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# Sperm Mediated Gene Transfer in Mammals; a Versatile Platform with Multiple Enhancements Techniques

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**Abstract**

In the field of animal transgenesis, many attempts have been made recently to simplify facilitate, and reduce the cost and labor required to do such tasks. Although several transgenesis techniques (such as DNA microinjection and somatic cell nuclear transfer) have been applied successfully to produce transgenic animals, these traditional techniques are so tedious and have several disadvantages. Retroviral mediated gene transfer has solved some of these usual problems but has, however, inevitable disadvantages represented most prominently by its biological hazard. Many researchers found that the most simple and non-cost effective way to produce transgenic animals is to focus on the natural ability of the sperm to "carry" the foreign DNA and to "fertilize" the oocyte. The most important breakthrough obtained in this aspect is the accumulated information that demonstrated the ability of foreign DNA to be internalized into the sperm head after simple incubation step. Accordingly, the only manipulation step is restricted into the head of the sperm. Then, nature will be allowed to fulfill its scheduled task of reproduction. This method known as sperm mediated gene transfer or SMGT. However, simple incubation of naked DNA with sperm head is not efficient enough to integrate the foreign DNA into the genome of the sperm. Thus, this review aims to pave the way for every effort to enable the researchers to undergo the transgenesis experiments in the routine laboratories. This is potentially can be done by testing the validity of the most modern enhancement approaches suggested on the original SMGT.

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Transgenesis is a dramatic line of technology for altering the characteristics of animals by directly modifying the genetic material. In general, it is as a procedure by which a gene or part of a gene from one individual is incorporated in the genome of the other one (Montaldo, 2006). It can be identified as a merely transfer of an exogenous gene into a host genome (Bacci, 2007). However, whatever the technique used to generate the transgenic animal, the general goal of transgenesis remain the same, which is "to add foreign genetic information to a genome" (Houdebine, 2003).

The generation of transgenic animals is a cumbersome process and remains problematic both in its methodology and impact (Dyck *et al.*, 2003). However, there are several methods and modern approaches of inserting a transgene in the mammals have been reviewed by many researchers, each review report an individual pattern of mammalian transgenesis (Bacci, 2007; Melo *et al.*, 2007; Wolf *et al.*, 2000), but, here are examples of the most currently used techniques in mammalian transgenesis such as pronuclear microinjection, retroviral mediated gene transfer, somatic cell nuclear transfer, and ovary mediated gene transfer but a special emphasis will be applied here on the simplest and the less cost effective technique, which is sperm mediated gene transfer (SMGT).

Just to think in the idea of simple incubation of sperm cell with the exogenous DNA may generate transgenic animal is very interesting for anyone who desire to generate transgenic animals. The original idea of sperm mediated transgenesis is simple, in which, just incubation of an ejaculated sperm cells with the exogenous DNA is however sufficient to transfect these sperm, then all the other steps are mimicry to the nature. Thus, this method is the simplest one in such away all manipulation steps are sometimes restricted to transfect sperm cells, and then researchers in this field will wait nature to fulfill its duty (Wall, 2002; Lavitrano *et al.*, 2006).

Practically, SMGT usually can be simplified by the incubation of either frozen of freshly collected sperm cells with, for however short period of time, the exogenous DNA suspension at 37 to 39°C in a suitable fertilization medium. During this time the exogenous DNA may penetrate the sperm cells (figure 1). The resultant transfected sperm are introduced into oocytes either *in vivo* or *in vitro* (Wheeler and Walter, 2001).

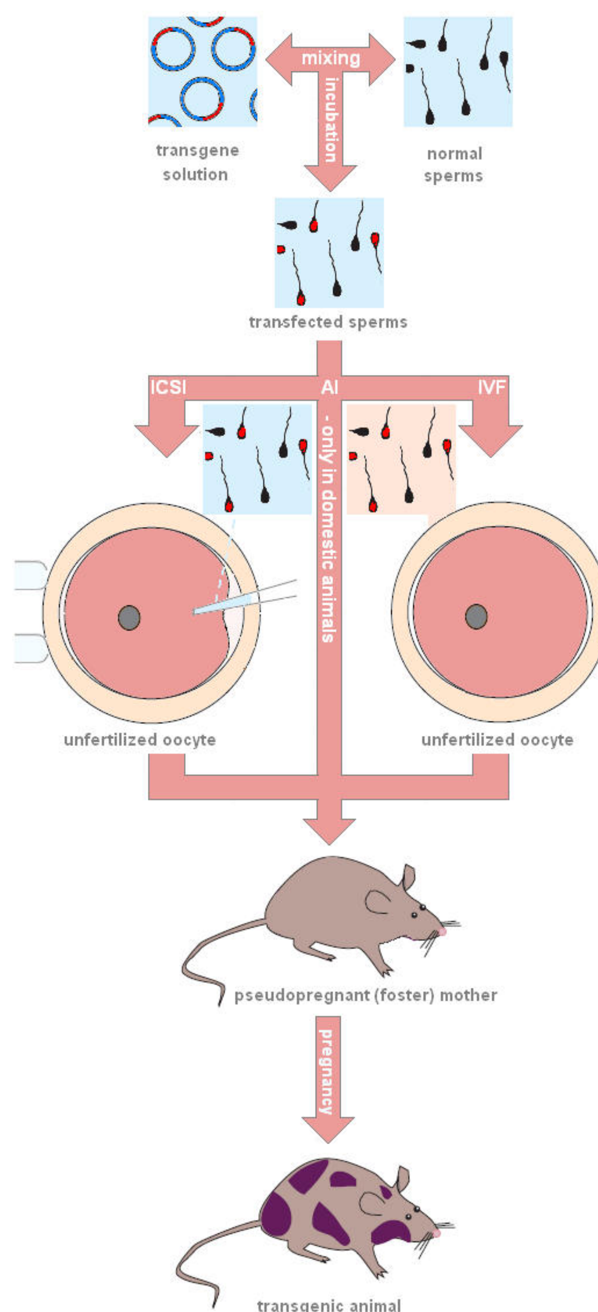


**Figure (1);** Illustration of sperm mediated gene transfer in transgenic animals. Simple incubation of sperm cells with exogenous DNA may permit the DNA to be inserted into the sperm cell. Then this sperm which hold this foreign DNA is used to fertilize oocytes either in vitro, such as in IVF, ICSI, or in vivo, such as in AI.

Traditional SMGT experiments are potentially characterized by lack of reproducibility (Wall, 1999). However, the sperm mediated gene transfer technique in mammalian systems is still a matter of controversy, since the viability of sperm outside the reproductive tract is not big enough to be manipulated efficiently to be transfected adequately (Sarmasik, 2003).

Since seminal fluid contains many inhibitors of exogenous DNA, the removing of sperm cells natural protection medium introduces many variable factors which may contribute into the efficiency of this technique (Niu and Liang, 2008). These variability of these factors in addition to species variability are forced the researchers however to enhance some conditions before undergoing any SMGT experiment.

The process of exogenous DNA integration into the sperm head is very crucial step (Celibi *et al.*, 2003). Thus, toward this step the sights of scientists are directed. Many research groups utilized many enhancement approaches to overcome the low and non-reproducible results of SMGT.

Several enhancements have been made in the original SMGT method (figure 2). These enhancement approaches have variable complexity ranging from just simple chemical reaction to the sophisticated steps that require special skills and devices such as intracytoplasmic sperm injection (ICSI) and artificial chromosomes (Chan *et al.*, 2000; Lai *et al.*, 2001; Moreira *et al.*, 2004; Osada *et al.*, 2005). But later have never reduced the cost of transgenesis technique since it utilizes micromanipulators. This in turn, may not represent the main purpose from which SMGT was developed, which is to reduce the cost, time and labor, that's why they may not deserve special attention (Lavitrano *et al.*, 2006). In this review, seven SMGT approaches are highlighted as most significant enhancements of SMGT and as follows:

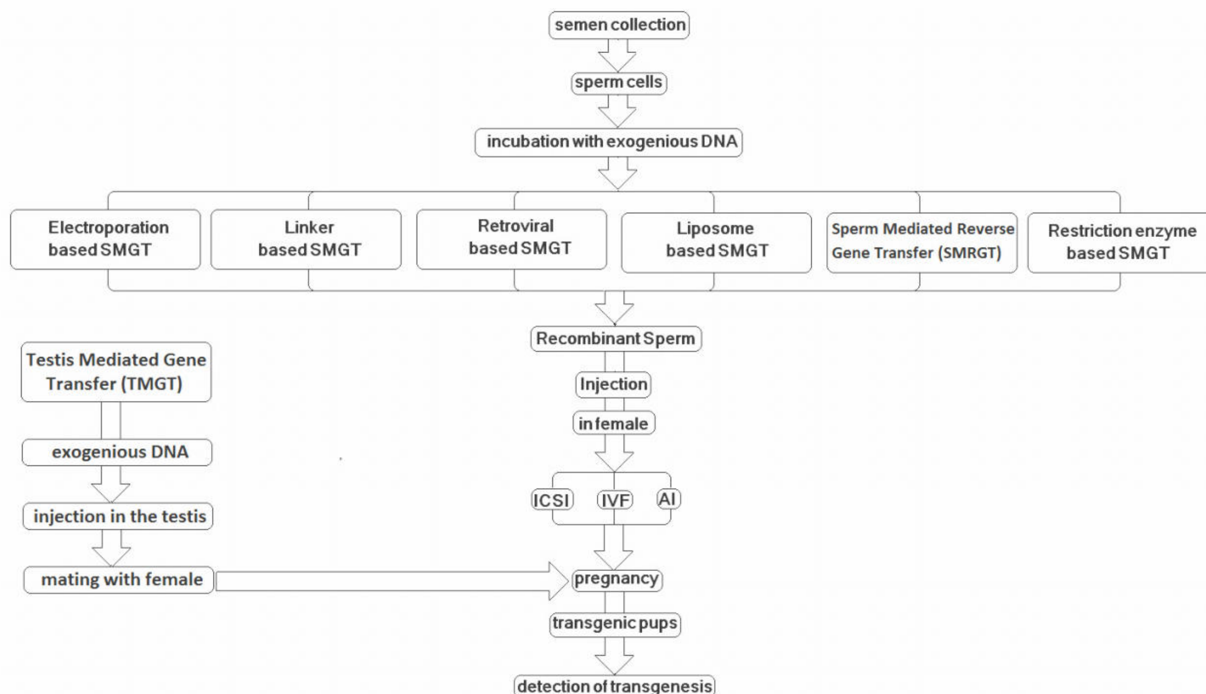


**Figure (2)**: shows the details of the most notable types of SMGT which they increased the efficiency of gene transfer through sperm vector to a significant extent. The variable routes of transgenesis that be taken place are per se differ in their efficiencies.

### 1. Testis Mediated Gene Transfer (TMGT)

Some scientists describe TMGT as an alternative and independent technique from SMGT (Niu and Liang, 2008), but others consider it as just a modification or simple variaiton of it (Collares et al., 2005; Lavitrano et al., 2006), because, in both cases, sperm cells are undertaking the process of gene transfer. The mechanism of TMGT is still under development but it can be simplified by direct injection of the transgene by a microsyringe into testes (figure 3).

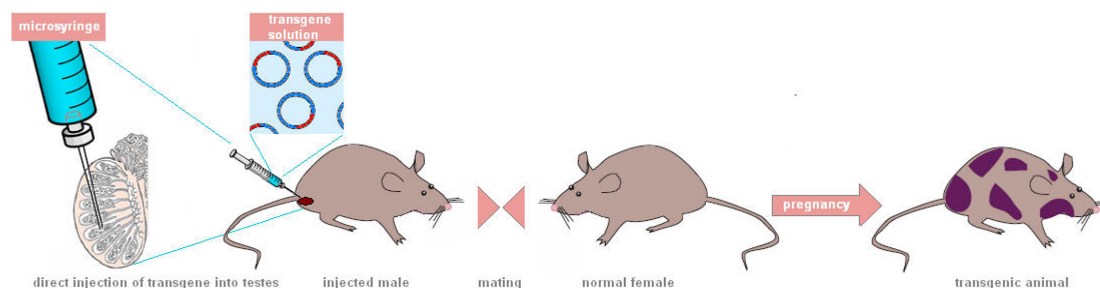


**Figure (3);** Illustration of testis mediated gene transfer (TMGT) technique. The injection of transgene is done on the corner of testes near the capus epididymis to a depth 5-6 mm. then the mice male that have the recombinant

 

gene are mated with normal female in order to transport the transgene from the testes of male to the oocyte of female. After natural mating followed by pregnancy period, the potentially expected transgenic offspring are generated.

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 (Niu and Liang, 2008; Yi-dong and Shu-long, 2008). Consequently, it become obvious that TMGT, as a derivative of the original SMGT, provides extreme simplicity compared with other SMGT derivatives represented by the absence of need to any manipulations either in sperm neither in fertilization (Celebi *et al.*, 2003).

The success of many papers in producing transgenic offspring having the transgene by TMGT doesn't mean that this method has an explicit ability on integrating the transgene into the genome. Rather, Celebi *et al* (2002) discovered the episomal state of the transgenic mice who generated by this method. This suggests that circle plasmid they injected is diluted out along with cell proliferation (Sato, 2005). Add to that, even the transgene ability to be integrated, high incidence of mosaicism has been observed (Yonezawa *et al.*, 2001).

Thus, this approach is still in its infancy and further improvement of TMGT itself is required. As for transgenic livestock generation, however TMGT is not a method of choice to produce livestock's animals (table 3).

**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 (figure 4). These electrical induced pores have the ability to be resealed spontaneously to get the transfected cell back into its normal state (Khan, 2010). Thus, the purpose of introducing electroporation in SMGT is, per se, to enhance the rate of DNA uptake by sperm cells (Lavitrano et al., 2006; Reith et al, 2000).

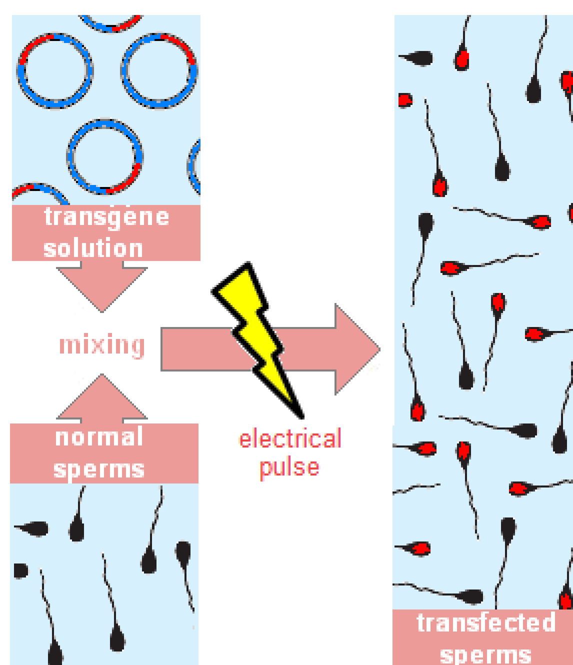


**Figure (4);** Illustration of electroporation based SMGT technique. Sperm cells are infected with the transgene solution with the aid of electrical pulse applied from an electroporator device.

There is several benefits of this method which can be considered as "significant" such as the method is fast, less costly then microinjection and SCNT, large number of cells can be treated, and, however, high percentage of transfected sperm cells can be produced (Khan, 2010). Several papers demonstrated the ability of electroporation to increase DNA integration ratio in the DNA of spermatozoa (Wall, 2002). Add to that, several researchers assured the feasibility, efficiency and the promising future of this method (Patil and Khoo, 1996; Heller *et al.*, 1996; Nishi *et al.*, 1996; Wall, 2002; Celebi *et al.*, 2003).

This method, which it also named "electrogene therapy", is a safe method because it does not require the viral vehicles, consequently, there is a high and promising ratio to apply this method on gene therapy (Nishi

*et al.*, 1996). In the same time, it was noted that this method of gene transfer may avoid several limitations and low transfection efficiency noticed in other methods (Heller *et al.*, 1996).

Despite the ability of this technique in increasing the uptake of exogenous DNA to spermatozoa and its increased efficiency in SMGT (Muller *et al.*, 1992), but, the increased electrical field strength had a deleterious effect on cell motility, causing clumping of spermatozoa at high voltages, so, this method require a careful optimization before its procedure is taken place (Gandolfi, 1998). Nevertheless, tremendous embryo lethality – despite its high transfection efficiency – is an usual consequences of this technique (Sciamanna *et al.*, 2000).

Financially, commercially available electroporators still cost effective (more then 40 000 $). Their high cost makes these devices unavailable at any routine labs.

### 3. Linker based SMGT (LB-SMGT)

In this approach, researchers used special molecules can be recognized by cellular receptors, such as antibodies, peptides, and proteins. They connected with exogenous DNA to form complexes able to penetrate cellular membrane through receptor mediated endocytosis pathway (Varga et al., 2000).

There are several manufactured peptides which have potential ability to play crucial role in this approach (Shwatrz et al., 1999). The most popular peptides are cationic peptides; the peptides rich in positively charged amino acids such as lysine and argentine since they counteract the negative charge of DNA molecules. This neutralization of the DNA charge abolishes the repulsion forces in DNA and packs it closely (Khan, 2010).

Another extremely interesting utilization of linker based SMGT came from the work done by Chang et al. (2002), they used positively charged monoclonal antibody and bound it with DNA through ionic interactions (figure 5). The antibody used by this group in mice and pigs can be recognized by sperm cells receptors of other mammalian species in precise manner (Epperly, 2007).

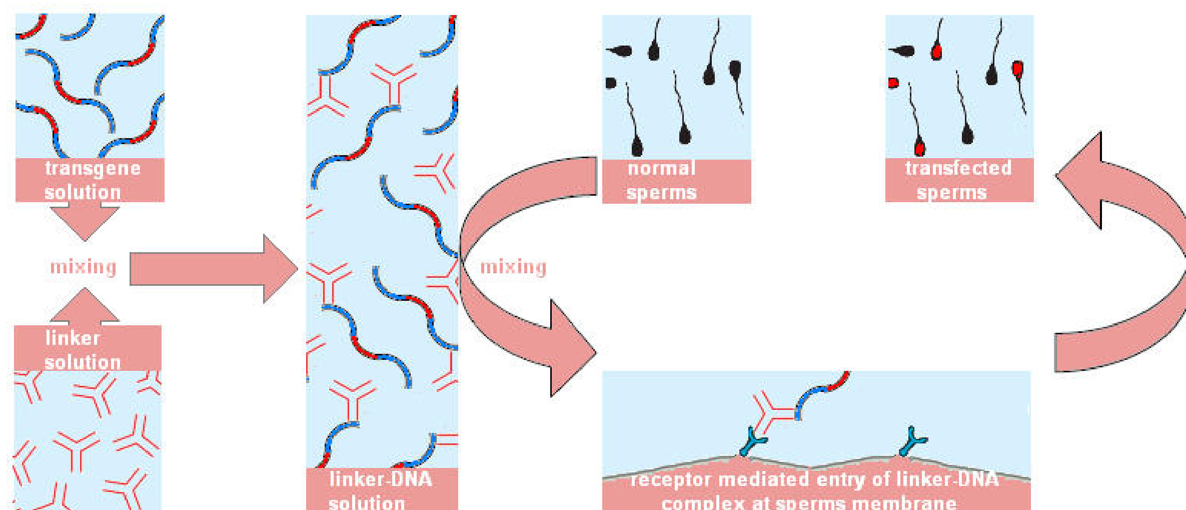


**Figure (5)** postulated illustration of linker based SMGT; after the binding of linker, such as antibody, with transgene it is recognized and internalized by specific receptors found at the surface of sperm cells.

Chang and his group (2002) firmly demonstrated that linker-based SMGT can be used to generate transgenic animals efficiently in many different species, especially in the farm livestock (Chang *et al.*, 2002). While others supported this results considering it as an effective way to improve the efficiency of SMGT (Epperly, 2007).

Very little papers concerning this approach are made till now, so, it is still not clear how far this technique is applicable keeping in mind the applicability of using "a common ligand" which has the ability to recognize the variable receptors in variable species. Therefore linker based SMGT is not universal and many papers shall be made to authenticate its versatility.

### 4. Retroviral based SMGT

One of the most promising areas used in the enhancement of the original SMGT is the retroviral based method. In this method, the most important derivative of retrovirus, e. g. lentivirus is used as a high efficient vehicle to facilitate the delivery the exogenous DNA into the head of the sperm cells (figure 6). Recently, some reviewers shed light on several useful retrovial based approaches that have been applied on SMGT (Niu and Liang, 2008).

The main advantages of using RMGT 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 (Khan, 2010; Wheeler and Walter, 2001). 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 (Stuhlman et al., 1984). The ability of retroviruses to be integrated naturally into target cell genome provides a powerful tool for stable transfer of the gene of interest (Chan, 1999). It makes gene transfer possible for species from which newly fertilized eggs cannot be readily obtained (Sarmasik, 2003).

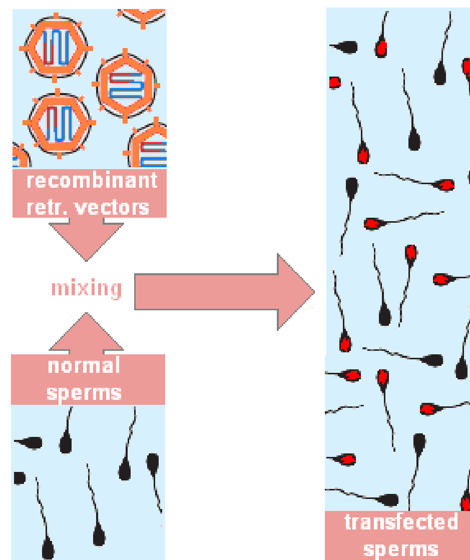


**Figure (6);**Retroviral based SMGT. This illustrated step is represented by infecting sperm cells with recombinant retroviral vectors.

In contrary with pronuclear microinjection which is very inefficient in livestock, RMGT has two interesting advantages make it very appealing for use in livestock. The first one, only a fraction of the resources needed for conventional pro-nuclear injection would be required, while the second is the simplicity of delivery, abolishing the need for specialized equipment (Whitelaw, 2003). Furthermore, Molecular genetic analysis of transgenics produced by RMGT usually show integration of a single proviral copy into a given chromosomal site, and the rearrangements of the host genome are normally confined only to the short direct repeats at the site of integration (Pease and Lois, 2006), while in pronuclear microinjection the transgene may integrate in a more randomized manner (Auerbach, 2004). In addition, the method is less invasive to the embryos, and technically less demanding. Delivering lentiviruses by co-incubation with denuded embryos obviates the need for micromanipulation and may be an easier option for many laboratories wanting to make transgenic animals. Furthermore, since the lentiviral delivery technique does not require visualization of the pronucleus, it has the potential to be extended to diverse mouse strains, as well as other animal species (Pease and Lois, 2006).

Despite many advantages that characterize RMGT, but nobody can demonstrate that this technique is “absolutely” the best one among other transgenesis technique (Wall, 2002). That’s, *per se*, because of several disadvantages of RMGT.

There are, however, disadvantages with the utilization of retroviruses in animal transgenesis; These disadvantages include: 1) the size of DNA to be transferred is limited by size, 2) the inserted gene don’t has the ability to express on itself in the second generation, which may, in turn, complicate the method and 3) many transgenics are mosaic, with potentially multiple insertion sites. (Wheeler and Walter, 2001), (4) Add to that, the biohazard emerged during the practical dealing retroviruses which put in mind several safety concerns (Cornetta *et al.*, 1991).

The capacity of retroviral vectors to carry the transgene is not enough to provide the space required to transfer the DNA fragment wanted in transgenesis (Thomas *et al.*, 2003). Size restriction imposed by the lentiviral genome represents the most obstacles toward using this virus as vehicles for gene transfer. In such away wild-type lentiviruses have a genome of about 8 kb, and the genetic load of these viruses (comprising the internal promoter, transgene and enhancer elements) should therefore be less than this size (Fassler, 2004).

The second potential disadvantage of RMGT is the complexity of the process as a consequence of the absence of transgene expression (Rhicahrd and Huber, 1993). Though “introducing” viral particles to oocytes

requires the least complicated embryo manipulation, but the packaging transgenes into virions takes many steps. For any gene transfer approach taken place through RMGT the transgene, both its structural and regulatory portions, must be built properly before proceeding to the next steps. Then the transgene must be introduced into the proviral genome by standard molecular cloning methodologies. The modified proviral genome is then transfected into the packaging cells, and the packaging cells should be grown to produce the recombinant viruses (Wall, 2002). Thus, the preparation of retroviral particles including the transgene of interest is a very laborious process, which may increase costs and requires more sophisticated technology (Sarmasik, 2003).

Generally, transgenic animals are generally mosaic and the trasngenes are not always expressed in the second generation (Dyck *et al.*, 2003). Infection of early embryos with retroviral vectors resulted in genetic mosaics represented by multiple insertion sites in different tissues (Chan *et al.* 1998). Retroviruses sometimes integrate within genes, which become inactivated. Repeated inserted sequences also modify gene activity when they are in their vicinity or within those genes (Houdebine, 2003).

The safety problems associated with retroviral vectors would not be omitted with respect to the disadvantages of RMGT (Temin, 1990; Cornetta *et al.*, 1991), since in many cases, cell culture systems used for production of replication-defective retroviral vectors may eventually produce replication-competent retroviruses after varying periods of incubation, because of the recombination of vector with helper viral sequences (Gunter *et al.*, 1993). Through history, several wise improvements of genetic manipulation of the lentivirus genome would ensure that the resultant vector would have a very high level of safety (Reeves and Cornetta, 2000; Kelly and Rushell, 2007), but, one would have to ask whether the current basic scientific understanding of retroviruses is sufficiently advanced to empower rational vector design (Smith, 2004).

Recently, Klymiuk and his colleges developed new genetic engineering strategies to reduce the biohazard of these natural vehicles (Klymuik *et al.*, 2010). But the potential problem still exist in terms of the long terminal repeats (LTRs); the flanking sequences the transgene of the recombinant retroviral genomes which have been reported to interfere with mammalian promoters, suppressing or misdirecting expression (Wolf *et al.*, 2000), or may lead to inavtivation of tumor suppressor genes or activation of proto-oncogenes (Ponder, 2001). This, in turn, makes the transgenic animals more susceptible to develop tumor (Harper *et al.*, 2004).

Some researchers are aware of the unwanted recombination event between the sequences of expression vector and a related sequences present in the same transgenic animal. If this taken place the pathogenic viruses are formed (Hellerman, 2002). While other researchers suggested to delete all the retroviral sequences in many experiments to ensure the safety of the process (Richards and Huber, 1993)!

### 5. Sperm-mediated "Reverse" Gene Transfer (SMRGF)

The mode of communication that naturally exist between sperm and its corresponding oocyte is not a random phenomenon, rather, it's precisely regulated process contributed by several factors, one of these factors is endogenous reverse transcriptase (Spadafora, 2008).

The interaction of exogenous molecules triggers an endogenous reverse transcriptase activity in spermatozoa. This activity reverse transcribe's exogenous RNA molecules (specifically, the human poliovirus RNA genome) into cDNA copies, which are transferred to embryos following IVF (Giordano et al., 2000).

Thus, Smith and Spadafora (2005) have called this phenomenon ''sperm-mediated reverse gene transfer'' or SMRGT. The discovery of functional RT in sperm cells provides the basis for SMRGT: in this process, the exogenous RNA is probably ''captured'' by the retrotransposon-mediated mechanism active in sperm cells, reverse-transcribed, further propagated through the embryo as non-integrated structures in tissues of founder individuals and transmitted to F1 progeny. It is demonstrated that reverse-transcribed sequences behave as functional genes, being correctly expressed in tissues of F0 and F1 animals (Pittoggi et al., 2006).

The role of this enzyme in SMGT is illustrated in model suggested by Smith and Spadafora (2005) and modernized three years later (Spadafora, 2008).

SMRGT is one of the few interesting mechanisms in finding a way to the ex-gene to be internalized into the genome. This event, if it is proved to occur in nature, it will has wide implications to human health and to evolutionary processes (Collares et al., 2005).

After the reverse transcription of exogenous RNA, the resulting cDNA molecules are located in and extrachromosomal place, while the ability of this segment to be integrated into the genome is a rare event (Collares et al., 2005). Consistent with extrachromosomal habit of these molecules are the negative results of various attempts to identify integration of the reverse transcribed cDNA copies (Smith and Spadofora, 2005). The utilization of the cost effective intracytoplasmic sperm injection equipment reduce the applicability of this technique and made it not readily to be made in small budget labs.

### 6. Liposome based (lipofection) SMGT

Another interesting approach is represented by utilizing of liposomes in order to facilitate the entry of exogenous DNA inside the sperm head (Lai et al., 2001) see figure (7).

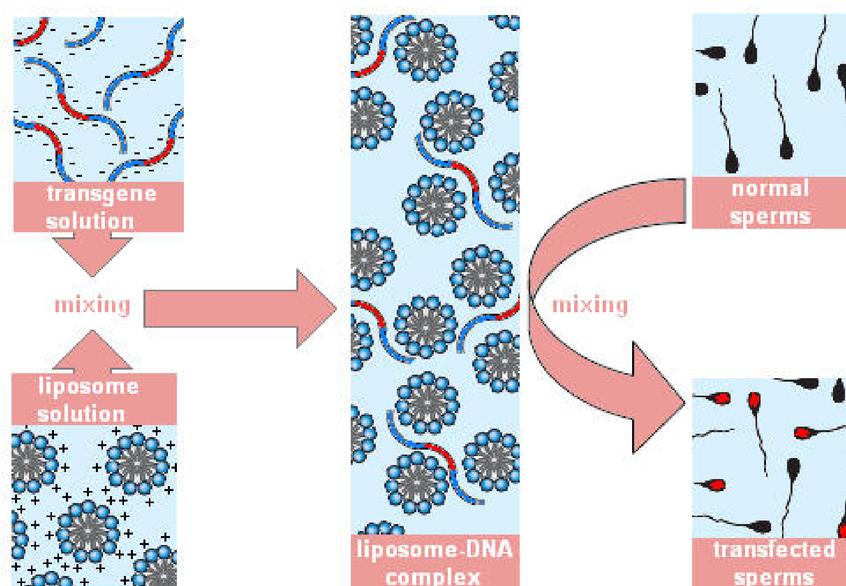


**Figure (7)**. Illustration shows liposome based SMGT. After mixing cationic liposome with the transgene of interest a complex of DNA – liposome is formed. Then, the resulting mixture is incubated with sperm cells for a while. During this process, the fusogenic nature of sperm cells is exploited for the penetration of such complexes inside the sperm cells to form recombinant sperm.

Currently available liposomes are spherical phospholipids vesicles, some of these structures have two faces hydrophilic head and hydrophobic tails, when the later moieties are used to associate with the hydrophobic moieties of the molecules to be transported, they tend to exclude water and encapsulate these molecules inside their structures (Kresina, 2001; Khan, 2010). But there is another type of liposomes known as cationic liposomes; they use ionic interactions or electrostatic attractions instead. These cationic liposomes are much more capable of being interacted with DNA compared with the uncharged counterparts (Reece, 2004). When the resulting complex is mixed with sperm cells in suitable solution (Figure6), such vesicles can fuse with the cell membrane and deliver DNA directly into the cytoplasm (Twyman, 2005).

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 (Niu and Liang, 2008). The most commercially known cationic liposomes are lipofectin or lipofectamine, DOTAP, and DOTMA (Invetrogen, Boehringer-Manheim, Evrogen). They are commonly used as transfection reagent in many gene transfer protocols.

Liposomes enjoy many features made them in many gene transfer protocols play very important role in the success of these experiments such as their simplicity, easy of use, long term storage and stability, low toxicity, in addition to their ability to protect the passenger DNA from degradation (Khan, 2010).

Despite the success rates came from the transfection of sperm head with several commercially available liposomes (Kim et al., 1997), they were unable alone to generate transgenic animal by this technique (Bachiller et al., 1991; Lai et al., 2001; Yonezawa et al., 2001).

It has been demonstrated significant reduction in sperm motility observed after treatment of murine sperm cells with liposome transfection reagent (Sasaki et al., 2000). The most potentially important factor which eliminates the affectivity of liposomes are represented by the lack of obvious ability of liposome to enable the transgene to integrate into the genome. Rather, it favors the episomal state (Kresina, 2008).

### 7. Restriction enzyme mediated integration SMGT (REMI-SMGT)

One method that proved to be of interest in species for which there is a need for a more powerful technique to increase the success of transgenesis is restriction enzyme mediated integration (REMI).

REMI SMGT is not a weird enhancement of the traditional SMGT but the combination of restriction enzymes made this technique very interesting with respect to the molecular mechanism by which the restriction enzyme enhance" the rate of integration.

This mechanism can be simplified by incubation of transgene located within a circular vector with its corresponding restriction enzyme; the enzyme that have only one sensitive site located out of the transgene sequence. After digestion of circular DNA, its linear counterpart is produced (figure8). The linearized transgene

 

and the same enzyme then incubated with liposome. The role of liposome here is just to pass the transgene and its corresponding enzyme through the cell membrane of the sperm cells (Sciamanna et al., 2000).

It is believed that once the exogenous DNA encounter the sperm genome its corresponding restriction begins to digest its sensitive sites that located on the hosting genome (figure 8), meanwhile the exogenous DNA will seize the opportunity in order to integrate itself into the genome of the sperm cell by cellular DNA repair mechanism.

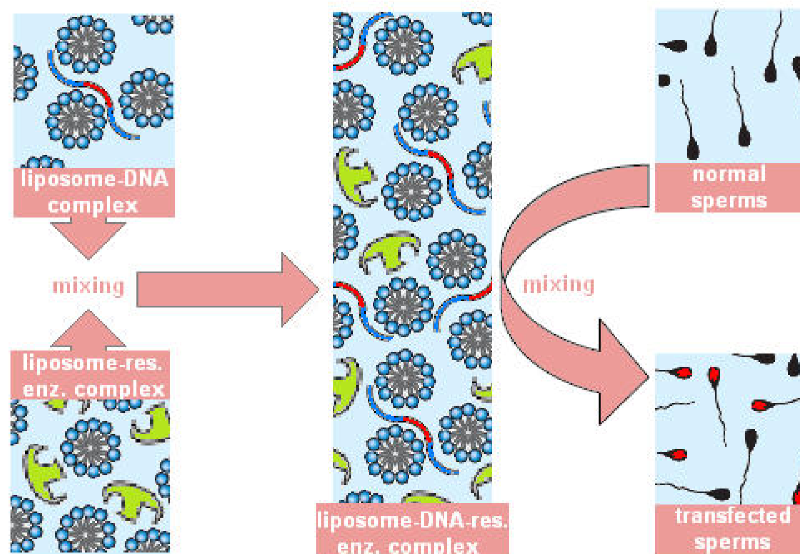


**Figure (8);** Speculated mechanism of restriction enzyme mediated integration sperm mediated gene transfer. The expected molecular mechanism of REMI SMGT is shown in the right portion of the figure represented in the nucleus of the sperm cell. In this mechanism, the corresponding restriction enzyme plays very important role in the integration of the transgene into the genome of the sperm, by mimicking a part of natural endogenous repair system.

The most advantageous feature of REMI may came from the fact that the foreign endonuclease that associated with foreign DNA have only one effect directed toward its genomic sensitive site rather than toward the linearized foreign DNA, this in turn confuse the host genome by potentially speculated repair mechanism by which the foreign DNA is integrated (Collares et al., 2005). In this cellular repair mechanism the host inserts the free cohesive ended foreign DNA within its original sequences. Consequently, the “natural repair machinery” of the host that has been exploited in order to enhance the rate on integration (Shemesh et al., 2000), still surrounded by some ambiguity.

Wall (2002) referred to the absence of any significant disadvantages in REMI SMGT. Nevertheless, despite the evident efficiency of several experiments that increases the rate of exogenous integration for several folds but this is not enough since there is a great necessity to repeat these experiments to make sure from the credibility of these results. However, the numbers of papers concerning REMI-SMGT is very little to judge how much this approach is efficient. Therefore further studies are in the way to elucidate much more details on the validity of this particular approach.

## Conclusion

Several enhancements have been made to increase the efficiency of this promising method such as using electroporation, linkers, retroviral vectors, and liposomes. But, according to many data, these approaches don’t have the molecular mechanisms that directly working on integrating the exogenous DNA during its incubation with sperm genomic DNA. Several researchers have further simplified SMGT by direct injection of foreign DNA into the testes of animals combined with electroporation or lipofection. Testis mediated gene transfer of TMGT, however, don’t have significant differences compared with the original SMGT because each of which relay’s upon sperm as a vehicles to carry the exogenous DNA. Thus, the problem of reduced integration still exists.

It has been reported that many enhancement approaches have increased the reproducibility of the original SMGT. Nevertheless, it becomes known to many researchers the obvious inefficiency of SMGT enhancement approaches to “integrate” the foreign DNA into the genome of the sperm. A surprising molecular trick that represented by implicating restriction enzymes in this arena has been made in SMGT. This trick has been made on SMGT by Israelite group at 2000 and 2009. This method is called restriction enzyme mediated integration SMGT or REMI-SMGT. But the unusual thing in this aspect is that nobody has tested the validity of this technique after this group. We think it is very necessary to see how much these technique are capable on

cheating the molecular repair mechanisms of sperm cell, since this tracking opens the door widely for more exploration of molecular manipulations of the sperm head for the sake of producing a transgenic animal with a minimum efforts and costs.

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