Sperm-mediated gene transfer: applications and implications

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Summary

Recent developments in studies of sperm-mediated gene transfer (SMGT) now provide solid ground for the notion that sperm cells can act as vectors for exogenous genetic sequences. A substantive body of evidence indicates that SMGT is potentially useable in animal transgenesis, but also suggests that the final fate of the exogenous sequences transferred by sperm is not always predictable. The analysis of SMGT-derived offspring has shown the existence of integrated foreign sequences in some cases, while in others stable modifications of the genome are difficult to detect. The appearance of SMGT-derived modified offspring on the one hand and, on the other hand, the rarity of actual modification of the genome, suggest inheritance as extrachromosomal structures. Several specific factors have been identified that mediate distinct steps in SMGT. Among those, a prominent role is played by an endogenous reverse transcriptase of retrotransposon origin. Mature spermatozoa are naturally protected against the intrusion of foreign nucleic acid molecules; however, particular environmental conditions, such as those occurring during human assisted reproduction, can abolish this protection. The possibility that sperm cells under these conditions carry genetic sequences affecting the integrity or identity of the host genome should be critically considered. These considerations further suggest the possibility that SMGT events may occasionally take place in nature, with profound implications for evolutionary processes. ***BioEssays*** **27:551–562, 2005. © 2005 Wiley Periodicals, Inc.**

SMGT: a short story

Introduction

In 1989, two independent reports made the claim that sperm cells could associate with exogenous DNA molecules (transgenes) and transfer these molecules during fertilization, resulting in genetically modified (transgenic) offspring.[1,2] This represented an important rediscovery of what in retrospect can now be viewed as a groundbreaking discovery: the spontaneous ability of sperm cells to bind DNA. The genesis of this rediscovery was reported previously[3] and essentially derived from early observations that intact, swimming spermatozoa were permeable to exogenous micrococcal nuclease that caused the degradation of sperm chromatin. The first report of mammalian sperm cells being able to act as vectors for foreign DNA[4] was published in 1971 but was ignored, only to be rediscovered after the publication of the 1989 work.

Thus, in 1989, a new mode of animal genetic manipulation came into existence. However, despite initial reports of success, the early history of sperm-mediated gene transfer (SMGT) was marred by difficulties of reproducibility, which eventually caused a good deal of skepticism over SMGT.[5,6] In subsequent years, however, many successful reports of SMGT have been published. Accordingly, in spite of the controversy that accompanied its first appearance, SMGT has gradually been perceived as a potentially promising method. SMGT may be able to provide efficient, rapid and low-cost protocols for animal transgenesis and, more futuristically, human germline gene therapy. Moreover, if sperm cells can act as vectors for foreign genetic sequences, it follows that the genome of sexually reproducing animals—including humans—may be exposed to alteration by exogenous genetic sequences carried by sperm cells, with important implications for evolutionary processes and for human health.[7]

SMGT experiments—approaches and outcomes

The scientific literature contains over seventy reports of the successful in vitro uptake of DNA constructs (transgenes) by animal sperm cells (see Table 1). Most of these reports provide evidence of postfertilization transfer and maintenance of transgenes, and several of the studies also report the subsequent generation of viable F_0 animals, the cells of which contain exogenous DNA sequences. In some cases, evidence is provided of transgene transmission to the progeny (F_1 or beyond). The mostcommon methodology, termed ‘‘DNA incubation‘ in Table 1 and the approach employed in the initial landmark experiments, is very straightforward: seminal plasma-free sperm cells are suspended in the appropriate medium and incubated with DNA. The resultant DNA-carrying sperm are then used to fertilize eggs, via in vitro fertilization (IVF) or artificial insemination (AI) or, in the case of aquatic animals, via waterborne (natural) fertilization. Other studies in Table 1 have used ‘augmentation’ techniques, such as electroporation or liposomes, to ‘force’ sperm to capture

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Table 1. A survey of successful sperm–DNA interaction and sperm-mediated gene transfer experiments

Sperm	Approach	Fertilization method	Latest stage in which transgenes found	Year	Reference
Rabbit	DNA incubation	AI	Embryos	1971	(4)
Sea urchin	DNA incubation	IVF	Embryos	1989	(1)
Mouse	DNA incubation	IVF	Viable animals + progeny	1989	(2)
Several farm animal species	DNA incubation	(n/a)	Sperm	1990	(8)
Mouse	DNA incubation	(n/a)	Sperm	1990	(9)
Bull, and insect species	DNA incubation	(n/a)	Sperm	1991	(10)
Mouse	Liposomes	IVF	Viable animals	1991	(11)
Bull	Electroporation	IVF	Embryos	1991	(12)
Mouse and chicken	DNA incubation	IVF & AI	Sperm	1991	(13)
Mouse	Liposomes	(n/a)	Sperm	1991	(14)
Pig	DNA incubation	(n/a)	Sperm	1991	(15)
Mouse, boar, bull & human	DNA incubation	(n/a)	Sperm	1992	(16)
Pig	DNA incubation	IVF	Embryos	1992	(17)
Pig	Electroporation	(n/a)	Sperm	1992	(18)
Zebrafish	DNA incubation	IVF	Viable animals + progeny	1992	(19)
Mouse	DNA incubation	(n/a)	Sperm	1992	(20)
Rooster	Liposomes	AI	Fetuses	1992	(21)
Mouse	DNA incubation	(n/a)	Sperm	1993	(22)
Chicken	DNA incubation	(n/a)	Sperm	1993	(23)
Salmon	Electroporation	IVF	Viable animals	1993	(24)
Salmon	Electroporation	(n/a)	Sperm	1994	(25)
Mouse	DNA incubation	IVF	Embryos	1995	(26)
Zebrafish	DNA incubation	(n/a)	Sperm	1995	(27)
Bull	DNA incubation	AI	Viable animals	1995	(28)
Mouse	Liposomes	(n/a)	Sperm	1995	(29)
Loach	Electroporation	IVF	Viable animals	1995	(30)
Various mammalian	DNA incubation	(n/a)	Sperm	1995	(31)
Various mammalian	DNA incubation	IVF	Embryos	1996	(32)
Xenopus	DNA incubation	IVF	Embryos	1996	(33)
Zebrafish	DNA incubation & electroporation	IVF	Viable animals	1996	(34)
Rabbit, bull, chicken	Liposomes	IVF	Viable animals	1996	(35)
Bull and pig	DNA incubation	AI	Viable animals	1996	(36)
Human	DNA incubation	IVF	Embryos	1997	(37)
Loach	Electroporation	IVF	Embryos	1997	(38)
Mouse	DNA incubation	(n/a)	Sperm	1997	(39)
Frog & mouse	DNA incubation	(n/a)	Sperm	1997	(40)
Mouse	DNA incubation	(n/a)	Sperm	1997	(41)
Abalone (mollusk)	Electroporation	IVF	Larvae	1997	(42)
Mouse	DNA incubation	(n/a)	Sperm	1997	(43)
Mouse & rat	TMGT	(n/a)	Sperm	1998	(44)
Mouse	DNA incubation	IVF	Fetuses	1998	(45)
Mouse	TransgenICSI	ICSI	Embryos	1999	(46)
Carp	Electroporation	Waterborne	Viable animals	1999	(47)
Mouse	TMGT	Natural	Viable animals + progeny	1999	(48,49)
Rhesus monkey	TransgenICSI	ICSI	Embryos	2000	(50)
Rhesus monkey	TransgenICSI	ICSI	Embryos	2000	(51)
Mouse	TMGT	Natural	Viable animals	2000	(52)
Xenopus laevis	DNA incubation	Waterborne	Viable animals + progeny	2000	(53)
Zebrafish	DNA incubation	Waterborne	Viable animals + progeny	2000	(54)
Bovine	Electroporation	IVF	Embryos	2000	(55)
Honey bee	DNA incubation	AI	Viable animals + progeny	2000	(56)
Mouse & pig	DNA incubation	IVF	Viable animals	2000	(57)
Salmon	Electroporation	Waterborne	Viable animals	2000	(58)
Pig	TransgenICSI	ICSI	Embryos	2001	(59)
Rabbit	Liposomes	IVF	Viable animals + progeny	2001	(60)
Rat	TMGT	Natural	Viable animals	2001	(61)
Mice	TMGT	Natural	Viable animals	2002	(62)
Pig	DNA incubation	AI	Viable animals	2002	(63)
Sea bream	Electroporation and TMGT	Waterborne	Viable animals	2002	(64)
Mouse	TMGT	(n/a)	Sperm	2002	(65)
Rat	Liposomes	AI	Viable animals	2002	(66)
Carp	DNA incubation	Waterborne	Viable animals	2002	(67)

(*Continued*)

Table 1. (*Continued*)

Sperm	Approach	Fertilization method	Latest stage in which transgenes found	Year	Reference
Pig & mouse	Antibody linked	Surgical AI/IVF	Viable animals	2002	(68)
Pig	TransgenICSI	ICSI	Embryos	2003	(69)
Mouse	TransgenICSI	ICSI	Embryos	2003	(70)
Rabbit	Liposomes	AI	Viable animals	2003	(71)
Rohu fish	Electroporation	Waterborne	Viable animals	2004	(72)
Chicken	Liposomes	AI	Viable animals	2004	(73)

transgenes (see Fig. 1 for a pictorial summary). More recent studies have introduced transgenes directly into the reproductive tract of male animals, either as naked DNA or encapsulated in liposomes. In this form of SMGT, known as testis-mediated gene transfer (TMGT), sperm pick up the transgenes in vivo and transmit them via natural mating or AI. Another recent innovation in SMGT has been the use of intracytoplasmic sperm injection (ICSI) to deliver transgene-containing sperm cells directly into the egg, a process known as 'transgenICSI'. These latest refinements to SMGT are considered in more detail later in this review.

SMGT: controversial aspects

Despite the successes reported in Table 1, SMGT has not yet become established as a reliable form of genetic manipulation. A significant aspect is that stable transgene integration has

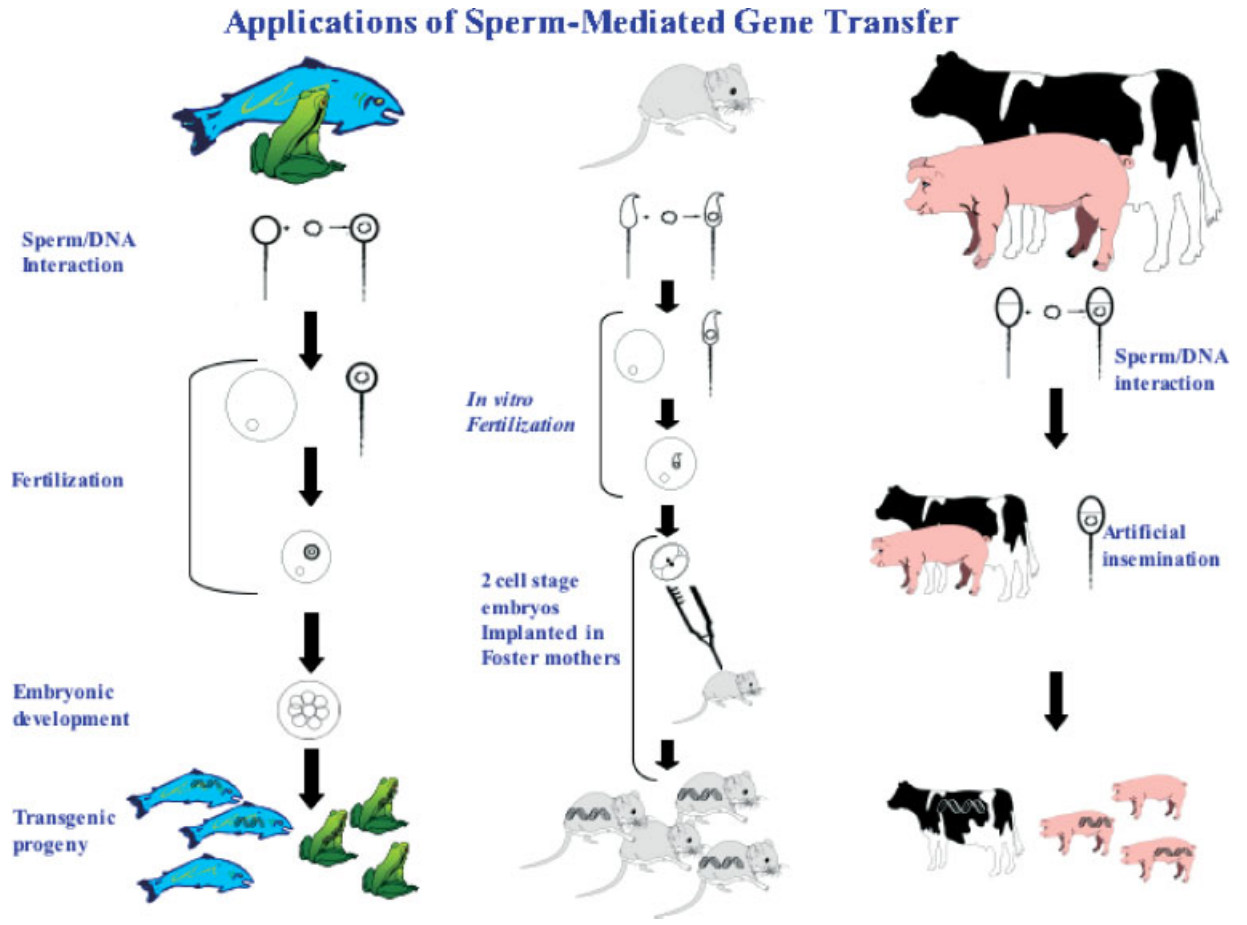

Figure 1. Routes to the production of genetically modified animals via SMGT.

rarely been detected with SMGT protocols. An additional, more conceptual skepticism is based on the assumption that major evolutionary chaos would result if sperm cells were able to act as vectors for exogenous genetic sequences. Given that the reproductive tracts contain 'free' DNA molecules (originating from natural cell death and breakage), it seems reasonable to expect sperm cells to be highly resistant to the risk of picking up such molecules.

Since SMGT can be demonstrated experimentally, as in the experiments in Table 1, and yet evolutionary chaos is not observed in nature, it follows that nature has erected formidable barriers against SMGT, though these barriers are not absolute. On this view, the inconsistent nature of SMGT experimental outcomes is readily explained: if there are powerful natural barriers against SMGT, then SMGT successes may be thought to represent unusual cases in which the barriers have failed. Such breaches may have resulted from the presence of some unknown critical factor(s) in the successful experiments. Indeed, at least two such barriers have been identified that antagonize the spontaneous, undesired intrusion of exogenous molecules in mammalian spermatozoa: (1) an ''inhibitory factor'' (IF-1), abundant in the seminal fluid or bound to the spermatozoa membrane in marine animals, that prevents the binding of foreign molecules, and (2) a sperm endogenous nuclease activity that is triggered in a dose-dependent manner upon interaction of spermatozoa with foreign molecules.[41] This activity causes the degradation of the exogenous sequences or, above a specific DNA threshold, an apoptotic-like suppression of the DNA-loaded spermatozoa.[41] It is reasonable to believe that these protections minimize any unintentional interaction between sperm and exogenous sequences and protect against the threat that every fertilization event may become a potentially mutagenic event. In the following section, we present a hypothetical model for the uptake of exogenous molecules by sperm cells that goes some way towards explaining the low frequency/variable uptake results in SMGT research to date.

Mechanisms of nucleic acid uptake by sperm

Several laboratories have invested significant efforts to improve SMGT and have contributed to developing our understanding of sperm interactions with nucleic acid molecules. As a whole, these studies confirm the original observation that sperm cells can play a role in transgenesis. Somewhat paradoxically, however, the expectation of practical benefits in biotechnology and efforts to optimize that output have shifted the focus away from the underlying molecular mechanisms of the SMGT process. Surprisingly, the possibility that SMGT is not only an experimental process for the generation of transgenic animals, but rather an adaptation of a naturally occurring phenomenon and a possible causative agent of genome reshaping, is not clearly perceived. Against this general trend, a small number of groups including ours has intensively pursued the elucidation of underlying mechanisms. We consider that acquiring this knowledge is of paramount importance, not only to achieve full experimental control of the SMGT process and improve animal transgenesis protocols, but also to disclose hidden aspects of a phenomenon that may have far-reaching implications if spontaneously occurring in nature. In an effort to clarify the molecular mechanism of SMGT, we have focussed particularly on the first and fundamental event of the process, namely the interaction between sperm cells and exogenous DNA. We surmised that the binding of exogenous DNA molecules to sperm cells and further internalization in nuclei does not occur haphazardly, but is a regulated process mediated by specific factors.[3] In the terminal steps of the process, internalized DNA molecules reach the nuclear scaffold, where a small fraction eventually undergoes recombination with the sperm chromosomal genome at a few selected sites, leading to rare integration events.[43] A set of chromatin sites with a distinctive conformation, accessible to nuclease digestion and complexed with histones rather than protamines[74] has been identified in the sperm chromatin, that may serve as potential integration sites. It is noteworthy that such ''accessible'' sites are enriched in LINE-1 retroelements. These findings (to which we will return below) began to uncover unexpected features of the sperm genome organization. Yet, the low frequency of integration events compared to the relatively high proportion of positive animals, together with the observation that, in most of these animals, the foreign DNA remains in an extrachromosomal form (see below), continued to foster the notion that important aspects of the underlying SMGT mechanisms remained persistently elusive.

A turning point in the understanding of the process came with the finding that the interaction of exogenous molecules triggers an endogenous reverse transcriptase (RT) activity in spermatozoa. Such RT activity is able to reverse transcribe exogenous RNA molecules (specifically, the human poliovirus RNA genome was used in the study that provided the first set of evidence) into cDNA copies, which are transferred to embryos following in vitro fertilisation (IVF).[75] That finding suggested for the first time that a sperm endogenous RT is implicated in the generation of newly reverse-transcribed sequences and, more generally, established the notion that the retrotransposon/retroviral machinery is involved in SMGT. Before examining the significance of this finding in more depth, we will briefly recall some background features.

Endogenous reverse transcriptase activities

RT activity of non-telomeric origin was historically associated with replication of retroviruses,[76,77] and later found to be also encoded by two major classes of repeated elements in higher eukaryote genomes: retroposons, such as those of the LINE (Long Interspersed Nuclear Element) family,[78] and endogen-

ous retroviruses,[79] collectively termed retroelements. Non-telomeric RT is the key function in retrotransposition of these elements. After completion of the human and murine genome sequencing, it is clear that as much as 45% of human and 37% of murine genomes are made up of retroelements.[80] That portion of the genome has been traditionally considered as a useless remnant of our genetic past and classified as "junk DNA".[81] However, growing evidence is challenging this view and suggests instead that the endogenous RT plays a role in the reshaping and rearrangement of genomes. An increasing body of evidence indicates that this enzyme is responsible for numerous genomic alterations and acts as a major driving force in evolution.[82] RT is expressed in elevated levels in embryos,[83,84] embryonic tissues[85] and tumors;[86,87] in contrast, no or very low levels of RT expression are typical of terminally differentiated cells. The mammalian genital tract,[88,89] germ cells[90] and gametes[91,92] are other preferential sites of expression of retroviral/retrotransposon genes. The endogenous RT activity is preferentially expressed in tissues with a high proliferation potential and may therefore be involved in pathway(s) regulating cell growth and differentiation. Consistent with this, we have recently found that inhibition of the endogenous RT activity arrests embryo development in early pre-implantation stages[93] and also modulates proliferation and differentiation in transformed cell lines.[94,95]

Sperm-mediated "reverse" gene transfer

As anticipated above, the RT activity detected in murine spermatozoa[75] is most likely encoded by a fraction of the sperm chromatin that is organized in an "active" nucleohistone (as opposed to protamine-associated) conformation and enriched in LINE-1 sequences.[74,96] The existence of an active RT in spermatozoa was unexpected. As a step towards assessing its functional activity and determining whether foreign RNA can be a suitable substrate for the sperm RT, we carried out IVF experiments with spermatozoa that were preincubated with a RNA vector marked with a beta-galactosidase (beta-gal) gene. In these experiments, the beta-gal-expressing RNA vector was taken up by sperm cells, reverse-transcribed, delivered to embryos upon IVF, and mosaic-propagated in founders and further in the F_1 progeny.[97] Moreover, the beta-gal protein was expressed in tissues from F_0 and F_1 animals. Thus, spermatozoa can reverse-transcribe exogenous RNA and generate transcriptionally competent sequences that are transmitted to offspring. We have called this phenomenon "sperm-mediated reverse gene transfer". The population of cDNA molecules that are reverse-transcribed from the beta-gal template exhibits peculiar features that distinguish them from other transgenes. First, these sequences are maintained at low copy number (i.e. <1 copy per genome). Second, they show a mosaic distribution in founder animals, are sexually transmitted from founders to the F_1 progeny, and are again mosaic propagated in the F1 offspring. These features strongly suggest that the reverse-transcribed sequences are not integrated in the host genome and are probably replicated independent from replication of the host genome. Consistent with this are the negative results of various attempts to identify integration of the reverse-transcribed cDNA copies, for example by constructing partial genomic libraries. Thus, available evidence suggests that retrotranscribed cDNA molecules are mostly maintained as autonomously replicating extrachromosomal structures, with integration in the host genome occurring rarely. A possible model for the process is schematised in Fig. 2: exogenous RNA molecules are thought to migrate in close contact with the sperm nuclear scaffold, where endogenous RT activity is localised.[75] Here, cDNA copies are generated by reverse transcription. We hypothesise that integration events occur at nuclear matrix-bound DNA sites that connect adjacent protamine-bound looped domains, corresponding to the "accessible" sites where the nucleosomal organisation was not replaced by protamines during spermatogenesis. Henceforth, extrachromosomal, autonomously replicating cDNA structures can be generated either by direct reverse transcription of the foreign RNA, or by the RNA transcribed from the integrated sequences, reminiscent of a provirus-type model. The finding that non-integrated DNA sequences are maintained in embryos and adult tissues[97] is not totally surprising, since extrachromosomal structures were previously demonstrated to be frequent guests of eukaryotic nuclei. In particular, transgenic sequences can generate extrachromosomal structures that are transmitted to the next generation, as documented in studies of transgenic mammals,[62,98] birds,[21] fish[19] and insects[56] obtained by SMGT and, in certain cases, by DNA microinjection.[99,100] The genesis of such extrachromosomal structures in transgenic animals, and their sexual transmission to the progeny, are unclear. According to the model, transgenic episomal DNA is thought to represent the product of an endogenous RT activity. Physiologically, regulated transposition events have been reported to occur in early cleavage stages.[101] Our findings that RT inhibition causes the irreversible arrest of embryonic development suggest that retrotransposon-encoded RT activity is required in some as yet unknown regulatory pathway in early developmental stages. The developmental arrest induced by RT inhibition is accompanied by a broad reprogramming of gene expression.[93] It is worth stressing that the sensitivity to RT inhibition is developmentally restricted to the late one- to the four-cell stages.[93] Growing work implicates retrotransposons in transcriptional interference or epigenetic control of cellular genes[102,103] and in RNAi-dependent chromatin-based gene silencing in yeast.[104] In addition to its basic significance to developmental regulaton, the discovery of functional RT in sperm cells provides the basis for sperm-mediated reverse gene transfer: in this process, the exogenous RNA is probably

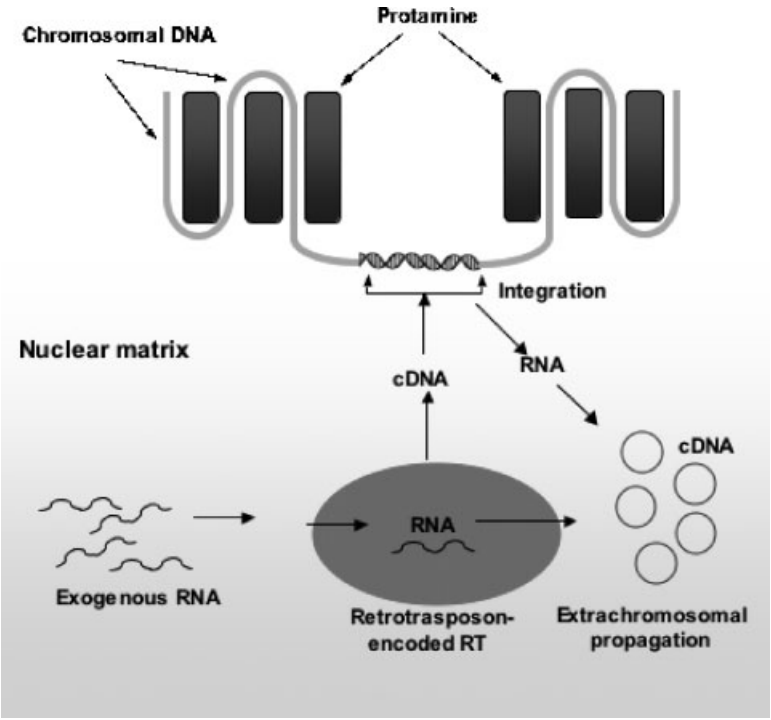

Figure 2. Hypothetical model for sperm-mediated "reverse" gene transfer. Exogenous RNA molecules are taken up by sperm cells, internalised in nuclei and reverse-transcribed in cDNA copies by the endogenous matrix-associated RT activity (represented by the brown circle) (for details see Ref. 75). A small proportion of the resulting cDNA is thought to eventually integrate into "accessible" sites of the sperm chromatin which retain a nucleosomal organisation (as from Pittoggi et al., see Ref. 74), and are probably located between adjacent protamine domains. Instead, the vast majority of cDNA copies appear to propagate in tissues of the offspring as extrachromosomal structures (blue circles). Extrachromosomal structures can either be generated by direct reverse transcription of the exogenous RNA, or from transcription of the integrated cDNA copy into RNA.

"captured" by the retrotransposon-mediated mechanism active in sperm cells, reverse-transcribed and further propagated through the embryo. We are currently working to establish whether this mechanism is triggered exclusively when sperm cells are incubated with RNA, or whether DNA can also induce a similar response. In the latter case, SMGT could be regarded as a retrotransposition-mediated phenomenon proceeding in two steps, i.e. exogenous DNA transcription into RNA, followed by reverse transcription and propagation of cDNA copies.

Applications of SMGT

Animal transgenesis

From 1989 to 2004, over 30 separate claims have been made for the production of viable transgenic animals using SMGT (Table 1). However, only a minority (~25%) of these reports include convincing demonstration of transmission to progeny (F_1 or beyond). Clearly, transmission of transgenes beyond the F_0 generation would be a prerequisite for usable animal transgenesis. The infrequency of transmission may be explained by the foregoing mechanism of sperm–DNA interaction, in that the transgenes may be present as episomes in the cells of the F_0 animals, being subsequently lost for the next generation.

The fact that several reports show transmission, and that some show DNA integration, suggests that there may be unknown factors that have operated to induce integration in some experiments. Interestingly, the clearest integration evidence has been obtained with transgenICSI and 'augmentation' protocols, such as liposome-mediated gene transfer. By contrast, few integrations have been shown to occur with direct sperm–DNA interaction. A possible interpretation of these results is that the plasma membrane of sperm plays a critical role: when the membrane is intact, as it usually is in direct interaction ('auto-uptake') protocols, the binding of DNA molecules to the cell surface likely triggers the mechanism of internalization and subsequent reverse transcription described above, yielding mostly the generation of episomal cDNA molecules. By contrast, the destruction or bypass (for example using liposomes) of the membrane, facilitates a direct interaction with the sperm chromatin, increasing the

probability of integration. Integration may occur early in the sperm nucleus (transgenic spermatozoa) or later after entry into oocytes, when the sperm nucleus is converted into a male pronucleus. In other words, a "sensor" might exist on the sperm surface: if the DNA interacts with the surface, then the end product will mostly be episomal, while the direct DNA loading on the chromatin after removal or bypass of the surface structures increases the yield of integration products. The identification of critical membrane functions modulating the outcome of genomic integration following SMGT awaits further research.

From the experiments reported in Table 1, it is clear that numerous animal species have proven amenable to SMGT, including mammals, birds, fish and insects. Thus, SMGT transgenesis demonstrates a very broad applicability across animal species. It is noteworthy that aquatic species are highly represented in reports of successful SMGT, and it may be the case that SMGT transgenesis will prove particularly useful for marine animal transgenesis. SMGT transgenesis offers the potential for mass-delivery of transgenes, utilizing simple and inexpensive technology, particularly when coupled with AI or natural mating, as opposed to IVF or ICSI. Despite the low frequency of stable genomic integration, the frequency of phenotypic modification and overall transgenesis (including episomally transmitted characters) can be as high as 80% in some experiments. Nevertheless, SMGT has not become an established method of transgenesis compared to pronuclear microinjection and embryonic stem cell (ESC) transgenesis, because the outcome of single experiments, at the present stage, is still difficult to predict in routine transgenesis work.

Recent technological advances from SMGT

An interesting recent development of SMGT has been the introduction of transgenes into testicular (sperm) stem cells in vivo (testis-mediated gene transfer, TMGT). In principle, TMGT removes the need to collect, manipulate or transfer eggs, thus providing a major streamlining of gene transfer. Preliminary results have been reported in mice, where transgene constructs were directly injected into the testis. For example, 60–70% of sperm were reported to carry the transgene following injection of naked DNA into the vas deferens,[44] with a follow-up report claiming detection of the transgene in the cells of 7.5% of offspring animals produced following fertilisation with the transgene-bearing sperm.[105] Similar results were reported using liposome-encapsulated transgene molecules injected close to the epididymis.[48,49,65]

Another recent development has been the combination of SMGT with microinjection (transgenICSI) in which sperm exposed to naked or liposome-encapsulated transgene molecules are microinjected into oocytes. Promising results have been reported with mice, with founder animals integrating and expressing the transgene.[46,70] Transgene uptake and expression following transgenICSI has also been reported in rhesus monkey embryos[50,51] and porcine embryos.[59,59] The success of transgenICSI provides further support for the notion that sperm are indeed able to act as transgene vectors. Reported rates of success (i.e. transgenics per transfer), although variable, are already fairly impressive, with a figure of around ca. 35% being fairly typical.[46,50,51,59,68–70]

Finally, a novel form of SMGT, termed linker based sperm-mediated gene transfer (LB-SMGT), has recently been reported.[68] This work employed sperm surface-specific monoclonal antibody (mAb C) complexed with DNA. Capable of specifically binding to the sperm of various species of animals, mAb C served as a linker molecule to attach transgenes to the surface of pig and mice sperm. Following fertilization, transgene DNA was detected in integrated form in the genome of viable pig and mouse offspring. Transmission to the F_1 generation occurred at an efficient rate, with 37.5% of pigs and 33% of mice testing positive for transgene presence. Transmission to the F_2 generation was also obtained for pigs. If this work can be replicated, LB-SMGT clearly holds promise for improving the production efficiency of large transgenic animals.

Gene therapy

As discussed above, transgenes in host cells following SMGT may, in most cases, be in an episomal form. This may open up an attractive future perspective for somatic gene therapy. The evidence summarised above indicate that non-integrated exogenous sequences are mostly propagated as extrachromosomal structures throughout embryonic development, maintained in the tissues of adult animals and sexually transmitted to the next generation, without disruption of the integrity of the host genome. Might this suggest that somatic gene therapy, aimed in particular at the embryo or foetus, may become a future possibility? Many severe genetic conditions, for which somatic gene therapy in humans would be highly desirable, kill or severely affect the living conditions of patients in the first months or years of life. Indeed, conceptually there exists an inverse correlation between the age of the patient and the efficacy of gene therapy. Therefore, the possibility of treatment prior to irreversible disorder-induced damage would be an ideal application of somatic gene therapy. Embryo somatic gene therapy would have other advantages compared with later-stage approaches,[106] including: (a) the possibility of correcting whole-tissue/organ alterations by genetically altering a small number of appropriate clonogenic cells (such cell-types are a feature of early foetal development); and (b) the avoidance of an immune response against transgene vectors, due to the preimmune status of the embryo and early foetus. The potential use of SMGT for embryo somatic gene therapy is theoretically appealing, considering that transgenes seem to persist best in the earlier stages of development, possibly due to their episomal status. However, the speculative nature of this possibility must be acknowledged.

Gene targeting and SMGT

Assuming that further developments of SMGT will make it a reliable tool for gene transfer, its unsuitability for gene targeting would still limit its use in the context of animal transgenesis. Gene targeting generally requires the ability to select for rare targeted outcomes against a background of random integration events. There is no conceivable way to select amongst sperm cells in the same way in which selection methods can be applied to embryonic stem cells and cultured somatic cells: therefore, using gene targeting with SMGT may prove inherently impossible. However, innovative lines of research from related fields may open up fruitful possibilities in SMGT. Research into the mechanism of gene targeting—homologous recombination (HR)—is yielding growing insight into the 'recombinase' enzymes that drive HR.[107] Speculatively, it may become possible in the future to co-transfer potent recombinase enzymes, or enzyme complexes, alongside/attached to transgene molecules during the transgene uptake stage in sperm cells, such that HR is stimulated before or soon after fertilization. This could then permit efficient gene targeting. The use of isogenic DNA constructs[108] and the insertion of repetitive DNA sequences[55] may favour the integration by HR and thus the coupling of gene targeting with SMGT.

Can exogenous DNA/RNA be taken up in nature or by accident?

In vitro studies indicate that exogenous nucleic acid uptake by sperm is strongly antagonised by IF-1,[3,31] an abundant glycoprotein in the seminal fluid of mammals and on the sperm surface in marine species. In mammals, IF-1 is probably lost during the movement of sperm in the female genital tract. In aquatic species, sperm are exposed to exogenous nucleic acids, derived from cell catabolism of animal and plant origin, present in seawater. Therefore, it is not surprising that the binding of IF-1 coats the sperm cell surface in these species. However, the exposure of sea urchin sperm to a low ionic strength medium (e.g. 0.8× diluted sea water) detaches IF-1, hence removing that protection and allowing exogenous nucleic acid molecules to interact with spermatozoa. Similar conditions may conceivably arise naturally in specific instances—for example, in sea water near the mouth of a river—which would allow millions of sperm cells to take up exogenous nucleic acids. Exogenous DNA or RNA molecules may undergo several possible fates after uptake into sperm cells in natural systems. First, they can be degraded by sperm nucleases, yielding no genotypic or phenotypic manifestation of SMGT:[41,70,74,109] indeed, sperm cells are highly unlikely to take up a full-length coding gene and regulatory sequences capable of conferring a sudden "gain of a function". However, DNA/RNA molecules surviving (or not subjected to) nuclease attack may either integrate into the sperm nucleus, or, more likely, remain as episomes as discussed above.

An alternative fate for exogenous DNA molecules is non-random genomic integration in sperm cells. As discussed above, the highly condensed structure of sperm chromatin is virtually inaccessible to foreign molecules, except at specific sites, characterised by an unusual chromatin organisation and specific sequence enrichment. Thus, the sperm chromatin offers an extremely restricted choice of possible integration sites, causing a non-random integration within the sperm genome.[43]

Implications of SMGT for human reproduction

SMGT studies have revealed a spontaneous ability of sperm cells of virtually all animal species, including humans, to take up exogenous nucleic acids and deliver them to oocytes at fertilisation. This may raise concern for the human offspring generated during assisted reproduction. Those handling germ cells in assisted reproduction practices are generally unaware of the concept that sperm cells may carry a potential threat to the genetic identity of the progeny. The traditional view that mature spermatozoa are metabolically inert cells, incapable of any other function but transferring the male genome into oocytes at fertilisation, is deeply rooted. Instead, the data accumulated in SMGT studies, summarized in this review, support the conclusion that the intrinsic "permeability" of mature spermatozoa, especially when subjected to extensive washes that remove the natural protecting IF-1 molecules, may represent vectors with high risk in assisted reproduction, because exogenous genetic material can internalize in sperm cells depleted of seminal fluid. In this light, the well-established IVF procedure and the newly developed ICSI technology currently used in human assisted reproduction can both expose unprotected sperm cells washed from the seminal fluid to the inadvertent intrusion of even minute amounts of exogenous DNA present in the environment.[3,31] At fertilization, these foreign sequences can be passed to oocytes, thereby endangering the offspring genetic integrity. Moreover, the finding that spermatozoa, including those of humans, are endowed with a RT activity that can reverse transcribe exogenous RNA molecules into cDNA copies should warn that the interaction with foreign RNA can be as risky an event as that with DNA.

An additional concern may lie when using semen from HIV serum-positive men in assisted reproduction. In these cases, the semen is thoroughly washed and cellular components different from spermatozoa are accurately removed in order to eliminate the HIV viral particles that are thought to be exclusively associated with the lymphocyte component of human semen. It is believed that once this component is removed, the spermatozoa can be used safely for fertilisation. However, experimental evidence contradicts this view and suggest instead that viral particles, such as HIV[110] and herpes,[111] can be taken up by sperm cells and, in some cases, passed to oocytes at fertilisation. Thus, spermatozoa

can be vectors not only of small-size nucleic acids but also of large structures such as viral particles.

Furthermore, our recent work on sperm RT indicates that a retroviral genome can be reverse-transcribed into transcriptionally active cDNA copies in murine sperm cells and transferred to embryos after fertilization. This may have far-reaching consequences for human health. It indicates another potential source of risk, in addition to the serum-positivity of the generated offspring, associated with IVF, as the vector sperm cell can potentially generate a "HIV-transgenic" individual carrying HIV genomic elements.

Evolutionary implications of SMGT

Based on the evidence summarised thus far, SMGT can be regarded as a potential source of mutation for the host genome. When genetic modifications occur under experimentally controlled conditions, the outcome is the generation of transgenic animals. When, in contrast, sperm-mediated gene transfer events take place occasionally in nature, due for example to the stochastic, unpredictable uptake of DNA molecules present in the environment, then any event occurring in germline cells may contribute to further evolutionary processes. It is impossible to establish at present whether SMGT has ever occurred in nature, and hence weight its evolutionary impact, but its occurrence cannot be ruled out, particularly in aquatic species under favourable environmental conditions.

As outlined above, stochastic SMGT events occurring in nature would be very unlikely to yield a "gain of a function" in the offspring, due to (i) the nuclease-dependent breakdown of large genetic sequences, and (ii) the higher statistical probability for any higher eukaryotic genome to release repeated DNA sequences (high or intermediate), rather than single-copy genes, as free molecules for the interaction with a sperm cell. Nevertheless, the integration of such sequences can alter the integrity of the host genome and influence the functional capability of endogenous genes, through inactivating integration or, on the contrary, providing activating sequences to host genes. In support of this view are the findings, discussed above, that spermatozoa[75,97] and early preimplantation embryos[93] harbor a functional RT able to promote retrotransposition.[101,112] Retrotransposition is proposed to form the primary mechanism in the dynamic remodeling of eukaryotic genomes.[113] RT-mediated genome reshuffling events are well described[113] and are reported to modulate gene expression by causing the functional knockout of genes or their ectopic activation. Retroelements are enriched in promoter sequences and recognition sites for transcription factors;[114,115] indeed, both of these classes of regulatory sequences have been identified in the sperm chromatin portion enriched in LINE-1 sequences and retaining an organisation similar to that of "active" somatic chromatin.[116] Hypothetically, SMGT may be viewed as a possible additional source for genome shuffling. As our understanding of the complete genome organisation and sequence progresses, it should become possible to address the evolutionary occurrence and relevance of any such event.

Conclusions

It is now well established that spermatozoa can play a role in transgenesis in virtually all species. Their ability to take up exogenous DNA molecules can be exploited to transmit novel genetic information to the offspring after fertilization. This potential is highlighted by the recent development of SMGT variant protocols, including transgenISCI, in which sperm cells, though being unviable and unable to fertilize oocytes on their own, act nevertheless as delivery vectors of exogenous sequences to oocytes. Efforts to dissect SMGT at the molecular level have revealed that the interaction of sperm cells with exogenous nucleic acid molecules is a well-regulated process mediated by specific factors. A sperm retrotransposon-encoded RT has been identified as a key factor in SMGT; this RT mediates the generation and propagation of biologically active genes that are reverse-transcribed from exogenous RNA sequences, or from RNA sequences transcribed from DNA sequences. Based on this finding, the underlying mechanism of SMGT is best viewed essentially as a retrotransposition-mediated process. Inasmuch as sperm cells are vectors of exogenous genetic information, there is little doubt that they have the potential to cause both genetic and phenotypic modification in individuals of a variety of species and are worthy of further methodoloigical investigation for optimal use in biotechnology. On these same grounds, however, sperm cells of the human species should be considered cautiously in assisted reproduction procedures, as an incompletely understood, non-mastered potential threat to human genomic integrity if carrying uncharacterised DNA or RNA. One serious concern is that sperm cells may act as vectors of sexually transmitted viral pathogens, and/or agents of genome mutations following the unintentional, undetected uptake of exogenous genetic information. These considerations further suggest the possibility, still speculative at this stage, that SMGT events may occasionally take place in nature. In such a scenario, nucleic acid-bearing spermatozoa may have an evolutionary impact. Considering the increasing relevance and growing applications of assisted reproduction and transgenic technologies in human health, these questions deserve careful investigation and full clarification.

References

1. Arezzo F. 1989. Sea-urchin sperm as a vector of foreign genetic information. Cell Biol Int Rep 13:391–404.
2. Lavitrano M, Camaioni A, Fazio VM, Dolci S, Farace MG, et al. 1989. Sperm cells as vectors for introducing foreign DNA into eggs—genetic transformation of mice. Cell 57:717–723.
3. Spadafora C. 1998. Sperm cells and foreign DNA: a controversial relation. Bioessays 20:955–964.

4. Brackett BG, Baranska W, Sawicki W. 1971. Uptake of heterologous genome by mammalian spermatozoa and its transfer to ova through fertilization. Proc Natl Acad Sci USA 68:353–357.
5. Smith KR. 1999. Sperm cell mediated transgenesis: A review. Anim Biotechnol 10:1–13.
6. Brinster RL, Sandgren EP, Behringer RR, Palmiter RD. 1989. No simple solution for making transgenic mice. Cell 59:239–241.
7. Smith KR. 2002. The role of sperm-mediated gene transfer in genome mutation and evolution. Med Hypotheses 59:433–437.
8. Castro FO, Hernandez O, Uliver C, Solano R, Milanes C, et al. 1990. Introduction of foreign DNA into the spermatozoa of farm animals. Theriogenology 34:1099–1110.
9. Wu GM, Nose K, Mori E, Mori T. 1990. Binding of foreign DNA to mouse sperm mediated by its MHC class-Ii structure. Am J Reprod Immunol 24:120–126.
10. Atkinson PW, Hines ER, Beaton S, Matthaei KI, Reed KC, et al. 1991. Association of exogenous DNA with cattle and insect spermatozoa in vitro. Mol Reprod Dev 29:1–5.
11. Bachiller D, Schellander K, Peli J, Ruther U. 1991. Liposome-mediated DNA uptake by sperm cells. Mol Reprod Dev 30:194–200.
12. Gagne MB, Pothier F, Sirard MA. 1991. Electroporation of bovine spermatozoa to carry foreign DNA in oocytes. Mol Reprod Dev 29: 6–15.
13. Gavora JS, Benkel B, Sasada H, Cantwell WJ, Fiser P, et al. 1991. An attempt at sperm-mediated gene transfer in mice and chickens. Can J Anim Sci 71:287–291.
14. Gorlova AV, Torchilin VP. 1991. Use of liposomes to associate foreign genetic material with spermatozoa. Bull Exp Biol Med 112:1309–1311.
15. Horan R, Powell R, McQuaid S, Gannon F, Houghton JA. 1991. Association of foreign dna with porcine spermatozoa. Arch Androl 26:83–92.
16. Camaioni A, Russo MA, Odorisio T, Gandolfi F, Fazio VM, et al. 1992. Uptake of exogenous DNA by mammalian spermatozoa—specific localization of DNA on sperm heads. J Reprod Fertil 96:203–212.
17. Horan R, Powell R, Gannon F, Houghton JA. 1992. The fate of foreign DNA associated with pig sperm following the in vitro fertilization of zona-free hamster ova and zona-intact pig ova. Arch Androl 29:199–206.
18. Horan R, Powell R, Bird JM, Gannon F, Houghton JA. 1992. Effects of electropermeabilization on the association of foreign DNA with pig sperm. Arch Androl 28:105–114.
19. Khoo HW, Ang LH, Lim HB, Wong KY. 1992. Sperm cells as vectors for introducing foreign DNA into zebrafish. Aquaculture 107:1–19.
20. Lavitrano M, French D, Zani M, Frati L, Spadafora C. 1992. The Interaction between exogenous DNA and sperm cells. Mol Reprod Dev 31: 161–169.
21. Rottmann OJ, Antes R, Hofer P, Maierhofer G. 1992. Liposome mediated gene transfer via spermatozoa into avian egg cells. J Anim Breed Genet 109:64–70.
22. Francolini M, Lavitrano M, Lamia CL, French D, Frati L, et al. 1993. Evidence for nuclear internalization of exogenous dna into mammalian sperm cells. Mol Reprod Dev 34:133–139.
23. Nakanishi A, Iritani A. 1993. Gene transfer in the chicken by sperm-mediated methods. Mol Reprod Dev 36:258–261.
24. Sin FYT, Bartley AL, Walker SP, Sin IL, Symonds JE, et al. 1993. Gene transfer in chinook salmon (Oncorhynchus tshawytscha) by electroporating sperm in the presence of pRSV-lacZ DNA. Aquaculture 117: 57–69.
25. Symonds JE, Walker SP, Sin FYT. 1994. Electroporation of salmon sperm with plasmid DNA—evidence of enhanced sperm DNA association. Aquaculture 119:313–327.
26. Chan PJ, Kalugdan T, Su BC, Whitney EA, Perrott W, et al. 1995. Sperm as a noninvasive gene delivery system for preimplantation embryos. Fertil Steril 63:1121–1124.
27. Patil JG, Khoo HW. 1995. Ultrastructural in-situ hybridization and autoradiographic detection of foreign DNA in zebrafish spermatozoa. Biochem & Mol Biol Int 35:965–969.
28. Schellander K, Peli J, Schmoll F, Brem G. 1995. Artificial insemination in cattle with DNA-treated sperm. Anim Biotechnol 6:41–50.
29. Sowa S, Graham JK, Barisas BG. 1995. Binding of protein-targeted liposomes to mammalian spermatozoa. FASEB J 9:A80.
30. Tsai HJ, Tseng FS, Liao IC. 1995. Electroporation of sperm to introduce foreign DNA into the genome of loach (Misgurnus anguillicaudatus). Can J Fisheries & Aquatic Sci 52:776–787.
31. Zani M, Lavitrano M, French D, Lulli V, Maione B, et al. 1995. The Mechanism of binding of exogenous DNA to sperm cells—factors controlling the DNA uptake. Exp Cell Res 217:57–64.
32. Chan PK, Kalugdan T, Cabrera M, Seraj IM, King A. 1996. Detection of exogenous DNA in blastocysts after continuous exposure to DNA carrier sperm. J Assist Reprod Genet 13:602–605.
33. Habrova V, Takac M, Navratil J, Macha J, Ceskova N, et al. 1996. Association of Rous sarcoma virus DNA with Xenopus laevis spermatozoa and its transfer to ova through fertilization. Mol Reprod Dev 44: 332–342.
34. Patil JG, Khoo HW. 1996. Nuclear internalization of foreign DNA by zebrafish spermatozoa and its enhancement by electroporation. J Exp Zool 274:121–129.
35. Rottmann O, Antes R, Hofer P, Sommer B, Wanner G, et al. 1996. Liposome-mediated gene transfer via sperm cells. High transfer efficiency and persistence of transgenes by use of liposomes and sperm cells and a murine amplification element. J Anim Breed Genet 113: 401–411.
36. Sperandio S, Lulli V, Bacci ML, Forni M, Maione B, et al. 1996. Sperm-mediated DNA transfer in bovine and swine species. Anim Biotechnol 7:59–77.
37. Cabrera M, Chan PJ, Kalugdan TH, 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. J Assist Reprod Genet 14:120–124.
38. Chen YL, Tsai HJ. 1997. Effect of electroporation conditions on loach sperm for successful gene transfer and early development. Fisheries Sci 63:527–532.
39. Lavitrano M, Maione B, Forte E, Francolini M, Sperandio S, et al. 1997. The interaction of sperm cells with exogenous DNA: A role of CD4 and major histocompatibility complex class II molecules. Exp Cell Res 233: 56–62.
40. Macha J, Stursova D, Takac M, Habrova V, Jonak J. 1997. Uptake of plasmid RSV DNA by frog and mouse spermatozoa. Folia Biol 43:123–127.
41. Maione B, Pittoggi C, Achene L, Lorenzini R, Spadafora C. 1997. Activation of endogenous nucleases in mature sperm cells upon interaction with exogenous DNA. DNA Cell Biol 16:1087–1097.
42. Tsai HJ, Lai CH, Yang HS. 1997. Sperm as a carrier to introduce an exogenous DNA fragment into the oocyte of Japanese abalone (Haliotis divorsicolor suportexta). Transgenic Res 6:85–95.
43. Zoraqi G, Spadafora C. 1997. Integration of foreign DNA sequences into mouse sperm genome. DNA Cell Biol 16:291–300.
44. Huguet E, Esponda P. 1998. Foreign dna introduced into the vas deferens is gained by mammalian spermatozoa. Mol Reprod Dev 51: 42–52.
45. Maione B, Lavitrano M, Spadafora C, Kiessling AA. 1998. Sperm-mediated gene transfer in mice. Mol Reprod Dev 50:406–409.
46. Perry ACF, Wakayama T, Kishikawa H, Kasai T, Okabe M, et al. 1999. Mammalian transgenesis by intracytoplasmic sperm injection. Science 284:1180–1183.
47. Sarangi N, Mandall AB, Bandyopadhyay AK, Venugopal T, Mathavan S, et al. 1999. Electroporated sperm mediated gene transfer in Indian major carps. Asia Pac J Mol Biol Biotechnol 7:151–158.
48. Sato M, Gotoh K, Kimura M. 1999. Sperm-mediated gene transfer by direct injection of foreign DNA into mouse testis. Transgenics 2:357–369.
49. Sato M, Yabuki K, Watanabe T, Kimura M. 1999. Testis-mediated gene transfer (TMGT) in mice: Successful transmission of introduced DNA from F0 to F2 generations. Transgenics 3:11.
50. Chan AWS, Luetjens CM, Dominko T, Ramalho-Santos J, Simerly CR, et al. 2000. TransgenICSI reviewed: Foreign DNA transmission by intracytoplasmic sperm injection in Rhesus monkey. Mol Reprod Dev 56:325–328.
51. Chan AWS, Luetjens CM, Dominko T, Ramalho-Santos J, Simerly CR, et al. 2000. 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 6:26–33.

52. Huguet E, Esponda P. 2000. Generation of genetically modified mice by spermatozoa transfection in vivo: Preliminary results. Mol Reprod Dev 56:243–247.
53. Jonak J. 2000. Sperm-mediated preparation of transgenic Xenopus laevis and transmission of transgenic DNA to the next generation. Mol Reprod Dev 56:298–300.
54. Khoo HW. 2000. Sperm-mediated gene transfer studies on zebrafish in Singapore. Mol Reprod Dev 56:278–280.
55. Rieth A, Pothier F, Sirard MC. 2000. Electroporation of bovine spermatozoa to carry DNA containing highly repetitive sequences into oocytes and detection of homologous recombination events. Mol Reprod Dev 57:338–345.
56. Robinson KO, Ferguson HJ, Cobey S, Vaessin H, Smith BH. 2000. Sperm-mediated transformation of the honey bee, Apis mellifera. Insect Mol Biol 9:625–634.
57. Sciamanna I, Piccoli S, Barberi L, Zaccagnini G, Magnano AR, et al. 2000. DNA dose and sequence dependence in sperm-mediated gene transfer. Mol Reprod Dev 56:301–305.
58. Sin FYT, Walker SP, Symonds JE, Mukherjee UK, Khoo JGI, et al. 2000. Electroporation of salmon sperm for gene transfer: Efficiency, reliability, and fate of transgene. Mol Reprod Dev 56:285–288.
59. Lai LX, Sun QY, Wu GM, Murphy CN, Kuhholzer B, et al. 2001. Development of porcine embryos and offspring after intracytoplasmic sperm injection with liposome transfected or non-transfected sperm into in vitro matured oocytes. Zygote 9:339–346.
60. Wang HJ, Lin AX, Zhang ZC, Chen YF. 2001. Expression of porcine growth hormone gene in transgenic rabbits as reported by green fluorescent protein. Anim Biotechnol 12:101–110.
61. Yonezawa T, Furuhata Y, Hirabayashi K, Suzuki M, Takahashi M, et al. 2001. Detection of transgene in progeny at different developmental stages following testis-mediated gene transfer. Mol Reprod Dev 60: 196–201.
62. Celebi C, Auvray P, Benvegnu T, Plusquellec D, Jegou B, et al. 2002. Transient transmission of a transgene in mouse offspring following in vivo transfection of male germ cells. Mol Reprod Dev 62:477–482.
63. Lavitrano M, Bacci ML, Forni M, Lazzereschi D, Di Stefano C, et al. 2002. Efficient production by sperm-mediated gene transfer of human decay accelerating factor (hDAF) transgenic pigs for xenotransplantation. Proc Natl Acad Sci USA 99:14230–14235.
64. Lu JK, Fu BH, Wu JL, Chen TT. 2002. Production of transgenic silver sea bream (Sparus sarba) by different gene transfer methods. Mar Biotechnol 4:328–337.
65. Sato M, Ishikawa A, Kimura M. 2002. Direct injection of foreign DNA into mouse testis as a possible in vivo gene transfer system via epididymal spermatozoa. Mol Reprod Dev 61:49–56.
66. Yonezawa T, Furahata Y, Hirabayashi K, Suzuki M, Yamanouchi K, et al. 2002. Protamine-derived synthetic peptide enhances the efficiency of sperm-mediated gene transfer using liposome–peptide–DNA complex. J Reprod Devel 48:281–286.
67. Zhong JY, Wang YP, Zhu ZY. 2002. Introduction of the human lactoferrin gene into grass carp (Ctenopharyngodon idellus) to increase resistance against GCH virus. Aquaculture 214:93–101.
68. Chang K, Qian J, Jiang M, Liu Y, Wu M, et al. 2002. Effective generation of transgenic pigs and mice by linker based sperm-mediated gene transfer. BMC Biotechnol 2:5.
69. Nagashima H, Fujimura T, Takahagi Y, Kurome M, Wako N, et al. 2003. Development of efficient strategies for the production of genetically modified pigs. Theriogenology 59:95–106.
70. Szczygiel MA, Moisyadi S, Ward WS. 2003. Expression of foreign DNA is associated with paternal chromosome degradation in intracytoplasmic sperm injection-mediated transgenesis in the mouse. Biol Reprod 68:1903–1910.
71. Wang HJ, Lin AX, Chen YF. 2003. Association of rabbit sperm cells with exogenous DNA. Anim Biotechnol 14:155–165.
72. Venugopal T, Anathy V, Kirankumar S, Pandian TJ. 2004. Growth enhancement and food conversion efficiency of transgenic fish Labeo rohita. J Exp Zool 301A:477–490.
73. Yang CC, Chang HS, Lin CJ, Hsu CC, Cheung JI, et al. 2004. Cock spermatozoa serve as the gene vector for generation of transgenic chicken (Gallus gallus). Asian Austral J Anim Sci 17:885–891.
74. Pittoggi C, Renzi L, Zaccagnini G, Cimini D, Degrassi F, et al. 1999. A fraction of mouse sperm chromatin is organized in nucleosomal hypersensitive domains enriched in retroposon DNA. J Cell Sci 112:3537–3548.
75. Giordano R, Magnano AR, Zaccagnini G, Pittoggi C, Moscufo N, et al. 2000. Reverse transcriptase activity in mature spermatozoa of mouse. J Cell Biol 148:1107–1113.
76. Temin HM, Mizutani S. 1998. RNA-dependent DNA polymerase in virions of Rous sarcoma virus. Rev Med Virol 8:6–8.
77. Baltimore D. 1970. RNA-dependent DNA polymerase in virions of RNA tumour viruses. Nature 226:1209–1211.
78. Ostertag EM, Kazazian HH. 2001. Biology of mammalian L1 retrotransposons. Ann Rev Genet 35:501–538.
79. Friedlander A, Patarca R. 1999. Endogenous proviruses. Crit Rev Oncog 10:129–159.
80. Deininger PL, Moran JV, Batzer MA, Kazazian HH. 2003. Mobile elements and mammalian genome evolution. Curr Opin Genet Dev 13: 651–658.
81. Orgel LE, Crick FH. 1980. Selfish DNA: the ultimate parasite. Nature 284:604–607.
82. Brosius J, Tiedge H. 1995. Reverse transcriptase: Mediator of genomic plasticity. Virus Genes 11:163–179.
83. Poznanski AA, Calarco PG. 1991. The expression of intracisternal-A particle genes in the preimplantation mouse embryo. Dev Biol 143: 271–281.
84. Packer AI, Manova K, Bachvarova RF. 1993. A discrete line-1 transcript in mouse blastocysts. Dev Biol 157:281–283.
85. Mwenda JM. 1993. Biochemical characterization of a reverse-transcriptase activity associated with retroviral-like particles isolated from human placental villous tissue. Cell Mol Biol 39:317–328.
86. Deragon JM, Sinnett D, Labuda D. 1990. Reverse-transcriptase activity from human embryonal carcinoma cells NTera2D1. EMBO J 9:3363–3368.
87. Martin SL. 1991. Ribonucleoprotein-Particles with Line-1 RNA in Mouse Embryonal Carcinoma Cells. Mol Cell Biol 11:4804–4807.
88. Kiessling AA, Crowell R, Fox C. 1989. Epididymis is a principal site of retrovirus expression in the mouse. Proc Natl Acad Sci USA 86:5109–5113.
89. DeHaven JE, Schwartz DA, Dahm MW, Hazard ES, Trifiletti R, et al. 1998. Novel retroviral sequences are expressed in the epididymis and uterus of Syrian hamsters. J Gen Virol 79:2687–2694.
90. Branciforte D, Martin SL. 1994. Developmental and cell-type specificity of line-1 expression in mouse testis - implications for transposition. Mol Cell Biol 14:2584–2592.
91. Nilsson BO, Kattstrom PO, Sundstrom P, Jaquemin P, Larsson E. 1992. Human oocytes express murine retroviral equivalents. Virus Genes 6:221–227.
92. Miller D. 2000. Analysis and significance of messenger RNA in human ejaculated spermatozoa. Mol Reprod Dev 56:259–264.
93. Pittoggi C, Sciamanna I, Mattei E, Beraldi R, Lobascio AM, et al. 2003. Role of endogenous reverse transcriptase in murine early embryo development. Mol Reprod Dev 66:225–236.
94. Mangiacasale R, Pittoggi C, Sciamanna I, Careddu A, Mattei E, et al. 2003. Exposure of normal and transformed cells to nevirapine, a reverse transcriptase inhibitor, reduces cell growth and promotes differentiation. Oncogene 22:2750–2761.
95. Spadafora C. 2004. Endogenous reverse transcriptase: a mediator of cell proliferation and differentiation. Cytogenet Genome Res 105:346–350.
96. Pittoggi C, Zaccagnini G, Giordano R, Magnano AR, Baccetti B, et al. 2000. Nucleosomal domains of mouse spermatozoa chromatin as potential sites for retroposition and foreign DNA integration. Mol Reprod Dev 56:248–251.
97. Sciamanna I, Barberi L, Martire A, Pittoggi C, Beraldi R, et al. 2003. Sperm endogenous reverse transcriptase as mediator of new genetic information. Biochem Biophys Res Commun 312:1039–1046.
98. Kuznetsov AV, Kuznetsova IV, Schit IY. 2000. DNA interaction with rabbit sperm cells and its transfer into ova in vitro and in vivo. Mol Reprod Dev 56:292–297.
99. Kiessling U, Becker K, Strauss M, Schoeneich J, Geissler E. 1986. Rescue of a Tk plasmid from transgenic mice reveals its episomal transmission. Mol Gen Genet 204:328–333.

100. Elbrecht A, Demayo FJ, Tsai MJ, Omalley BW. 1987. Episomal maintenance of a bovine papilloma virus vector in transgenic mice. Mol Cell Biol 7:1276–1279.
101. Prak ETL, Dodson AW, Farkash EA, Kazazian HH. 2003. Tracking an embryonic L1 retrotransposition event. Proc Natl Acad Sci USA 100:1832–1837.
102. Speek M. 2001. Antisense promoter of human L1 retrotransposon drives transcription of adjacent cellular genes. Mol Cell Biol 21:1973–1985.
103. Kashkush K, Feldman M, Levy AA. 2003. Transcriptional activation of retrotransposons alters the expression of adjacent genes in wheat. Nat Genet 33:102–106.
104. Schramke V, Allshire R. 2003. Hairpin RNAs and retrotransposon LTRs effect RNAi and chromatin-based gene silencing. Science 301:1069–1074.
105. Huguet E, Esponda P. 2000. Generation of genetically modified mice by spermatozoa transfection in vivo: Preliminary Results. Mol Reprod Dev 56:243–247.
106. Waddington SN, Kennea NL, Buckley SM, Gregory LG, Themis M, et al. 2004. Fetal and neonatal gene therapy: benefits and pitfalls. Gene Ther 11Suppl 1:S92–S97.
107. Vasquez KM, Marburger K, Intody Z, Wilson JH. 2001. Manipulating the mammalian genome by homologous recombination. Proc Natl Acad Sci USA 98:8403–8410.
108. Riele HT, Maandag ER, Berns A. 1992. Highly efficient gene targeting in embryonic stem cells through homologous recombination with isogenic DNA constructs. Proc Natl Acad Sci USA 89:5128–5132.
109. Ward MA, Ward WS. 2004. A model for the function of sperm DNA degradation. Reproduction, Fertil Dev 16:547–554.
110. Baccetti B, Benedetto A, Burrini AG, Collodel G, Ceccarini EC, et al. 1994. HIV-particles in spermatozoa of patients with AIDS and their transfer into the oocyte. J Cell Biol 127:903–914.
111. Bobroski L, Bagasra AU, Patel D, Saikumari P, Memoli M, et al. 1998. Localization of human herpesvirus type 8 (HHV-8) in the Kaposi's sarcoma tissues and the semen specimens of HIV-1 infected and uninfected individuals by utilizing in situ polymerase chain reaction. J Reprod Immunol 41:149–160.
112. Ostertag EM, DeBerardinis RJ, Goodier JL, Zhang Y, Yang N, et al. 2002. A mouse model of human L1 retrotransposition. Nat Genet 32:655–660.
113. Moran JV, DeBerardinis RJ, Kazazian HH. 1999. Exon shuffling by L1 retrotransposition. Science 283:1530–1534.
114. Tomilin NV. 1999. Control of genes by mammalian retroposons. Int Rev Cytol 186:1–48.
115. Jordan IK, Rogozin IB, Glazko GV, Koonin EV. 2003. Origin of a substantial fraction of human regulatory sequences from transposable elements. Trends Genet 19:68–72.
116. Pittoggi C, Magnano AR, Sciamanna I, Giordano R, Lorenzini R, et al. 2001. Specific localization of transcription factors in the chromatin of mouse mature spermatozoa. Mol Reprod Dev 60:97–106.