Sperm-mediated gene transfer (SMGT) is based on the ability of spermatozoa to bind exogenous DNA and transfer it into oocytes by fertilization. However, SMGT is still undergoing optimization to improve its efficiency to produce transgenic animals. The acrosome reaction is necessary for spermatozoa to carry the exogenous DNA into oocytes. In this study, the effect of the acrosome reaction on the efficiency of spermatozoa carrying exogenous DNA was evaluated. The results showed that the efficiency of the acrosome reaction was significantly higher (p<0.05) after incubation with 50 μmol/L progesterone compared to incubation without progesterone. It was significantly higher (p<0.05) in the 20, 40, and 60 min of progesterone treatment groups than in the 0 min treatment group. The spermatozoa were further incubated with cyanine dye Cy5 labeled DNA (Cy5-DNA) for 30 min at 37°C, and positive fluorescence signals were detected after the acrosome reaction was induced by progesterone at concentrations of 0 and 50 μmol/L for 40 min. The percentage of positive Cy5-DNA signals in spermatozoa was 96.61±2.06% and 97.51±2.03% following exposure to 0 and 50 μmol/L progesterone, respectively. The percentage of partial spermatozoa heads observed following combination with Cy5-DNA was 39.73±3.03% and 56.88±3.12% following exposure to 0 and 50 μmol/L progesterone, respectively. The ratio of positively stained spermatozoa combined with exogenous DNA showed no reduction after the acrosome reaction. These results suggest that the acrosome reaction might not be the key factor affecting the efficiency of SMGT.

**Key words:** spermatozoa, SMGT, acrosome reaction, exogenous DNA

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**Effect of the acrosome reaction on the efficiency of sperm-mediated DNA transfer**

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**Abstract**

Sperm-mediated gene transfer (SMGT) is based on the ability of spermatozoa to bind exogenous DNA and transfer it into oocytes by fertilization. However, SMGT is still undergoing optimization to improve its efficiency to produce transgenic animals. The acrosome reaction is necessary for spermatozoa to carry the exogenous DNA into oocytes. In this study, the effect of the acrosome reaction on the efficiency of spermatozoa carrying exogenous DNA was evaluated. The results showed that the efficiency of the acrosome reaction was significantly higher (p<0.05) after incubation with 50 μmol/L progesterone compared to incubation without progesterone. It was significantly higher (p<0.05) in the 20, 40, and 60 min of progesterone treatment groups than in the 0 min treatment group. The spermatozoa were further incubated with cyanine dye Cy5 labeled DNA (Cy5-DNA) for 30 min at 37°C, and positive fluorescence signals were detected after the acrosome reaction was induced by progesterone at concentrations of 0 and 50 μmol/L for 40 min. The percentage of positive Cy5-DNA signals in spermatozoa was 96.61±2.06% and 97.51±2.03% following exposure to 0 and 50 μmol/L progesterone, respectively. The percentage of partial spermatozoa heads observed following combination with Cy5-DNA was 39.73±3.03% and 56.88±3.12% following exposure to 0 and 50 μmol/L progesterone, respectively. The ratio of positively stained spermatozoa combined with exogenous DNA showed no reduction after the acrosome reaction. These results suggest that the acrosome reaction might not be the key factor affecting the efficiency of SMGT.

**Key words:** spermatozoa, SMGT, acrosome reaction, exogenous DNA

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**Introduction**

In comparison to other techniques, such as DNA microinjection, nuclear transfer, and retroviral infection, sperm-mediated gene transfer (SMGT) is a simpler technique by which transgenic animals can be produced (Kues and Niemann 2004, Smith and Spadafora 2005, Bacci et al. 2009, Petersen 2017). The ability of spermatozoa to accept exogenous DNA and transfer it to the oocyte during fertilization was first reported in rabbits in 1971 (Brackett et al. 1971). Since then, this technique has been used by several laboratories. A variety of transgenic animal species have been successfully produced by SMGT, including fish (Patil and Khoo 1996), chickens (Nakanishi and Iritani 1993), mice (Maione et al. 1998), and swine (Lavitrano et al. 1997).

Despite the use of SMGT to produce transgenic animals, the efficiency of the technology is still relatively