Sperm-mediated gene transfer in poultry
1. The relationship with cock sperm viability

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ABSTRACT

The cock spermatozoa were used in this study as vectors for gene transfer. The objectives of the study were to assess the efficacy of the sperm to uptake exogenous DNA in relation to sperm viability. Two trials were carried out. Trial 1 was achieved to assess the effects of semen dilution (4 µl diluent/1 µl semen), heat incubation (exposure of semen to 37°C for 30 minutes) and the addition of lipofectin and their combinations on sperm characteristics. The sperm motility in the heat incubated or the diluted semen was 79% and was significantly less than that estimated for the un-treated semen (90%). However, no significant effects of heat incubation or semen dilution were mostly observed in the percentages of live, dead and abnormal sperm. The combination of heat incubation and semen dilution resulted in more reduction in the sperm motility compared to that occurred by either one. The addition of lipofectin to the diluted semen then heat incubated significantly detracted the motility and the percentage of live sperm, and increased the percentages of dead and abnormal sperm, and the effect was positively associated with lipofectin concentration. Trial 2, was designed to assess the effects of lipofectin (5%) addition on the fusion of DNA into the sperm and sperm viability. The exogenous DNA used was the plasmid pUC18, and two specific primers (forward and reverse) were used to recognize the existence of exogenous DNA in the sperm through the PCR analysis. It was observed that the plasmid DNA was successfully internalized into the sperm treated and un-treated with lipofectin. However, the DNA fragment recognized in the sperm incubated with lipofectin was apparently of higher yield. It was concluded that lipofectin stabilizes and facilitates the fusion of DNA into the sperm. The sperm motility and percentage of live sperm reached to 56.7% and 65.1% in the diluted semen incubated with lipofectin (5%)- DNA mixture, vs 75% and 90.6% in the diluted semen incubated with DNA only. This indicates that the addition of lipofectin to the sperm, for some extent, depressed their viability and subsequently the availability for fertilization. Because of the necessity of using lipofectin to enhance the fusion of DNA into the sperm, therefore it may be used at low concentration (5%, in this experiment).

Keywords: *Sperm-mediated gene transfer, cock spermatozoa, lipofectin, sperm viability.*

INTRODUCTION

Sperm cells can spontaneously incorporate exogenous DNA and transfer it to the eggs during fertilization, thus the exogenous DNA integrates the germ line. Then, the exogenous gene transmits through the germline into the subsequent generations. The incorporation of DNA into the sperm is mediated by DNA receptors located on spermatozoa which are specific DNA-binding proteins of 30-35 KD (Lavitrano *et al.*, 1992), and is antagonized by

glycoproteins present in the seminal fluid (Zani *et al.*, 1995). The DNA uptake by sperm cells was shown in eight different species (Castro *et al.*, 1990), where mature sperm cells were incubated with cloned DNA. The portion of DNA that has been associated with spermatozoa varied from about 1% in buffalo to about 25% in roosters. The ability of mature sperm to associate DNA molecules was influenced by sperm motility. The more the sperm motility, the more the DNA incorporated into spermatozoa. Also, DNA/sperm ratio was a parameter determining number of DNA molecules incorporated into the sperm. The more the DNA/sperm ratio, the more the number of DNA molecules associated with each sperm. The sperm-DNA association kinetics was considered rapid, where it involved up to 40 minutes in different species (Castro *et al.*, 1990; Camaioni *et al.*, 1992; and Lavitrano *et al.*, 1992). Nakanishi and Iritani (1993) used fluorescence *in situ* hybridization to recognize the sites at which exogenous DNA incorporates into the fowl sperm. Three patterns of DNA association with the sperm were observed, where DNA-sperm association took place in the posterior head region, in the anterior head region or in the whole head region. Zoraqi and Spadafora (1997) demonstrated that the DNA internalized into the sperm is extensively rearranged and undergone recombination with the sperm genomic DNA.

DNA-sperm binding can take place under widely different environmental conditions (Atkinson *et al.*, 1991). Unless particular experimental conditions are used, the number of DNA-uptaken sperm cells is quite low, as $1:10^6$ exposed cells (Camaioni *et al.*, 1992). It was found that the efficacy of binding the DNA molecules to the sperm dramatically increases when the DNA molecules are conjugated with liposomes, and the efficiency of DNA uptake correlates with concentration of liposomes (Zoraqi and Spadafora, 1997; and Glick and Pasternak, 2003). Liposomes could be toxic to cells. The fertility of cock spermatozoa was detracted when treated with lipofectin (Rottman *et al.*, 1992; and Squires and Drake, 1993). However, Trefil *et al.*, (1996) observed continuous egg fertility for three weeks of hens inseminated with lipofectin-treated spermatozoa, and the fertility reached to 52.3% during the third week. Also, the toxicity of lipofectin on mouse spermatozoa was reduced when the DNA and lipofectin molecules were conjugated together at a ratio of 1:3 (Bachiller *et al.*, 1991). The fluorescence *in situ* hybridization used by Nakanishi and Iritani (1993) showed that 51.6% of the exogenous DNA-lipofectin-treated sperm retained the exogenous DNA. The fertility of eggs was 47% for the DNA incubated with sperm, 23% for the electroporated sperm and 67% for the lipofectin-treated sperm. Fellgner *et al.* (1987) and Sato *et al.* (2003) indicated that lipofectin stabilizes exogenous DNA and keeps it intact, confirming the DNA stability in the liposomal-DNA complex.

From the point of view of poultry geneticists, gene transfer provides means to increase the genetic variation by the introduction of a new genetic material directly onto the genome (Shuman and Shoffner, 1986). Besides, it permits the transfer of favorable traits from low-performing stocks to high-performing stocks, without the transmission of less favorable genes. It also permits the gene flow between vastly different organisms, thus transcending the limitations of sexual reproduction. Although the sperm-mediated gene transfer seems applicable in avian species, methods stabilizing the foreign DNA and increasing the efficiency of integration of DNA into the sperm chromatin are not yet settled. In addition, it would be presumed to apply the artificial insemination

to make the sperm mediation of gene transfer practicable. Therefore, the viability of the sperm population must not be depleted to make possible the commercial use of sperm cells as vectors for gene transfer. Also, because sperm characteristics correlate with their fertilizing capacity, maintaining their viability would always be of much interest. In addition, fowl semen is low in volume, but is highly concentrated with spermatozoa. An eject of a cock varies from 0.2 to 0.5 ml and has an average density of about 3×10^9 sperm/ml (Hafez, 1974). Therefore, the dilution of semen is common to increase its volume and to increase the number of hens inseminated per an eject. Sexton (1977) developed a phosphate buffered semen diluent known as Beltsville Poultry Semen Extender (BPSE). It was reported that a dilution rate of 1:4 and a weekly insemination dose of 20 million sperm is considered the maximum extension rate of chicken semen diluted in the BPSE.

There is a lack of literature concerning the viability of sperm in relation to the appropriate conditions targeting the sperm for gene transfer. Therefore, the objectives of this study are to examine the efficacy of the sperm to uptake exogenous DNA under different dilution and lipofectin treatments and to investigate the effects of DNA incubation with sperm on their viability.

MATERIALS AND METHODS

Semen collection and exogenous DNA

Adult males, 10 months old, of a local broiler male line were used in this experiment. Semen samples were individually collected from five males following the dorsal-abdominal massage described by Lake (1957). The exogenous DNA used was the plasmid pUC18 (Fig. 1), described by Yanisch-Perron *et al.* (1985).

Experimental design

Two trials were carried out to achieve the aims of the study. Trial 1, was proposed to study the effects of semen dilution (4 µl diluent/1 µl semen), heat incubation (exposure of semen to 37°C for 30 minutes using water path) and the addition of lipofectin and their combinations on sperm characteristics. Trial 2, was designed based on the results obtained in trial 1, where imperical knowledge on the semen dilution and the concentration of lipofectin added to the semen have become available. So, trial 2, was proposed to assess the effects of lipofectin on the DNA incorporation into the sperm and sperm viability.

Trial 1

Individual semen samples were collected five times, three days apart. In each, the individual semen samples were immediately mixed after collection forming pooled semen. The pooled semen was then divided, as equally as possible, into seven parts for seven different treatments. Part 1, was kept un-treated (control). Part 2, was heat incubated. Part 3, was diluted with BPSE. Part 4, was diluted with BPSE, then heat incubated. Parts 5, to 7, were all diluted with BPSE, mixed with lipofectin reagent which was added by 5, 10 and 20 µg/100 µl diluted semen for parts 5, 6, and 7 respectively, and then all were heat incubated.

Trial 2

The semen was individually collected from the same birds for three times, three days apart. In each, the individual samples were immediately mixed after collection to form pooled semen. The pooled semen was then divided, as equally as possible, into three parts. Part 1, was kept un-treated (control). Parts 2, and 3, were diluted with the BPSE (4 µl BPSE/1 µl semen). Part 2, was then mixed with the pUC18 plasmid (2.5 µg plasmid/100 µl diluted

semen), then heat incubated. Part 3, was incubated with a mixture of the pUC18 plasmid and lipofectin (2.5 µg plasmid + 5 µg lipofectin/100 µl diluted semen), then heat incubated. After heat incubation in treatments 2 and 3, sperm were washed by adding 500 µl of BPSE to each treatment and mixed thoroughly, then centrifuged at 4000 rpm for 5 minutes. The supernatant was removed and the pellets (sperm cells) were re-washed using the same procedure. The un-treated sperm (control) was also subjected to washing procedures and used as a negative control. The DNA was then extracted from the washed sperm of all treatments. The DNA of all treatments, along with the plasmid DNA which was used as a positive control, were subjected to polymerase chain reaction (PCR) for the detection of exogenous DNA.

DNA analysis

DNA extraction

DNA was extracted from the washed sperm of treatments 2 and 3 of trial 2, as well as from the un-treated sperm (control), according to the procedures of Jerzy *et al.* (2003). Two hundred µl of the semen were digested with 2 µl of proteinase K (25 mg/ml) and 25 µl of 10% sodium dodecyl sulfate (SDS). All components were then thoroughly mixed and incubated at 37°C for 60 minutes.

The phenol : chloroform:isoamylalcohol mixture was added to the digested sperm at a ratio of 1:1; this means that 227 µl of the mixture was added to the digested sperm. Then the total volume was mixed and centrifuged at 12000 xg for 5 minutes, where the upper phase (water phase) was transferred into another tube. Then, 2.5 volumes of 96% ethanol and 1/10 volume of 3 M sodium acetate (pH=5.2) were added, and mixed thoroughly.

The mixture was then incubated overnight at -20°C, and then centrifuged on 12000 xg at 4°C for 15 minutes. The DNA was collected as pellets, washed with 70% ethanol, dried and dissolved in 30 µl of double-distilled water and kept at -20°C until use.

Table (1): Lengths (b) and sequences of the forward and reverse primers, and the primer recognition site on the plasmid pUC18.

Primer	Length	Sequence	Recognition site
Forward	18	5'-TGACGCCGGGCAAGAGCA-3'	1130-1147
Reverse	20	5'-GGCCGAGCGCAGAAGTGGTC-3'	1531-1550

Table (2): The polymerase chain reaction (PCR) components.

Reaction components	Amount
DNA sample	1 µl (100 ng)
Master Mix (Fermentas)[1]	10 µl (2x)
Forward primer	2 µl (0.2 µmol)
Reverse primer	2 µl (0.2 µmol)
Distilled water	5 µl
Total volume	20 µl

[1], Fermentas Inc. 520 Cohnelley Drive, Hanover, MD 21076, USA.

DNA concentration

The DNA solution of each treatment of trial 2, was diluted in distilled water at a rate of 1 µl DNA solution/100 µl distilled water. Then, the DNA concentration was determined using the spectrophotometer, according to Sambrook *et al.* (1989).

Recognition of exogenous DNA in the sperm

The polymerase chain reaction (PCR) was applied to the sperm treatments of trial 2, along with the two control treatments, to recognize the existence of the plasmid DNA in the sperm. Two specific primers, forward and reverse, were used (Table 1), to prime a specific DNA substrate of 420 bp long on the *rep* (pMB1) of the plasmid (Fig.1). The PCR analysis was performed in 20 µl reaction solution (Table 2). Samples were subjected to initial denaturation by heating at 95°C for 5 min., then denatured in 35 cycles at 95°C for one min., followed by the annealing at 65°C for one min. and at 72°C for 2 min. for extension reaction and the final extension was at 72°C for 7 min. The PCR products were mixed with 2 µl loading dye and loaded to a 2 % agarose gel. The gel was prepared by melting one gram agarose in 50 ml 1x TAE buffer and inoculated with 1µl (10 mg/ml) ethedium bromide. The loaded products were then subjected to electrophoresis at 50 volt for 1.5 hr. Thereafter, the DNA fragments were visualized on the gel using ultraviolet light.

Sperm motility

Individual motility of the sperm was assessed, where a drop of semen of each of the given treatments was mixed with 200 µl of BPSE warmed to 37°C. The mixture was then put on a plate hotted to 37°C under light microscope. The individual motility was numerically estimated according to Morisson *et al.* (1997).

Sperm viability

The parameters of spermatozoa viability included percentages of live, dead and live but abnormal sperm. Forty microliters of semen samples of each treatment were added to 150 µl of the staining solution (eosin 16 g/l and nigrosin 60 g/l in BPSE) and put onto a slide. Two minutes later, smears were performed for each sample and spermatozoa were observed, using a microscop with an oil immersion objective. Live spermatozoa were seen white in color because they were eosin-impermeable. However, dead spermatozoa were pink because they became eosin-permeable. Three hundred spermatozoa per sample were observed and the percentages of live, dead and abnormal spermatozoa were calculated (Sorensen, 1979).

Statistical analysis

The statistical analysis system (SAS, 1999), was applied to the data set of each trial for the analysis of the effect of different treatments on semen characteristics. Multiple range test of Duncan (1955), was used to separate means, whenever significance of treatment effect was shown.

RESULTS AND DISCUSSION

Trial 1

The effects of heat incubation, dilution and lipofectin treatments on sperm characteristics are presented in Table (3). The sperm in un-treated semen (control) showed 90% motility and the percentages of live, dead and abnormal sperm were 94.8, 1.6, and 3.4%, respectively. The percentage of live sperm is higher, and the percentages of dead and abnormal sperm are even lower than those obtained by Morisson *et al.* (1997), in two lines of Rhode Island Red chickens. The percentages of live, dead and abnormal sperm averaged, overall the two lines, 90.5, 4.85 and

4.75%, respectively. The sperm motility in the heat-incubated semen was 79%, and was significantly less than that estimated for the control treatment (90%). However, no significant differences were found in the percentages of live, dead and abnormal sperm between both treatments. When BPSE was used as a diluent, it significantly reduced the individual motility to 79% compared to 90% for the un-treated semen (control). The dilution of semen with BPSE did not significantly influence the percentages of live, dead and abnormal sperm. The heat incubation of the BPSE-diluted semen resulted in more depression in sperm motility than that occurred by either the dilution or heat incubation, however it did not affect the live and abnormal sperm percentages.

The estimates of live, dead and abnormal sperm percentages obtained for all treatments that have undergone incubation and/or dilution, although were, to some extent, significantly different from those of the control; they were still in agreement with those reported by Morisson *et al.* (1997).

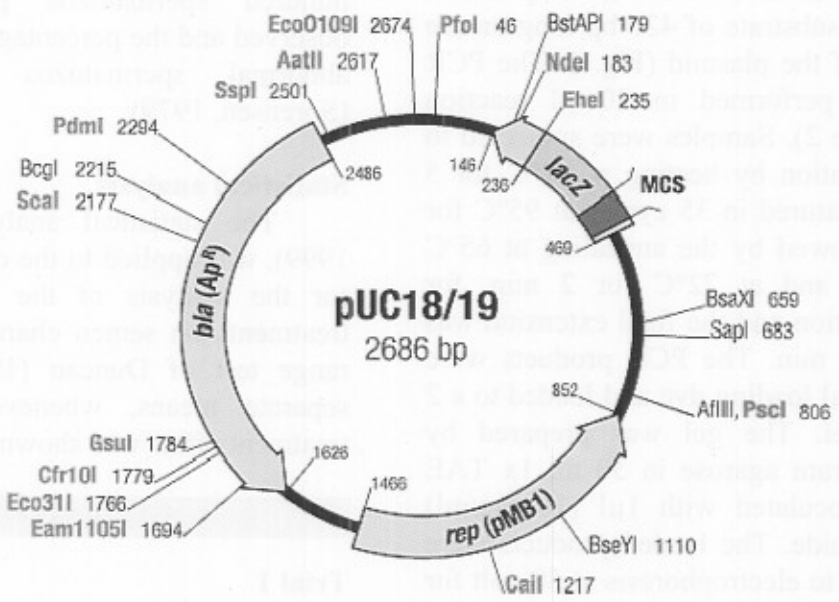

Fig. (1): Schematic diagram of the plasmid pUC18.

The addition of lipofectin to the BPSE-diluted and heat-incubated semen treatments drastically and significantly detracted the motility, and the detraction was positively associated with lipofectin concentration. The more the concentration of lipofectin, the more the detraction in motility. The motility was 53.3% for the BPSE-diluted, 5% lipofectin-, and heat-incubated semen and reached to 20% for the BPSE-diluted, 20% lipofectin, and heat-incubated semen. Also, the percentage of live sperm has significantly been reduced and that of dead sperm has apparently been increased in the BPSE-diluted, lipofectin-, and heat-incubated semen treatments.

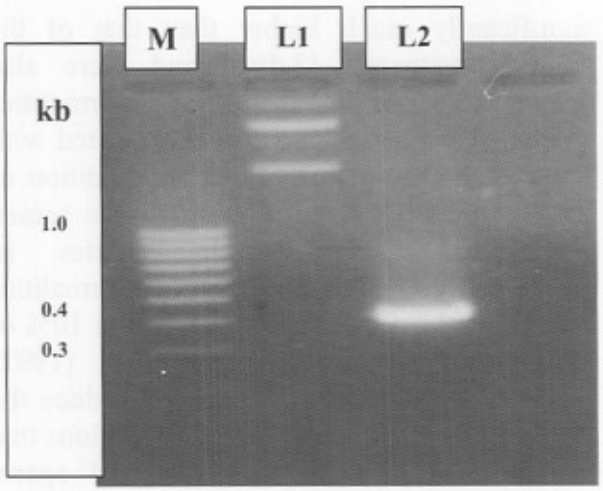

Fig. (2): Profile of the gel electrophoresis of the positive control treatment used in the experiment. Lane 1: the plasmid pUC18, not subjected to PCR analysis. Lane 2: the PCR product of the plasmid pUC18, where the specific primers primed the DNA substrate (420 bp) of the pMB1 replicon. M appriviates for the DNA marker with known molecular size.

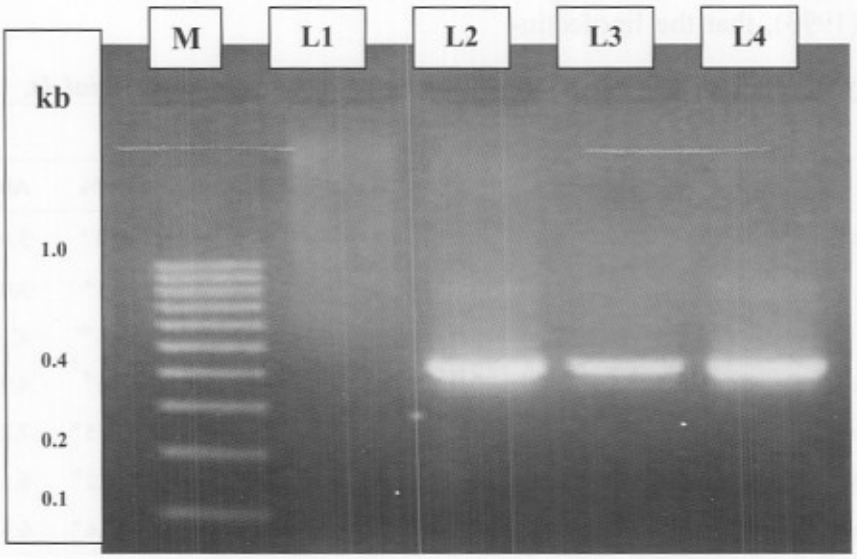

Fig. (3): Photograph of the electrophoretic gel of the DNA in the different treatments. Lane 1: the un-treated sperm DNA (negative control). Lane 2: the PCR product of the un-treated plasmid DNA, where the specific band (420 bp) on the rep pMB1 was amplified (positive control). Lane 3: the PCR products of sperm incubated with the plasmid DNA. Lane 4: the PCR products of sperm incubated with the plasmid DNA-lipofectin complex. M appriviates for the DNA marker with known molecular size.

The more the concentration of lipofectin, the less the percentage of live sperm and obviously the more the percentage of dead sperm. The percentages of live and dead sperm reached 28.3 and 65.3% in the BPSE-diluted, 20% lipofectin-, heat-incubated semen *vs* 94.8, and 1.6% in the un-treated semen. The percentages of abnormal sperm for semen treated by different lipofectin concentrations varied from 6.1 to 7.2%. These estimates were

significantly much higher than that of the control treatments (3.4%), and were also somewhat higher than the sperm abnormalities estimated for semen samples not treated with lipofectin. This indicates that the addition of lipofectin at 5% concentration to the semen would cause sperm abnormalities as significantly much as the sperm abnormalities caused by the addition of lipofectin at 10% or 20% concentration. Rottmann *et al.* (1992) reported that liposomes in general reduce the fertility of cock spermatozoa. It is obvious that the increase in dead and abnormal sperm percentages in the semen treated with lipofectin would reduce the semen fertilizing capacity. This result agrees with the finding of Squires and Drake (1993), that the lipofectin-incubated cock spermatozoa showed low fertility after insemination.

The results, in general, denote that the heat incubation of semen (the exposure to 37°C for 30 min.) and/or the dilution with BPSE did not result in much depression in the sperm characteristics. Because of the necessity of using lipofectin to enhance the fusion of DNA into the sperm, therefore, it may be used at low concentration (5%). Bachiller *et al.* (1991) reported a reduction in toxicity of lipofectin on mouse spermatozoa when used with low concentrations. However, Zoraqi and Spadafora (1997) reported that the efficiency of DNA uptake by cells is correlated with the concentration of liposomes.

Table (3): Summary of fowl sperm characteristics in different treatments (Trial 1).

Treatment	$\bar{X}$ ±SE Motility, %	Live, %	Dead, %	Abnormal, %
Non-treated semen (control)	90.0 ±1.6 [a]	94.8 ±0.8 [ab]	1.6 ±0.4 [c]	3.4 ±0.5 [cb]
Heat-incubated semen	79.0 ±2.9 [b]	95.7 ±0.7 [a]	1.2 ±0.5 [c]	3.0 ±0.4 [c]
BPSE [1]-diluted semen	79.0 ±3.3 [b]	93.1 ±0.9 [ab]	2.6 ±0.5 [de]	4.3 ±0.5 [abc]
BPSE-diluted, heat-incubated semen	70.0 ±2.7 [c]	91.8 ±1.4 [b]	3.2 ±0.6 [d]	4.9 ±1.5 [abc]
BPSE-diluted, 5% lipofectin-, heat-incubated semen	53.3 ±3.3 [d]	69.8 ±0.2 [c]	22.9 ±0.5 [c]	7.2 ±0.4 [a]
BPSE-diluted, 10% lipofectin-, heat-incubated semen	33.3 ±3.3 [e]	65.2 ±0.5 [d]	28.7 ±0.2 [b]	6.1 ±0.2 [ab]
BPSE-diluted, 20% lipofectin-, heat-incubated semen	20.0 ±0.0 [f]	28.3 ±0.6 [c]	65.3 ±0.6 [a]	6.3 ±1.2 [ab]

[1], BPSE = Beltsville poultry semen extender.

[a-f], means of different treatments, within trait, with different superscripts are significantly different ($P \leq 0.05$).

Trial 2

1. Recognition of exogenous DNA in sperm cells and the effect of lipofectin

The DNA plasmid was recognized using two primers (forward and reverse), specific to bind a DNA substrate of 420 bp existed in the pMB1 replicon on the plasmid. Figure (2) presents the plasmid DNA before and after PCR analysis. It shows that the primers successfully primed the DNA substrate; therefore it is used as a positive control. The products of PCR for the two experimental treatments were electrophorated on a gel along with the PCR products of the two control treatments (Fig. 3). It is observed that the plasmid DNA was successfully internalized into the sperm in both treatments, since the region on the exogenous DNA recognized by the primers was successfully amplified. However, the fragment recognized in the DNA isolated from the sperm incubated with the plasmid DNA-lipofectin mixture was

apparently of higher yield than that recognized in the DNA of the sperm incubated with the plasmid not treated with lipofectin, although the DNA concentration used in both treatments was high (100 ng/ml). This suggests that more molecules of the plasmid DNA were fused into the sperm cells in the presence of lipofectin. Nakanishi and Iritani (1993) reported that the treatment of sperm cells with lipofectin to enhance the exogenous DNA uptake resulted in having 51.6% of the sperm retained exogenous DNA *vs* 6.3% for the sperm not treated with lipofectin. It was suggested that lipofectin molecules interact spontaneously with DNA forming a lipid-DNA complex that can be easily fused in the plasma membrane. Zangenberg *et al.* (1999) attributed the low yield of PCR products to the degradation of the DNA samples. Sato *et al.* (2003) presumed that seminal plasma of chicken males contains DNase activity, although birds have no accessory glands. The enzyme degrades the exogenous DNA, unless the DNA is conjugated with the liposome in a complex. This explains the existence of the fragment amplified from the DNA isolated from sperm treated with the naked exogenous genes, of a low yield, compared to that amplified from the DNA of the sperm treated with the exogenous DNA-lipofectin mixture. Where the DNase targets the naked exogenous DNA, whereas the exogenous DNA conjugated with liposome molecules becomes stabilized. In this concern, Sato *et al.* (2003) supported the use of lipofectin reagents to sustain the mediation of the sperm as vectors for gene transfer. Therefore, it is concluded that lipofectin stabilizes and facilitates the fusion of DNA into the sperm cells through protecting it from the digestion by DNase. However, the addition of lipofectin may somewhat cause a retardation on sperm viability. Therefore, the use of lipofectin at a concentration as low as possible, just enough to protect the DNA molecules (5% in this experiment) may not, for some extent, influence the stability of the sperm population viability, which is still of much interest to retain the fertilizing capacity.

2. *The interaction between sperm uptake of DNA and sperm viability*

Table (4) presents the sperm characteristics. It is obvious that the incubation of plasmid DNA with the BPSE-diluted semen significantly detracted motility to 75%, compared to 90% for the control. But the detraction was significantly similar to that caused by the incubation of semen at 37°C for 30 minutes (79%). However, the reduction in motility was even more and reached to 56.7% when the plasmid DNA-lipofectin (5%) mixture was heat incubated (37°C for 30 min.) with the BPSE-diluted semen. The results of sperm motility in both treatments indicate that the introduction of DNA to the sperm did not disturb their motility and the depression in motility was obviously attributed to the heat incubation of the semen. However, the drastic reduction in sperm motility in the lipofectin-incubated semen obviously transcended the heat incubation effect to include the effect of lipofectin as well. Nakanishi and Iritani (1993) reported a slight reduction in chicken sperm motility due to the addition of lipofectin. Castro *et al.* (1990) demonstrated a positive association between sperm motility and the incorporation of DNA into spermatozoa.

Table (4): Summary of fowl sperm characteristics for samples incubated with plasmid DNA (Trial 2).

Treatment	$\bar{X}$ ±SE			
	Motility, %	Live, %	Dead, %	Abnormal, %
Non-treated semen (control)	90.0 ±1.6 [a]	94.8 ±0.8 [a]	1.6 ±0.4 [c]	3.4 ±0.5 [b]
Heat-incubated semen	79.0 ±3.0 [b]	95.7 ±0.7 [a]	1.2 ±0.5 [c]	3.0 ±0.4 [b]
BPSE-diluted, plasmid DNA, heat-incubated semen	75.0 ±2.9 [b]	90.6 ±0.9 [b]	5.4 ±0.7 [b]	4.0 ±0.3 [b]
BPSE-diluted, 5% lipofectin-DNA mixture, heat-incubated semen	56.7 ±3.3 [c]	65.1 ±0.9 [c]	25.4 ±0.5 [a]	9.5 ±0.4 [a]

[1], BPSE = Beltsville poultry semen extender.
[a-c], means of different treatments, within trait, with different superscripts are significantly different ($p \leq 0.05$).

The percentage of live sperm in the BPSE-diluted, plasmid DNA-incubated semen (90.6%) was significantly less than that in the control (94.8%), and also less than that in the heat- incubated semen (95.7%). The treatment of the semen with lipofectin concentrated at 5% significantly resulted in much less percentage of live sperm (65.1%). On the contrary, the percentage of dead sperm was significantly higher in both treatments compared to the control (5.4% and 25.4% *vs* 1.6%, respectively). This indicates that the introduction of DNA to the sperm depressed their viability and the depression was much more pronounced with the addition of lipofectin. The percentage of abnormal sperm in the semen diluted with BPSE and incubated at 37°C for 30 min. with plasmid DNA was not significantly different from that of the control (4.0% *vs* 3.4%). However, it was significantly much higher (9.5%) when lipofectin at 5% concentration accompanied the exogenous DNA during the incubation with the BPSE-diluted semen. This again indicates the relative damage in the sperm due to the incorporation with exogenous substrates (DNA and lipofectin in this study), although the effect of lipofectin was more percepted. The results reported in this study may explain the causes of the reduction in fertility of cock spermatozoa reported by Rottmann *et al.* (1992), when liposomes were incubated with sperm cells. However, Nakanishi and Iritani (1993) stated that lipofectin addition to chicken sperm did not affect fertility, although it slightly reduced motility.

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الملخص العربي

نقل الجين عن طريق السائل المنوي في الدواجن. ١- العلاقة مع حيوية الحيوانات المنوية

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تم دراسة استخدام الحيوان المنوي لذكور الدجاج كوسيط لعملية نقل الجينات، وكانت أهداف الدراسة هي تقييم فعالية الحيوانات المنوية في الاتحاد مع الجينات وعلاقة ذلك بحيويتها. لهذا الغرض تم تصميم تجربتين. التجربة الأولى كانت لتقييم تأثيرات تخفيف السائل المنوي (٤ ميكرولتر مخفف لكل ١ ميكرولتر سائل منوي)، التعريض الحراري للسائل المنوي (٣٧°م لمدة ٣٠ دقيقة)، إضافة الليبوفكتين وتأثيراتهم المشتركة على حيوية الحيوانات المنوية. وجد أن حركة الحيوانات المنوية في أي من معاملة التعريض الحراري أو التخفيف (٧٩%) نقل معنويا عن حركة الحيوانات المنوية غير المعاملة (٩٠%). لم تظهر أي تأثيرات لأي من التخفيف أو التعريض الحراري على نسبة كلٍّ من الحيوانات المنوية الحية، الميتة أو الشاذة. لم يختلف التأثير المشترك للتخفيف والتعريض الحراري عن تأثير أي منهما منفردا. إضافة الليبوفكتين أدى إلى انخفاض حركة الحيوانات المنوية ونسبة الحيوانات المنوية الحية وزيادة نسبة كلٍ من الحيوانات المنوية الميتة والشاذة وإزداد التأثير بزيادة تركيز الليبوفكتين. في التجربة الثانية تم تقييم تأثير إضافة الليبوفكتين بتركيز ٥% إلى السائل المنوي على نفاذ الدنا إلى الحيوانات المنوية وعلى حيويتها. تم استخدام البلازميد pUC18 كنموذج للجين المطلوب نقله، وتم استخدام بادئين وراثيين primers خاصين بالتعرف على البلازميد pUC18 عند إجراء تحليل PCR للدنا المستخلص من حيوانات منوية معاملة بالبلازميد فقط، حيوانات منوية معاملة بالـبلازميد مع الليبوفكتين ، وحيوانات منوية غير معاملة (كنترول). وجد أن الدنا قد نفذ إلى داخل الحيوانات المنوية سواء كانت معاملة بالليبوفكتين أو غير معاملة. لكن المقطع الذي تم التعرف عليه من الحيوانات المنوية المعاملة بالليبوفكتين كان أكثر وضوحا وسمكا من المقطع الذي تم التعرف علية من الحيوانات المنوية غير المعاملة بالليبوفكتين مما يدل على أن الليبوفكتين حافظ على ثبات الـدنا وساعد على بقائه سليما خلال نفاذه للحيوانات المنوية. كانت حركة الحيوانات المنوية ونسبة الحيوانات المنوية الحية ٥٦,٧% و ٦٥,١% في الحيوانات المنوية المعاملة بالليبوفكتين (٥%) مقابل ٧٥% و ٩٠,٦% للحيوانات المنوية غير المعاملة بالـليبوفكتين . هذه النتائج تدل على أن أضافة الليبوفكتين يؤدي إلى تدهور حيوية الحيوانات المنوية وبالتالي مدى إتاحتها للإخصاب. نظرا لضرورة إضافة مركب الليبوفكتين حيث يساعد على نفاذ الدنا إلى داخل الحيوانات المنوية لذلك يمكن استخدامه بأقل تركيز يمكن أن يؤدي الغرض من استخدامه (٥% في هذه التجربة).