض بمعدل 1.2% وثامرا. كان هناك حدث زياده في عدد البيض الذي تم إنتاجه خلال أول تسعة يوم من الخلق في المجموع معنوية بين كنترول عن الكنترول وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك حدث زياده في عدد البيض التي تم إنتاجه خلال أول تسعة يوم من الخلق في المجموع معنوية بين كنترول عن الكنترول وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والكنترول باستخدام تكنيك SMGT وثامرا. كان هناك فروق معنوية بين المجموع المعاملة والKEN.