EFFECTS OF ELECTROPORATION ON QUALITY OF ROOSTER SEMEN

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Summary

Semen was collected from Taiwan commercial local chickens, the diluted sperm suspension were placed in the Gene Pulser cuvette for electroporation. The motility, mortality and abnormality of spermatozoa were evaluated. The fertility and hatchability were also investigated. The results showed that smaller motility and greater mortality or abnormality than the control were found when the capacitances were increased either for spermatozoa treated with small capacitances (0.25, 1, 3 and 25 μ FD) or treated with high capacitances (125, 250, 500 and 960 μ FD). In general, greater field strengths also resulted in smaller motility and greater mortality or abnormality of spermatozoa. Although the electroporation decrease the fertility there were no effect on the hatchability.
(**Key Words** : Electroporation, Semen Quality, Chicken)

Introduction

Electroporation is one method to introduce various molecules into cells of diverse types, and was used as a tool to investigate gene expression and regulation, cell physiology, and cell membrane signal transduction. The idea of using sperm as a transformation vector is not new. As early as 1971, such a method was reported for rabbits (Brackett et al., 1971), although remarkably this publication appeared to excite little interest. Recently, Tomkins and Houghton (1988) electroporation to induce acrosome reaction of human sperm. Rickords et al. (1990) used electroporation to investigate the penetration of bovine sperm. Development of germ-line transformation for gene transfer would be particularly attractive. In this paper, we describe the effects of electroporation on the chicken spermatozoa. We hope that this work can provide some information for the researchers who are interested in the transgenic chicken.

Materials and Methods

Semen collection and dilution

The semem was collected from 18 Taiwan commercial local chickens by standrad procedures of abdominal massage method, then diluted to 4~5X 10^8/ml with 0.1 M Millonig phosphate buffer (1.8 g $NaH_2PO_4 \cdot H_2O$, 23.5 g $NaHPO_4 \cdot 7H_2O$, 5.0 g NaCl to 100 ml with dH_2O, pH 7.4, 440 mOsmols) (Glauert, 1975) at 4℃ for electroporation.

Electroporation and evaluation of semen quality

Diluted sperm suspension (0.8 ml) was placed in the Gene Pulser cuvette (Bio-Rad, CA). The cuvette containing sperm was then moved in the Gene Pulser chamber, and pulsed once. The following conditions of electroporation were tested:

A. Small capacitance: 0.25, 1, 3 and 25 μ FD; Field strength (E): 0.25, 1.25, 2.50, 3.75, 5.00 and 6.25 KV/cm.
B. Large capacitance: 125, 250, 500, 960 μ FD; Field strength (E): 0.25, 0.50, 0.75, 1.00 and 1.25 KV/cm.

The motility, mortality and abnormality of spermatozoa were evaluated according to standard procedures (Herman and Madden, 1953) after electroporation. The fertility and hatchabiltiy of spermatozoa treated with electroporation (E = 2.5 KV/cm, 1 μ FD; E = 2.5 KV/cm, 25 μ FD; E = 0.5 KV/cm, 250 μ FD) were also investigated at the fifth day and twenty-first day after artificial insemination, in total 51 hens.

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Results and Discussion

Rooster semen (0.3-0.6 ml) per ejaculate was collected, and the concentration of semen was 3.2-4.5 X 10^9/ml. Excellent motility, 80% or more, of the spermatozoa was employed for the tests.

The choice of electroporation buffer is reported to be important (Chu et al., 1987; Knight and Scrutton, 1986; Malinin et al., 1989; Tomov et al., 1988). We found the Millonig phosphate buffer give good performance. Because components of the buffer were simplified, excluding Ca^{2+}, Mg^{2+}; the survival rates of spermatozoa were great enough; it wasn't a necessary to add other components or nutrients; because the electric restriction of buffer was small, so the period of electroporation was brief; and damage to spermatozoa was slight.

Motility

The motility of spermatozoa of the control group was 82.7%. After electroporation, no matter with small capacitances (0.25 to 25 μ FD) or large capacitances (125 to 960 μ FD), the greater the field strengths given, the motility was smaller. The effects of increasing capacitance would be more obviously to diminish motility (figure 1 and 2).

At small capacitances, 25 μ FD treatments gave the smallest motility. When E = 6.25 KV/cm, the rate of motility was only 35.0%. At the same time, 25 μ FD treatments produced the greatest mortality. When E = 6.25 KV/cm, the mortality rate was 21.9%; the same result was found at large capacitances with the motility at 960 μ FD treatments smaller than for the others.

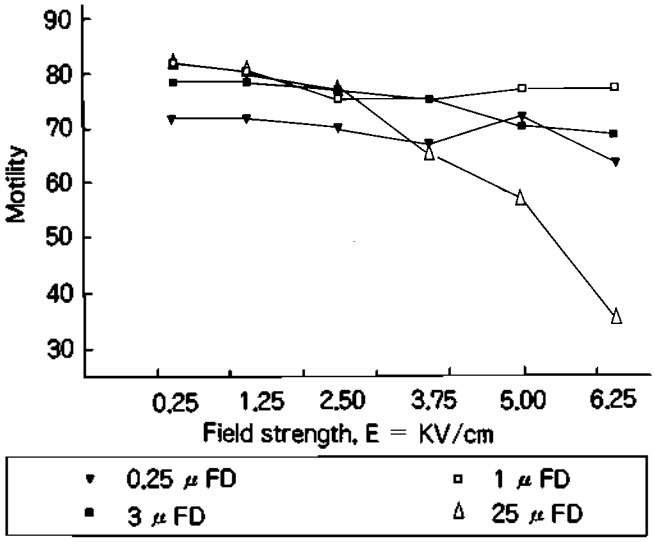

Figure 1. Motility of rooster spermatozoa treated with small capacitance electroporation.

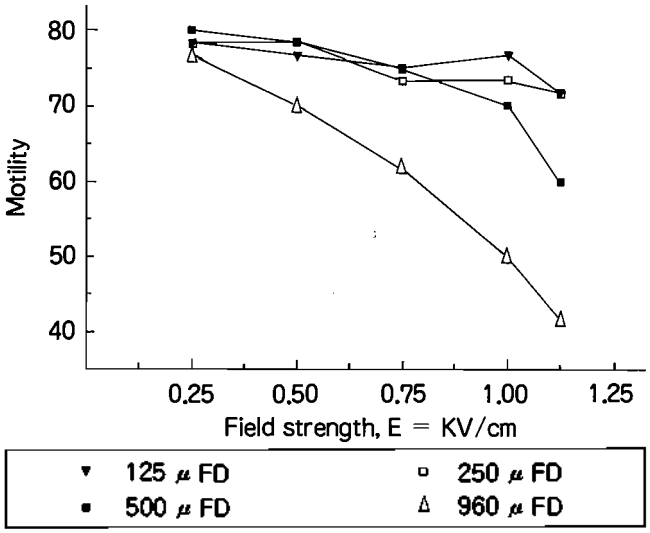

Figure 2. Motility of rooster spermatozoa treated with large capacitance electroporation.

Mortality

In the control group, the average mortality of spermatozoa was 6.2%. At small capacitance, the rates of mortality increased from 5.5% to 21.9%, according to the increased capacitance (figures 3 and 4). The same results were obtained at large capacitance, with the mortality rates increased from 8.8% to 20.5%, and greater than that with small capacitance even more.

The field strength affected the ability of gene transfer and cell mortality. The relationship of field strength and transfer ability was positive relative to a limited range. The transfer efficiency decreased rapidly when the field strength was greater enough to break the structure of the cell; at this time, most cells were dead (Chu et al., 1987).

The other factor that affected the mortality was the duration of electroporation. Under the same field strength, increased duration accompanied increased capacitance. The greater capacitance produced higher resistance and the duration of electroporation became increased also. At small capacitance, for example, E = 5.0 KV/cm; 0.25, 1, 3, 25 μ FD treatments had the duration time 0.1, 0.1, 0.1, 0.4 msec respectively, and the mortality were 8.2%, 7.2%, 9.6%, 14.1%; at high capacitances, for example, E = 1.0 KV/cm, 125, 250, 500 and 960 μ FD treatments had durations 2.3, 4.2, 7.4, 14.5 msec, and the mortalities were 14.5%, 9.0%, 13.2% and 18.8% respectively.

We conclude that greater resistances were produced by larger capacitances and the duration increased also, the resistibility of sperm cells decreased consequently, so as to increase mortality.

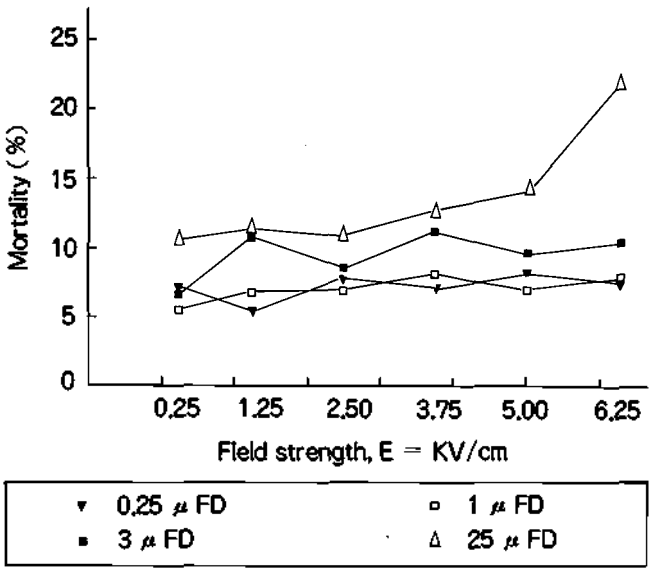

Figure 3. Mortality of rooster spermatozoa treated with small capacitance electroporation.

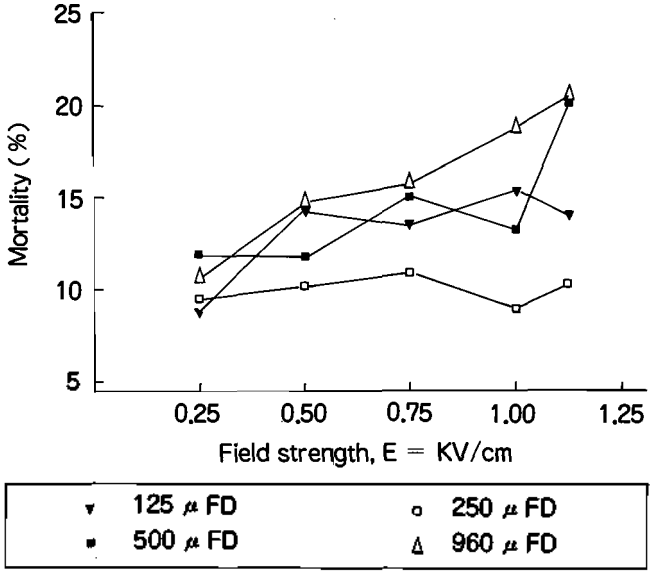

Figure 4. Mortality of rooster spermatozoa treated with large capacitance electroporation.

Abnormality

The fertility decreased when abnormality increased. In general, the fertility was unaffected unless abnormality exceeded 15-20%. The results showed that no matter whether large or small capacitance, increased abnormality followed increased field strength. The effects were more obvious when the conditions involved large capacitances, especially (figure 5 and 6). The average abnormality of the control group was 8.9%. Variation 7.7-32.2% were found in various treatments.

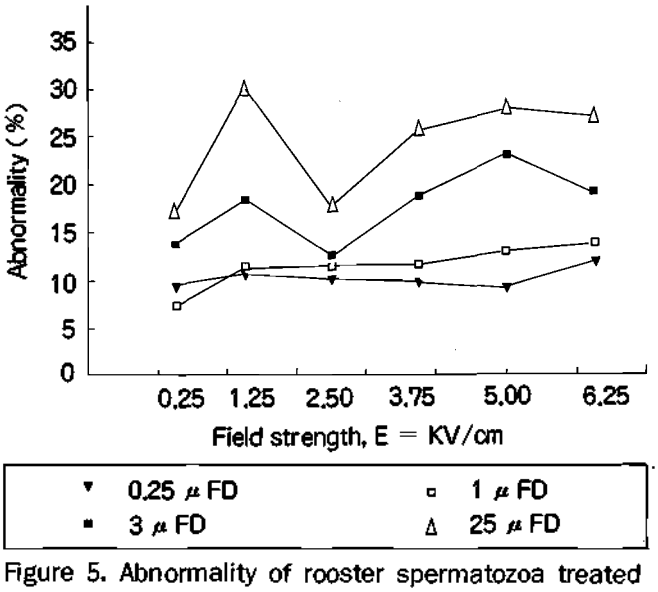

Figure 5. Abnormality of rooster spermatozoa treated with small capacitance electroporation.

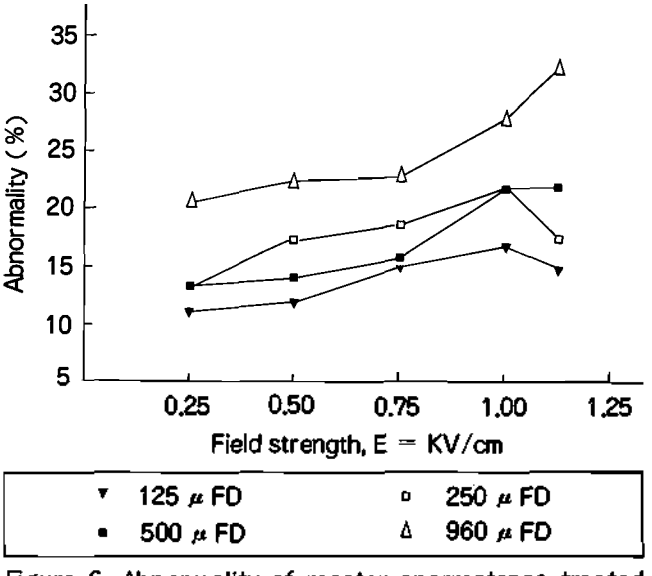

Figure 6. Abnormality of rooster spermatozoa treated with large capacitance electroporation.

The rate of abnormality of rooster spermatozoa increased after electroporation. The reason was that the stress induced by the huge electric stimulus occurred in a brief period and at a large field strength. Small capacitance treatments (0.25, 1, 3, 25 μ FD) produced the maximum of abnormality rate at E = 1.25 KV/cm (10.7 %, 11.4%, 18.5%, 30.3% respectively) (figure 5), then decreased rapidly, but increased again with increasing field strengths. E = 1.25 KV/cm became a threshold value when rooster spermatozoa suffered in high electric

field ? No other reports is possible at this moment.

Fertility and hatchability

The fertility of spermatozoa treated with electroporation were 52.58% (E = 2.5 KV/cm, 1 μ FD), 51.39% (E = 2.5 KV/cm, 25 μ FD) and 58.49% (E = 0.5 KV/cm, 250 μ FD), respectively (table 1). The fertility of spermatozoa treated with electroporation was smaller than that of the control group ($p<0.05$). Hence electroporation of sperm decreased the fertility.

TABLE 1. FERTILITY AND HATCHABILITY OF ROOSTER SPERMATOZOA TREATED WITH ELECTROPORATION

Treatment	No. of hens	No. of eggs	Fertility (%)	Hatchability (%)
1 μ FD, E = 2.5 KV/cm	15	697	52.58 ± 4.77^b	77.61 ± 3.05
25 μ FD, E = 2.5 KV/cm	15	725	51.39 ± 4.37^b	77.71 ± 4.13
250 μ FD, E = 0.5 KV/cm	15	614	58.49 ± 3.92^b	82.00 ± 3.10
Control	6	94	77.65 ± 4.85^a	75.76 ± 6.79

1. Sperm concentration: 5×10^8 cells / ml, volume of artificial insemination: 200 μl/hen (1×10^8 spermatozoa).
2. Values of fertility and hatchability are Mean ± SE.
3. Data in the same column with the different letters are significantly ($p<0.05$).

Although the electroporation decreased the fertility, there were no effect on hatchability. The hatchability of treatment at E = 0.5 KV/cm, 250 μ FD was slightly greater than that of the control group, but not significantly ($p>0.05$) (table 1).

The fertility rates of rooster spermatozoa treated with electroporation were significantly less than that of control group. The reason may be the decreased motility and the increased of mortality after electroporation. According to observations by scanning microscopy (data not shown), the spermatozoa treated with electroporation at a large field strength showed serious damage, including seriously cracked or broken sperm head.

There remain many questions for us to answer from these tests, and it also make us interested in making a transgenic chicken.

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