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ORIGINAL PAPER

Sperm mediated gene transfer in livestock

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INTRODUCTION

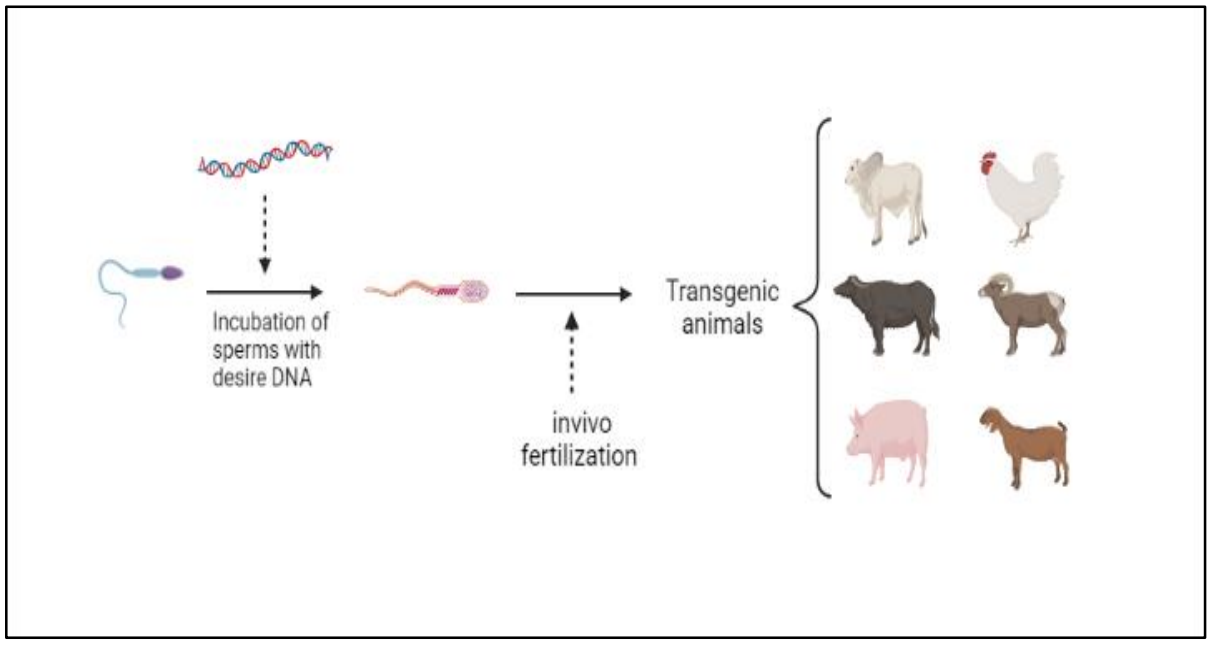
The sperm-mediated gene transfer (SMGT) as an alternate method for generating transgenic animals. As spermatozoa can absorb desired foreign genes as DNA molecules *in vitro*, they can transfer them to the oocyte during fertilization. A result of this process is the incorporation of new genetic material into an embryo's genome, resulting in a change in how some genes are expressed in offspring and subsequent generations. Spermatozoa have a very specialised and controlled method for absorbing DNA. Transgenesis methods for animals are currently being improved, but SMGT has been proven effective. Despite its shortcomings, the SMGT method offers significant biotechnology and medicinal potential. Multitransgenic animals could be used for xenotransplantation or as disease models in humans. The sperm mediated gene transfer technique in mammalian systems remains controversial, since sperm outside the reproductive tract cannot be effectively manipulated to be transfected. Since seminal fluid contains several inhibitors of exogenous DNA, removing sperm cells' natural protection medium introduces many variables which may affect its efficiency. These variability of these factors in addition to species variability are forced the researchers however to enhance some conditions before undergoing any SMGT experiment. Hence, the present review discuss different methods of SMGT used in different livestock species.

Methods of SMGT:

1. Testis Mediated Gene Transfer (TMGT)

The injection of transgene is done on the corner of the capus epididymis and the male are mated with normal female in order to transport the transgene from the testes of male to the oocyte of female for prodcution of transgenic offspring. TMGT is not cost effective, low technically demanded, not require special techniques and equipments, easily to be understood since everything is natural except the recombinant testes that

have the directly injected transgene. However, the transgene ability integration showed high incidence of mosaicism in some studies. Thus, this approach is not a good choice to produce transgenic livestock.



Methodology of sperm mediated gene transfer technique

2. Electroporation based SMGT

Simply, electroporation is a technique by which a series of short electric pulses are conducted by gene pulser device to generate transient pores in the cell membrane to allow the transgenes to enter the cells. These electrical induced pores have the ability to be resealed spontaneously to get the transfected cell back into its normal state. Thus, the purpose of introducing electroporation in SMGT is to enhance the rate of DNA uptake by sperm cells. There is several benefits of this method such as fast, large number of cells can be treated. Despite the ability of this technique in increasing the uptake of exogenous DNA but the increased electrical field strength had a deleterious effect on cell and spermatozoa.

3. Linker based SMGT (LB-SMGT)

In this method, special molecules, such as antibodies, peptides, and proteins are connected with exogenous DNA to form complexes able to penetrate cellular membrane through receptor mediated endocytosis pathway. There are several manufactured peptides which have ability to play crucial role in this approach. The most popular peptides are cationic peptides. In few studies, positively charged monoclonal antibodies are bonded them with DNA through ionic interactions, which is another incredibly intriguing use of linker-based SMGT. Hence, linker-based SMGT can be used to generate transgenic animals efficiently in many different species, especially in the farm livestock.

4. Retroviral based SMGT

In this method, lentivirus is used as a vehicle to facilitate the delivery of the exogenous DNA into the sperm cells. The main advantages of using this method is arise from the stability of the integration of the viral genome into the host and to the technical feasibility of introducing a virus to embryos at several developmental stages. These vectors are particularly characterized by their ability to be applied as suitable gene vehicles in that they infect a variety of cell types and introduce genes at high efficiency. The ability of retroviruses to be integrated naturally into target cell genome provides a powerful tool for stable transfer of the gene of interest.

5. Liposome based (lipofection) SMGT

In order to enhance the entry of exogenous DNA inside the sperm head, another intriguing strategy is represented by the use of liposomes. A DNA-liposome complex is created by combining cationic liposome with the desired transgene. After that, sperm cells are added to the resulting mixture and incubated. In order to generate recombinant sperm, these complexes must be able to enter sperm cells due to the sperm cells' ability to be fusogenic. Liposomes that made up of cationic lipids can interact with the negatively charged nucleic acid molecules to form complexes forcing the nucleic acid to be associated with their structures. This technique is simple, easy to use and low toxicity, in addition to their ability to protect the DNA from degradation.

6. Restriction enzyme mediated integration SMGT (REMI-SMGT)

REMI, or restriction enzyme-mediated integration, transforms cells using a combination of plasmid DNA that has been linearized by a restriction enzyme which can produce compatible cohesive ends in the genome.This mechanism can be simplified by incubation of transgene located within a circular vector with its corresponding restriction enzyme.After digestion of circular DNA, its linear counterpart is produced. The linearized transgene and the same enzyme then incubated with liposome to pass the transgene and its corresponding enzyme through the cell membrane of the sperm cell..However,further studies are needed to validite this particular approach.

CONCLUSION

SMGT usually can be simplified by the incubation of either frozen or freshly collected sperm cells with, for however short period of time with DNA. During this time the exogenous DNA may penetrate the sperm cells. Animal traits can be changed by transgenesis technology, which directly modifies the genetic code. Generally speaking, it is a process whereby a gene or a portion of a gene from one individual is incorporated into the genome of the other.. The resultant transfected sperm are introduced into oocytes either *in vivo* or *in vitro*.The major benefits of the SMGT technique were found to be its high efficiency, low cost and ease of use compared with other methods. Furthermore, SMGT does not require embryo handling or expensive equipment. Sperm-mediated gene transfer could also be used to generate multigene transgenic animals that

would be of benefit as large animal models for medical research, for agricultural and pharmaceutical applications and, in particular, for xenotrans- plantation, which requires extensive genetic manipulation of donor pigs to make them suitable for grafting to humans. Hence, SMGT will become a core area to produce transgenic animals in future.