Genetic transformation of quails spermatogonia in vitro

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INTRODUCTION

Spermatogonia are precursors of male germ cells. This type of cell is considered as a promising target for the introduction of recombinant DNA. The cells development after transfection in the gonads of male recipients would allow a population of transformed mature germ cells - sperm to be obtained, which can be used for female insemination in order to obtain transgenic offspring.

The aim: . We have studied the transformation efficiency of quail spermatogonia in vitro using various methods of lipofection and electroporation.

MATERIAL AND METHODS

Spermatogonia cells were isolated from the testes of 1-week-old quail males.

For transfection of spermatogonia in vitro, the plasmid pZsGreen1-N1 (Addgene, # 54702) coding the ZsGreen gene under the CMV promoter was used.

Lipofectamine3000 Reagent Kit (Invitrogen, # L3000001) was used for lipofection. Electroporation was performed using the Neon transfection system (Invitrogen).

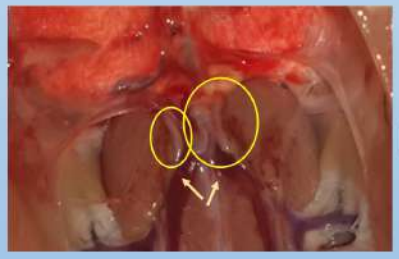
Electroporation conditions were chosen by voltage (from 850 to 1700 V), pulse exposure time (10, 20, 30 μs), and number of breakdowns (1, 2, 3 pulses). Each experiment was carried out in 3 replicates.

RESULTS

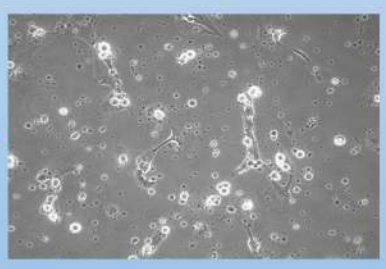
The effectiveness of spermatogonia transfection by the lipophilic agent was 1.6±0.3%. The efficiency of spermatogonia transformation using electroporation was higher. The optimal electroporation parameters were 1100 V and 20 μs and 1 pulses. The percentage of transformed cells reached 9.2±0.8%. When using other parameters, this indicator was lower by 10-80% ($p<0.05$).

CONCLUSION

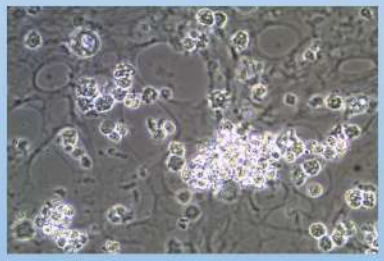
Based on the data obtained, the use of electroporation can be recommended for the efficient transfection of the quails spermatogonia in vitro.



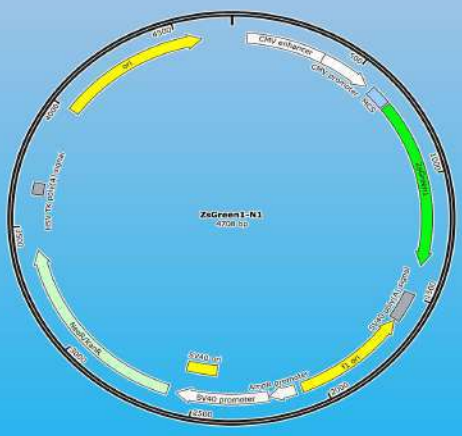
1 week quail's testes



Culture after transferred into new dish
(Separate cells of spermatogonia are visible)



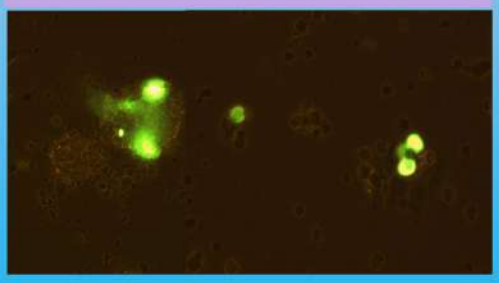
7-days culture



Lipophilic transfection

Tissue culture vessel	Amount of DNA, μL*	Volume of TurboFect, μL	Effectiveness, %
96-well plate	20	0,3	0,8
96-well plate	20	0,4	1,2
96-well plate	20	0,5	1,6
96-well plate	20	0,6	1,4

Electroporation



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