# Sperm-Mediated Transgenesis: Practical Implications of a Biological Process

Corrado Spadafora and Rodolfo Lorenzini

*Graft* 2001; 4; 68

# Sperm-Mediated Transgenesis: Practical Implications of a Biological Process

*Corrado Spadafora and Rodolfo Lorenzini*

Spermatozoa are traditionally regarded as metabolically inert cells because they lack most fundamental biochemical and molecular functions, such as DNA replication, gene expression and protein synthesis. This view has been somewhat corroborated by their peculiar morphology, characterized by an extremely reduced cytoplasmic compartment and a large nucleus containing the genomic DNA tightly packed in a condensed chromatin structure, connected to a long flagellum. These observations inspired the conclusion that the only possible role of these cells is to act as vectors of their own genome in fertilization.

An unexpected feature of spermatozoa was first revealed in the late 1970s. While probing the chromatin of sea urchin sperms with micrococcal nuclease, it was realized that chromatin degradation was triggered simply by adding the nuclease to the intact spermatozoa suspension.[1] That simple observation first suggested that foreign molecules can spontaneously penetrate through the plasma and nuclear membranes in close contact with the sperm chromosomal DNA. That original observation also suggested that spermatozoa may possibly be able to similarly take up exogenous DNA and transfer it to oocytes during fertilization, thereby acting as vectors not only of their own genome but also of foreign genes, disclosing a potential use in transgenesis.

In 1989, we published an article[2] reporting that:

- mouse epididymal spermatozoa can spontaneously take up plasmid DNA;
- genetically transformed offspring can be generated in in vitro fertilization (IVF) assays; and
- the exogenous DNA sequences are transmitted from founders to F1 animals.

That article aroused controversy within the scientific community when several groups reported their failure to reproduce our results.[3] In our reply, while confirming our original findings, we had to accept the evidence that transgenesis mediated by spermatozoa was not as simple and straightforward a process as we had originally thought.

Over the past ten years, our efforts have concentrated on clarifying the molecular steps of the sperm-mediated transgenesis process, starting with the earliest fundamental event, i.e., the interaction of foreign DNA with spermatozoa. Many other laboratories have developed further sperm-mediated transgenesis studies, showing that DNA can bind spermatozoa of virtually all species and hence can be transferred to oocytes, yielding genetically-modified animals with variable efficiencies. If fully developed, these protocols may allow the transformation of a large number of individuals, avoiding the demanding DNA microinjection in each zygote.

In this brief review, we summarize both published experiments of sperm-mediated transgenesis in mammals and results from basic studies of sperm/DNA interaction. The possible involvement of retroviral functions in this mechanism and their potential implications for xenotransplantation are discussed.

## Current State of Sperm-Mediated Transgenesis

Genetically-modified embryos and/or born animals of virtually all species, from Echinoids to Mammals, have been obtained by inducing the interaction of foreign DNA with spermatozoa. Here we will focus on work with mammals. For a review on sperm-mediated transgenesis in other species (see ref. 4).

Corrado Spadafora, Ph.D.
Institute of Biomedical Technology, CNR
Via G.B. Morgagni 30/E
00161 Rome, Italy
Tel.: 39.06.49903117 or
39.06.49902972
Fax: 39.06.49387120
email: cspadaf@tin.it

DNA injection in the vas deferens of mice has allowed the interaction between the exogenous DNA and spermatozoa to take place within the reproductive male tract and the ensuing generation of modified offspring.

The results obtained by adapting different protocols of sperm-mediated transgenesis in mammals are summarized in Table 1. Direct DNA uptake by sperm cells has allowed the generation of modified mice,[2] rabbits,[5] pigs[6] and cattle.[6,7] Transgene expression and transmission to the progeny has been reported in mice[2] and rabbits.[5] DNA injection in the vas deferens of mice has allowed the interaction between the exogenous DNA and spermatozoa to take place within the reproductive male tract and the ensuing generation of modified offspring.[8] Sperm electroporation has been used to improve the efficiency of DNA uptake by sperm in cattle.[9] Sperm lipofection, combined with restriction enzyme-mediated integration (REMI), has been reported as an effective approach for the production of transgenic cattle. The simultaneous lipofection of NotI-linearized plasmid DNA and NotI restriction enzyme improved the integration of plasmid DNA into NotI double-strand breaks generated in the sperm genome. Two calves obtained using this method express a GFP reporter gene in lymphocytes.[10] Two recent reports demonstrate the high efficiency of combining the use of spermatozoa as exogenous DNA vectors and the technology of intracytoplasmic sperm injection (ICSI). Transgenic mice[11] and monkey embryos[12] expressing GFP reporter genes have also been produced by injecting the sperm/DNA complex into oocytes.

***The mechanism of interaction between spermatozoa and the exogenous DNA.*** Most efforts in our group have focused on the molecular mechanism underlying the interaction between sperm cells and foreign DNA. The binding of exogenous DNA always occurs in the subacrosomal segment of the sperm head, regardless of the rounded or hook-shaped morphology of sperm cells,[4] as can be seen for the murine spermatozoa shown in Figure 1. The interaction only occurs in epididymal or ejaculated and thoroughly washed spermatozoa, because it is strongly antagonized in the presence of seminal fluid;[4] a glycoprotein (termed IF-1), abundant in the seminal fluid of mammals and on the surface of lower eukaryote spermatozoa, exerts a powerful inhibitory effect on DNA interaction,[4] thus protecting spermatozoa from undesired intrusions in nature. The binding of exogenous DNA to sperm cells is mediated by a class of proteins of 30-35 kDa that act as DNA-binding substrates and assemble stable complexes with the DNA; assembly of these protein/DNA complexes is strongly inhibited by the IF-1 glycoprotein.[4]

A constant proportion (15-22%) of sperm-bound DNA is further internalized in nuclei;[4] this observation suggests that the internalization is not a consequence of passive transfer, but is rather mediated by a regulatory mechanism. The finding that CD4 plays a key role in internalization further supports this view, since spermatozoa from CD4 knock-out mice, in spite of their ability to bind DNA, fail to internalize it into nuclei.[4] Once internalized, the exogenous sequences sequentially reach the nuclear scaffold and trigger endogenous nuclease activities in a DNA dose-dependent manner; the latter cause the exogenous DNA to rearrange[4] and undergo a process of recombination which eventually yields its integration into the sperm genome.[13] From these findings, the interaction between DNA and spermatozoa is emerging as a complex and well-regulated process, whose function in nature is not yet understood.

***The retroviral connection.*** The surprising finding that foreign DNA sequences integrate in the sperm genome[13] suggested to us that sperm chromatin is not a uniformly compact structure, thoroughly inaccessible to foreign molecules. That idea prompted us to search for "loosely packaged" domains in the sperm chromatin. By exploiting the activities of the endogenous nucleases present in spermatozoa,[4] we have isolated and characterized a nuclease-sensitive fraction from murine sperm chromatin.[14] This fraction contains poorly methylated DNA[15] which is associated with histones and lacks protamines—the major component of the sperm bulk chromatin.[14] These structural features are analogous to those of transcriptionally "active" domains in somatic chromatin. By sequence analysis, the nuclease-sensitive fraction is highly enriched in DNA sequences showing extensive retroposon/retroviral homology, particularly to the L1-derived ORF2 gene encoding the reverse transcriptase (RT) enzyme.[14] Based on these findings, we sought to establish whether the retroposon/retroviral sequences are set in a "transcriptionally competent" state in spermatozoa.

To clarify that issue, we have used exogenous RNA molecules in the reaction with spermatozoa. We have found that a RT activity is actually triggered in mature spermatozoa upon their interaction with foreign RNA: the exogenous RNA molecules are indeed retrotranscribed in cDNA fragments which are further delivered to embryos during fertilization.[16]

Table 1 | **SPERM-MEDIATED GENE TRANSFER IN MAMMALS**

| | | Offspring | | | | |
|---|---|---|---|---|---|---|
| Species | Examined Stage | # Analyzed | # Positive | % Transgenic | Remarks | Ref. |
| Mouse | Adults | 250 | 75 | 30 | CAT expression/F1 transgenic progeny | 2 |
| Mouse | Fetuses | 1755 | 30 | 7.4 | | 4 |
| Mouse | 1-cell to blastocyst | 50 | 46 | 92 | No transgenic adults were obtained | 4 |
| Mouse | Adults | 57 | 11 | 20 | ICSI of sperm/DNA complex—GFP expression | 11 |
| Mouse | Adults | 53 | 4 | 7.5 | DNA injection in vas deference—GFP expression | 8 |
| Rabbit | 1-2 cell embryos | 23 | 9 | 39 | Phenotypic expression of SV40 DNA (no molecular analysis) | 4 |
| Rabbit | Adults | 44 | 32 | 72 | LacZ expression in embryos—no transgene transmission to F1 progeny | 5 |
| Rabbit | Blastocysts | 57 | 11 | 19,3 | LacZ expression | 5 |
| Pig | Adults | 139 | 8 | 5.7 | | 6 |
| Pig | Blastocysts | 82 | 5 | 6 | | 6 |
| Bovine | Blastocysts | 677* | 149* | 22* | | 6 |
| Bovine | Blastocysts | 30 | 3 | 10 | | 7 |
| Bovine | Adults | 41 | 1 | 2,4 | | 7 |
| Bovine | Embryos | 188* | 41* | 22* | Sperm electroporation | 9 |
| Bovine | Adults | 2 | 2 | 100 | Liposome REMI-mediated sperm transgenesis. GFP expression in lymphocytes | 10 |
| Monkey | Embryos-Adults | – | – | – | GFP expression in embryos—born animals were not transgenic | 12 |

*This was the best obtained result from a variety of tested experimental conditions.

. . . retroviruses could play an active role in sperm-mediated transgenesis, both in the transformation of founders and in the transmission of foreign genes to the F1 progeny.

Work in progress in our laboratory further suggests that the retroviral machinery is activated when spermatozoa interact with foreign RNA/DNA. We believe that retroviruses could play an active role in sperm-mediated transgenesis, both in the transformation of founders and in the transmission of foreign genes to the F1 progeny.

***Potential implications for xenotransplantation.*** Transgenic technologies have broad possibilities of application in biomedicine and biotechnology. In the field of xenotransplatation, transgenic animals, particularly pigs are expected to assume growing importance as donors of organs to compensate for the currently inadequate supply of human organs. However, concerns have recently been raised over the potential threat that bursts of human-adapted heterologous retroviruses may infect human recipients and cause genetic transformations in both somatic and germ cells. In this respect, it has been recently shown that cross-species infection is a real threat, as porcine endogenous retroviruses (PERV) actually infect SCID mice after grafting of pancreatic islets.[17] These concerns would be even more serious if organs were derived from transgenic animals obtained by sperm. As summarized above, endogenous retroviral functions are activated in spermatozoa upon DNA interaction, at least in murine animal models used in our laboratory. It may be envisaged that transgenic animals obtained by sperm-mediated gene transfer may express a stimulated endogenous retroviral machinery, and hence a higher retroviral load. These circumstances should be carefully evaluated and experimentally controlled when pursuing the possibility of using animals produced by sperm as organ donors for xenotransplantation.

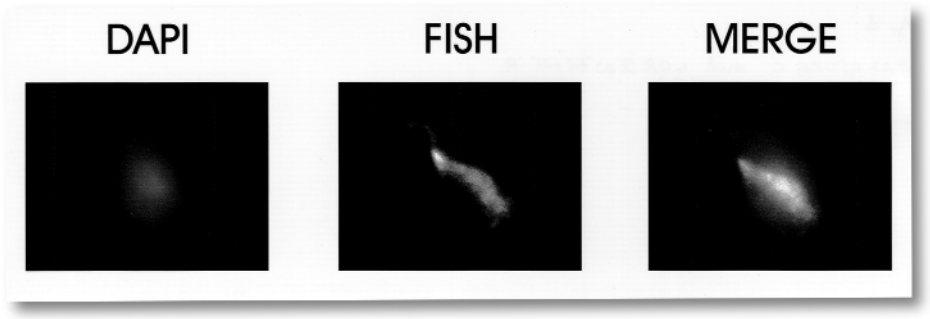


Figure 1. Fluorescent in situ hybridization (FISH) of plasmid DNA on mouse sperm nuclei. Nuclei were purified from spermatozoa preincubated with plasmid DNA and hybridized using a biotin-dUTP labeled DNA probe; foreign DNA was localized with FITC-labeled avidin (middle panel) and sperm chromosomal DNA was stained with DAPI (left panel). Merged DAPI and FISH signals are shown in the right panel.

. . . cross-species infection is a real threat, as porcine endogenous retroviruses (PERV) actually infect SCID mice after grafting of pancreatic islets.

## References


1. Geraci G, Noviello L. Dnase hydrolysis of chromatin DNA in intact sea urchin sperm cells. Effect of the ionic strength on the digestion parameters. **Cell Diff 1979;8:203**.
2. Lavitrano M, Camaioni A, Fazio V et al. Sperm cells as vectors for introducing foreign DNA into eggs: Genetic transformation of mice. **Cell 1989; 57:717**.
3. Brinster RN, Sandgren EP, Behringer RR et al. No simple solution for making transgenic mice. **Cell 1989; 59:239**.
4. Spadafora C. Sperm cells and foreign DNA: A controversial relation. **BioEssays 1998; 20:955**.
5. Kuznetsov AV, Kuznetsova IV Schit UR. DNA interaction with rabbit sperm cells and its transfer into ova in vitro and in vivo. **Mol Reprod Dev 2000; 56:292**.
6. Sperandio S, Lulli V, Bacci ML et al. Sperm-mediated DNA transfer in bovine and swine species. **Anim Biotechnol 1996; 7:59**.
7. Schellander K, Peli J, Schmall F et al. Artificial insemination in cattle with DNA-treated sperm. **Anim Biotechnol 1996; 6:41**.
8. Huguet E, Esponda P. Generation of genetically modified mice by spermatozoa transfection in vivo: preliminary results. **Mol Reprod Dev 2000; 56:243**.
9. Gagné MB, Pothier F, Sirard MA. Electroporation of bovine spermatozoa to carry foreign DNA in oocytes. **Mol Reprod Dev 1991; 29:6**.
10. Shemesh M, Gurevich M, Harel-Markowitz E et al. Gene integration into bovine sperm genome and its expression in transgenic offspring. **Mol Reprod Dev 2000; 56:305**.
11. Perry AC, Wakayama T, Kishikawa H et al. Mammalian transgenesis by intracytoplasmic sperm injection. **Science 1999; 284:1180**.
12. Chan AW, Luetjens CM, Dominko T et al. Foreign DNA transmission by ICSI: Injection of spermatozoa bound with exogenous DNA results in embryonic GFP expression and live rhesus monkey births. **Mol Hum Reprod 2000; 6:26**.
13. Zoraqi G, Spadafora C. Integration of foreign DNA sequences into mouse sperm genome. **DNA Cell Biol 1997; 16:291**.
14. Pittoggi C, Renzi L, Zaccagnini G et al. A fraction of mouse sperm chromatin is organized in nucleosomal hypersensitive domains enriched in retroposon DNA. **J Cell Sci 1999; 112:3537**.
15. Pittoggi C, Zaccagnini G, Giordano R et al. Nucleosomal domains of mouse spermatozoa chromatin as potential sites for retroposition and foreign DNA integration. **Mol Reprod Dev 2000; 56:248**.
16. Giordano R, Magnano AR, Zaccagnini G et al. Reverse transcriptase activity in mature spermatozoa of mouse. **J Cell Biol 2000; 148:1107**.
17. Van Der Laan LJW, Lockey C, Griffeth BC et al. Infection by porcine endogenous retrovirus after islet xenotransplantation in SCID mice. **Nature 2000; 407:501**.