DOI: 10.22055/ivj.2019.184521.2134

Effects of sperm parameters and incubation conditions of sperms with foreign DNA in ovine sperm transfection

Khosro Hoseini Pajooh[1*] and Parviz Tajik[2]

[1] Assistant Professor, Department of Biotechnology, Iranian Research Organization for Science and Technology, Tehran-Iran
[2] Professor, Department of Theriogenology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

Received: 06.05.2019 Accepted: 27.11.2019

Abstract

Sperm-mediated gene transfer (SMGT) based on the intrinsic ability of sperms to bind and take-up of exogenous DNA was introduced in 1989. From that time on, it has been a challenging topic. One of the serious challenges was the motility of transfected sperms and hence their ability to fertilize the oocyte. The present study aimed to determine the effects of media and incubation conditions and also sperm parameters on the transfection of ovine spermatozoa by foreign DNA. In this study, the effects of various incubation temperature (5, 20 and 37 ºC), three different media (PBS, TCM and DMEM), presence of FBS in medium, viability and motility of sperms and sperm capacitation in DNA absorption rate and intensity were assessed by using rhodamine-labeled EGFP plasmid. Results showed that incubation of spermatozoa with plasmid in 37ºC leads to more transfection rate but various incubation media and presence or absence of FBS had no significant effect on DNA uptake rate and intensity. Motility rate and capacitation of sperms had no significant effects too. However, in sperms with a damaged membrane, the DNA uptake rate increased significantly. All of the spermatozoa with true DNA absorption (post acrosome absorption) were immotile and none of the examined treatments in this study could produce motile transfected spermatozoa. Regarding the results of this study, it seems that membrane-damaged spermatozoa incubated with foreign DNA can be used for SMGT-ICSI to produce transgenic animals.

Key words: Ovine, SMGT, sperm, transfection, incubation conditions

[*] **Corresponding Author**: Khosro Hoseini Pajooh, Assistant Professor, Department of Biotechnology, Iranian Research Organization for Science and Technology, Tehran-Iran, Iran, E-mail: pajooh@irost.ir

Refrences

Anzar, M., & Buhr, M. (2006). Spontaneous uptake of exogenous DNA by bull spermatozoa. *Theriogenology, 65*(4), 683-690 .

Arias, M. E., Sanchez-Villalba, E., Delgado, A., & Felmer, R. (2017). Effect of transfection and co-incubation of bovine sperm with exogenous DNA on sperm quality and functional parameters for its use in sperm-mediated gene transfer. *Zygote, 25*(1), 85-97. doi:10.1017/s096719941600037x

Atkinson, P. W., Hines, E. R., Beaton, S., Matthaei, K. I., Reed, K. C., & Bradley, M. P. (1991). Association of exogenous DNA with cattle and insect spermatozoa in vitro. *Molecular Reproduction and Development, 29*(1), 1-5 .

Blash, S., Melican, D., & Gavin, W. (2000). Cryopreservation of epididymal sperm obtained at necropsy from goats. *Theriogenology, 54*(6), 899-905.

Buhr, M. M., Canvin, A. T., & Bailey ,J. L. (1989). Effects of semen preservation on boar spermatozoa head membranes. *Gamete Research, 23*(4), 441-449. doi:10.1002/mrd.1120230409

Cabrera, M., Chan, P., Kalugdan, T., & King, A. (1997). Transfection of the inner cell mass and lack of a unique DNA sequence affecting the uptake of exogenous DNA by sperm as shown by dideoxy sequencing analogues. *Journal of Assisted Reproduction and Genetics, 14*(2), 120-124. doi:10.1007/bf02765781

Camaioni, A., Russo, M., Odorisio, T., Gandolfi, F., Fazio, V., & Siracusa, G. (1992). Uptake of exogenous DNA by mammalian spermatozoa: specific localization of DNA on sperm heads. *Reproduction, 96*(1), 203-212 .

Canovas, S., Gutierrez-Adan, A., & Gadea, J. (2010). Effect of exogenous DNA on bovine sperm functionality using the sperm mediated gene transfer (SMGT) technique. *Molecular Reproduction and Development, 77*(8), 687-698. doi:10.1002/mrd.21205

Eghbalsaied, S., Ghaedi, K., Hosseini, S., Tanhaie, S., Forouzanfar, M., Hajian, M., . . . Nasr Esfahani, M. (2009). Selection of the Most Appropriate Medium for Assessing Motility and DNA Uptake of Bovine Spermatozoa. *Yakhteh Medical Journal,, 10*(4), 266-271 .

Eghbalsaied, S., Nasr-esfahani, M. H., Ghaedi, K., & Mozafari, N. (2008). Lipofectamine Does Not Enhance Bovine Sperm Transfection Efficiency *Yakhteh Medical Journal,* 10(1),48

Gandolfi, F. (1998). Spermatozoa, DNA binding and transgenic animals. *Transgenic Res, 7*(3), 147-155 .

García-Vázquez, F., Gutiérrez-Adán, A., & Gadea, J. (2009). Evaluation of binding sperm-exogenous DNA in ejaculate and epididimary porcine spermatozoa. *Archivos de Medicina Veterinaria, 41*(2), 131-138 .

García-Vázquez, F. A., García-Roselló, E., Gutiérrez-Adán, A., & Gadea, J. (2009). Effect of sperm treatment on efficiency of EGFP-expressing porcine embryos produced by ICSI-SMGT. *Theriogenology, 72*(4), 506-518.

Gordon, J. W., & Ruddle, F. H. (1981). Integration and stable germ line transformation of genes injected into mouse pronuclei. *Science, 214*, 1244-1246.

Hassanane, M. S ,.El Makawy, A. I., Helalia, S. M., Abdoon, A. S., Khalil, K. M. A., Ghanem, T. A., . . . Shen, W. (2017). First study of sperm mediated gene transfer in Egyptian river buffalo. *Journal of Genetic Engineering and Biotechnology, 15*(2), 475-482.

Holt, W. V., & North, R. D. (1984). Partially irreversible cold-induced lipid phase transitions in mammalian sperm plasma membrane domains: Freeze-fracture study. *Journal of Experimental Zoology, 230*(3), 473-483.

Horan, R., Powell, R., McQuaid, S., & al., e. (1991). *Archives of Andrology, 26*, 83-92 .

Hoseini pajooh, K., Tajik, P., Karimi poor, M., & Behdani, M. (2014). Tranfection of ram spermatozoa with pEGFP carrying human lysozyme gene. *Journal of Veterinary Resarch, 69*(3), 245-254 .

Hoseini Pajooh, K., Tajik, P., Karimipoor, M., & Behdani, M. (2016). Techniques for augmentation of exogenous DNA uptake by ovine spermatozoa. *Iranian Journal of Veterinary Research, 17*(1), 25-30 .

Hoseini Pajooh, K., Tajik, P., & Karimipour, M. (2014). Dynamics of interaction between ram sperm with plasmid carrying human lysozyme gene in SMGT. *Journal of Comparative Pathobiology, 11*,(3), 1331-1344 .

Kadze, R., Chan, P .J., Jacobson, J. D., Corselli, J. U., & King, A. (2002). Temperature variable and the efficiency of sperm mediated transfection of HPV16 DNA into cells. *Asian journal of andrology, 4*(3), 169-173 .

Lavitrano, M., Camaioni, A., Fazio, V. M., Dolci, S., Farace, M. G., & Spadafora, C. (1989). Sperm cells as vectors for introducing foreign DNA into eggs: genetic transformation of mice. *Cell, 57*(5), 717-723 .

Lavitrano, M., Forni, M., Bacci, M. L., Di Stefano, C., Varzi, V., Wang, H., & Seren, E. (2003). Sperm mediated gene transfer in pig: Selection of donor boars and optimization of DNA uptake. *Molecular Reproduction* and *Development, 64*(3), 284-291.

Lavitrano, M., French, D., Zani, M., Frati, L., & Spadafora, C. (1992). The interaction between exogenous DNA and sperm cells. *Molecular Reproduction* and *Development, 31*(3), 161-169 .

Li, L., Shen, W., Min, L., Dong, H., Sun, Y., & Pan, Q. (2006). Human lactoferrin transgenic rabbits produced efficiently using dimethylsulfoxide-sperm-mediated gene transfer. *Reprod Fertil Dev, 18*(6), 689-695.

Pereyra-Bonnet, F., Fernández-Martín, R., Olivera, R., Jarazo, J., Vichera, G., Gibbons, A., & Salamone, D. (2008). A unique method to produce transgenic embryos in ovine, porcine, feline, bovine and equine species. *Reproduction, Fertility and Development, 20*(7), 741-749.

Pereyra-Bonnet, F., Gibbons, A., Cueto, M., Sipowicz, P., Fernandez-Martin ,R., & Salamone, D. (2011). Efficiency of Sperm-Mediated Gene Transfer in the Ovine by Laparoscopic Insemination, In Vitro Fertilization and ICSI. *Journal* of *Reproduction* and *Development, 57*, 188-196.

Perry, A. C. F., Wakayama, T., Kishikawa, H., Kasai, T., Okabe, M., Toyoda, Y., & Yanagimachi, R. (1999). Mammalian Transgenesis by Intracytoplasmic Sperm Injection. *Science, 284*(5417), 1180-1183. doi:10.1126/science.284.5417.1180

Rubessa, M., Lotti, S. N., Kandel, M. E., Popescu, G., & Wheeler, M. B .(۲۰۱۹) .SLIM microscopy allows for visualization of DNA-containing liposomes designed for sperm-mediated gene transfer in cattle. *Molecular Biology Reports, 46*(1), 695-703.

Sanchez-Villalba, E., Arias, M. E., Loren, P., Fuentes, F., Pereyra-Bonnet, F., Salamone, D., & Felmer, R. (2018). Improved expression of green fluorescent protein in cattle embryos produced by ICSI-mediated gene transfer with spermatozoa treated with streptolysin-O. *Anim Reprod Sci, 196*, 130-137. doi:10.1016/j.anireprosci.2018.07.005

Sciamanna, I., Piccoli, S., Barberi, L., Zaccagnini, G., Magnano ,A. R., Giordano, R., . . . Spadafora, C. (2000). DNA dose and sequence dependence in sperm-mediated gene transfer. *Molecular Reproduction* and *Development, 56*(2 Suppl), 301-305.

Shadanloo, F., Najafi, M. H., Hosseini, S. M., Hajian, M., Forouzanfar, M., Ghaedi, K., . . . Esfahani, M. H. N. (2010). Sperm status and DNA dose play key roles in sperm/ICSI-mediated gene transfer in caprine. *Molecular Reproduction and Development, 77*(10), 868-875.

Shannon, P., & Vishwanath, R. (1995). The effect of optimal and suboptimal concentrations of sperm on the fertility of fresh and frozen bovine semen and a theoretical model to explain the fertility differences. *Animal Reproduction Science, 39*(1), 1-10.

Simões, R., Binelli, M., Nicacio, A. C., Milazzotto, M. P., Feitosa, W. B., Visintin, J. A., & Assumpção, M. E. O. A. (2007). Use of spermatozoa as vectors of exogenous dna for in vitro production of bovine transgenic embryos. *Reproduction, Fertility and Development, 19*(1), 320-321.

Vasicek, D., Vasickova, K., Parkanyi, V., Rafay, J., & Ondruska, L. (2010). Effective generation of genetically modified rabbits by sperm mediated gene transfer. *World Rabbit Science, 15*(3), p. 161-166 .

Wang, H. J., Lin, A. X., & Chen, Y. F. (2003). Association of rabbit sperm cells with exogenous DNA. *Animal Biotechnology, 14*(2), 155-165 .

Wu, Y., Liu, C. J., Wan, P. C., Hao, Z. D., & Zeng, S. M. (2009). Factors affecting the efficiency of producing porcine embryos expressing enhanced green fluorescence protein by ICSI-mediated gene transfer method. *Animal Reproduction Science, 113*(1-4), 156-166

Zelphati, O., & F C Szoka, J. (1996). Mechanism of oligonucleotide release from cationic liposomes. *PNAS, 93*(21), 11493-11498 .

Zhao, Y., Yu, M., Wang, L., Li, Y., Fan, J., Yang, Q., & Jin, Y. (2012). Spontaneous uptake of exogenous DNA by goat spermatozoa and selection of donor bucks for sperm-mediated gene transfer. *Molecular Biology Reports., 39*(3), 2659-2664.