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Rederivation of transgenic and gene-targeted mice by embryo transfer

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Abstract

Research on genetically engineered mice provides insights into the etiology, therapy, and genetic basis of human diseases. An important variable that affects the results of mouse studies is the health status of the animals. Pathogen burdens may confound observations and obscure underlying mechanisms. Mouse resource centers frequently rederive infected mouse strains. We review our experience on the use of a well-established technique, embryo transfer to rederive infected mouse strains. The following mouse pathogens were eliminated by embryo transfer: Mouse Parvovirus, Mouse Hepatitis Virus, Mouse Rotavirus, Mouse Encephalomyelitis Virus, Mouse Adenovirus, *Helicobacter* species, endoparasites, and ectoparasites. We rederived transgenic mouse lines, gene-targeted mouse lines, and lines with spontaneous mutations. In the majority of strains, fertilized eggs for embryo transfer were obtained by mating superovulated egg donors with males of the desired genotype. A total of 309 embryo transfers were performed to rederive 96 mouse strains. The pregnancy rate was 76%; 1996 pups were born, of which 43% carried the desired genotype. We performed 44 additional embryo transfers to rederive 15 other strains. The pregnancy rate was lower (45%) and none of the 135 pups carried the desired genotype. Although we successfully eliminated the pathogens in all transfers, we were unable to obtain pups with the desired genotype in 15 of 111 mouse lines. Multiple factors affect the efficiency of rederivation by embryo transfer. They include the response to superovulation by embryo donors, the number and age of stud males, the yield of fertilized eggs, the number of embryo transfers, and genotyping.

Introduction

Use of genetically engineered mice to understand human disease processes is increasing. It is important that these studies are conducted in specific pathogen free (SPF) mouse models. Animals need to be free of pathogen infections if physiological changes attributed to genetic alterations are to be extrapolated to other species. If the mouse models are pathogen infected their response to infection may mask genetic changes and/or complicate the interpretation of results. Viral, bacterial, and parasitic infections can interfere with research results in animal models (National Research Council, 1991, reviewed in Homberger et al., 1999). Consequently, it is frequently desirable to rederive mouse stocks to SPF status. This is often required to import animals into clean barrier facilities, to replace populations after a spontaneous infection of unknown etiology, or to eliminate pathogens that may interfere with research. We have extensive experience in pathogen free rederivation of mouse stocks. In our hands, the use of embryo transfer is a reliable and effective method of rederivation.

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The pathophysiological response to infection may obscure the consequences of genetic alterations. For example, viral infections cause immune system dysfunction (Jacoby et al., 1996; Homberger 1997). In addition to provoking physiological changes, the clinical presentation of viral infections can mimic phenotypes caused by experimental genetic changes in mice. For example, hindlimb paralysis occurs in alpha A-crystallin transgenic mice (De Rijk et al., 2000) and in mice infected with Murine Hepatitis Virus (MHV) (Glass et al., 2002) or Theilers Mouse Encephalomyelitis Virus (TMEV) (Tsunoda et al., 1996). In some cases, an infection is responsible for producing a phenotype in a gene-targeted mouse that would otherwise appear normal. Engle et al. (2002) reported that double mutant mice for Tgfb1 and Rag2 develop colitis and colon cancer if they are infected with *Helicobacter hepaticus* but remain free of inflammatory lesions and carcinoma in the absence of *H. hepaticus* organisms. Sensitive behavioral tests are also influenced by experimental viral infections. Reduced activity in open-field behavior tests occurred in v-Ha-ras transgenic mice (Colombo et al., 2000) and in mice experimentally infected with human influenza virus (Shi et al., 2003). Just as the presence of a pathogen can induce a phenotypic alteration, so can the treatment of mice for parasites. A common approach to pinworm infection is to treat mice with the anti-helmitic drug Ivermectin. Paradoxically, although this treatment effectively eliminates pinworm and its consequences, Ivermectin treatment alters the outcome of mouse behavior tests (Davis et al., 1999). Research on physiological changes attributed to experimentally induced genetic changes needs to be distinct from changes induced by pathogen infections. Careful health assessment of these animals is required to determine whether changes are due to genetic manipulation.

Rederivation of pathogen-infected mice by embryo transfer to pathogen free recipients is an effective method of disease elimination. Reetz et al., (1988) eliminated MHV and *Pasteurella pneumotropica* from six inbred strains. Morrel (1999) reported the elimination of MHV, rotavirus (Epizootic Diarrhea of Infant Mice Virus: EDIM), reo-3 virus, TMEV, and Mouse Adenovirus (MAD). Selecting embryos with intact zona pellucida (z.p.) for embryo transfer is important because the z.p. prevents contact between infectious viral particles and the cell membrane. Incubation of eggs with and without z.p. in Mouse Parvovirus (MPV) suspensions demonstrated that the z.p. prevents infection from occurring (Mohanty & Bachman, 1974). Similarly, both Reetz et al. (1988) and Carthew et al. (1985) incubated embryos with or without z.p. in MHV suspensions and observed that the z.p. prevents MHV infection. Indirect evidence suggests that the z.p. also protects embryos from TMEV since inoculation of pregnant female mice with the virus on days 1–3 of gestation (while preimplantation embryos are protected by the z.p.) does not result in virus positive fetuses although positive fetuses are found when virus is inoculated on gestational days 4 and 5 (after the z.p. is shed) (Abzug & Tyson, 2000). Hill and Stalley (1991) showed that *Mycoplasma pulmonis* could be eliminated from z.p. intact eggs by overnight culture in mouse embryo media, which does not support *M. pulmonis* growth. Serial dilution of preimplantation embryos collected from infected mice reduces the concentration of infectious organisms below the threshold of infection in the transfer fluid (Stringfellow, 1998). As a consequence, pups, born after transfer of washed embryos to SPF surrogate mothers, enjoy the same pathogen free status as their mothers.

We summarize our results on the effectiveness of mouse embryo transfer to rederive 111 genetically altered mouse strains with enzootic pathogen infections. Our results confirm the elimination of MHV, EDIM, TMEV, and MAD by embryo transfer. In addition, we show that this method effectively eliminates MPV, *Helicobacter* spp., *M. pulmonis*, endoparasites (pinworm), and ectoparasites (fur mites). We discuss our success rate and the factors that influence it. We compare embryo transfer rederivation with alternatives.

Methods and materials

Animals

Adult (6 weeks to 6 months old) (C57BL/6J X DBA/2J) F1 male and female mice were obtained from the Jackson Laboratory (Bar Harbor, Maine). Adult CD-1 male and female mice were obtained from Charles River Laboratory (Wilmington, Delaware). We used CD-1 mice to

produce pseudopregnant recipients until year 2000 and used (C57BL/6J X DBA/2J)F1 mice since then. Vasectomized males were housed one per cage and females were housed five per cage prior to embryo transfer. Vasectomized males were mated randomly with two or three females naturally cycling through estrus to produce pseudopregnant recipients for embryo transfers. Weanling (3 weeks old) egg donors (C57BL/6J, C3H/HeJ, DBA/2J, and 129S1/SvlmJ) were obtained from the Jackson Laboratory. Egg donors were superovulated and mated with infected males to produce fertilized mouse eggs. If there was a need to maintain the unique mixed genetic background of a mouse strain then both egg donors and stud males were obtained from the infected mouse colony. Static microisolater caging (Allentown Caging, Allentown, New Jersey) and ground corncob bedding were used for all animals. Access to water and food was *ad libitum*. The animals were fed Purina 5008 rodent diet. Animal rooms were climate controlled to provide temperatures of 22–23°C and relative humidity of 15% on a 12-h light/dark cycle (lights on at 0600). All procedures were approved by the University of Michigan Committee on Use and Care of Animals. Animal care was provided in accordance with the principles and procedures outlined in the National Research Council *Guide for the Care and Use of Laboratory Animals*.

Egg collection and washing

Superovulation was used to increase the numbers of eggs per donor and to reduce the total number of egg donors required. At 15:00, egg donors were injected intraperitoneally with 5 I.U. pregnant mare's serum gonadotropin obtained from the National Hormone and Peptide Program (National Institute of Diabetes and Digestive and Kidney Diseases). After 46 h, egg donors were injected intraperitoneally with 5 I.U. of human chorionic gonadotropin (HCG, Sigma Chemical Company). After HCG treatment, egg donors were taken from their pathogen free mouse room to the quarantine room and singly mated with infected stud males. Whenever possible, 3–6 males were mated so that there were enough fertilized eggs for transfer to multiple recipients. The following morning (09:00–11:00) egg donors were euthanized in the quarantine room and submerged in 3.3% bleach during transportation to the egg collection laboratory (5–8 mins). This treatment is expected to inactivate viruses, bacteria, parasites and their eggs (D. Ringler, personal communication). The eggs were stripped of cumulus granulosa cells by hyaluronidase treatment as described (Hogan et al. 1994). Fertilized eggs with intact zona pellucida were selected for subsequent washing and transfer. Eggs were sequentially washed five times in 1.0 ml drops of sterile M2 medium (Sigma Chemical Co., St. Louis, Missouri) with changes of sterile transfer pipets between drops. Eggs were washed through four drops of 0.075 ml $C0_2$ equilibrated BOMC medium (Invitrogen, Carlsbad, California) supplemented with 5000 I.U./ml penicillin and streptomycin (Invitrogen) with transfer pipet changes between drops. The eggs were incubated in BOMC at 37°C, 5% $C0_2$, 95% relative humidity until surgical transfer. The washing steps diluted the original volume of fluid containing the eggs by a factor of 1×10^{21}, which exceeds the 1×10^{20} dilution, recommended by the International Embryo Transfer Society (Stringfellow, 1998). For the elimination of *Mycoplasma pulmonis*, embryos were incubated overnight and transferred the following day (Hill & Stalley, 1991).

Embryo transfer and genotyping

Pseudopregnant females were identified by the presence of a copulation plug after mating with vasectomized males. Embryo recipients were anesthetized with ketamine/xylazine as described by Zeller et al. (1998). Egg transfer into oviducts was performed as described in Hogan et al. (1994). Each recipient received 20–25 one-cell egg divided between the two oviducts. When eggs were cultured overnight, 16–20 two-cell eggs were divided between the oviducts. Recipients were housed one or two per cage. When pups were 2 weeks of age, they were ear tagged (National Band and Tag, Newport, Kentucky) for tracking purposes and 5-mm tail tip biopsies were collected for DNA extraction. Each DNA sample was genotyped by a PCR assay for a specific transgene or targeted gene. All genotyping assays were performed by the research laboratories that

submitted animals for rederivation. When a genetic test was unavailable for the desired genetic background, offspring were bred to verify the transmission of the desired phenotype. This was often the case for mouse strains carrying spontaneous mutations that were molecularly uncharacterized or for which the genetic etiology had not been established.

Pathogen testing

Serum samples were collected from dams 6–8 weeks after the embryo transfer procedure. Immuncomb tests (Charles River Laboratory) were used on site (University of Michigan Unit for Laboratory Animal Medicine) to detect antibodies to MHV, Sendai virus, and *Mycoplasma pulmonis*. Cecal flotation was used to detect the presence of *Aspicularis tetraptera* (pinworm). *Myobia musculi* (fur mites) were detected by examining skin and fur. All other assays (MPV, EDIM, TMEV (GDVII), MAD, and *Helicobacter* spp.) were performed offsite as part of Health Assessment Plus testing at Charles River Laboratories.

Criteria for success

Three criteria were used to determine whether a mouse strain was successfully rederived as a SPF mouse line. (1) Viable animals of the desired genotype were produced. For transgenic or gene-targeted mouse strains crossed to standard inbred mouse lines, at least one male or female pup with the transgene was produced. For strains in mixed genetic backgrounds, at least one male and one female pup were produced to establish a breeding pair. (2) Embryo recipients were pathogen free 6–8 weeks after embryo transfer. (3) Subsequent surveillance of pups and their offspring demonstrated they continued to be pathogen free.

Results

MHV elimination

Over a 3-year period 42 embryo transfers were performed to successfully eliminate MHV from nine mouse strains backcrossed to C57BL/6 (two gene-targeted and seven transgenic lines). Fertilized eggs were obtained for transfer by mating pathogen free C57BL/6 females with infected stud males. In the successful rederivations, 81% of the embryo recipients became pregnant and delivered an average of seven pups (Table 1). MHV was eliminated in every case; however, pups with the desired genotype were not obtained from three strains.

MPV elimination

Over a 2-year period, 77 embryo transfers were performed to eliminate MPV from 47 mouse strains. One of these was a gene-targeted strain on a mixed 129/Sv genetic background, the other 46 were transgenic strains backcrossed to C57BL/6. Fertilized eggs were obtained by mating commercially procured pathogen-free C57BL/6 females to infected transgenic males. In order to maintain the unique mixed genetic background of the gene-targeted strain, infected embryo donors were superovulated and mated with infected stud males to obtain fertilized eggs. In the successful rederivations, 84% of the embryo recipients became pregnant and delivered an average of 10 pups (Table 2). MPV was eliminated in every case; however, pups with the desired genotype were not obtained from nine of the mouse strains.

Elimination of Helicobacter *species*

We rederived two mouse strains that were infected with *Helicobacter* species, as detected by diagnostic testing at Charles River Laboratory.

Table 1. Mouse hepatitis virus elimination by mouse embryo transfer

Egg donor strain	Strains rederived	Embryo transfer recipients	Number of pregnancies	Number of pups born	Pups with desired genotype
C57BL/6J	9	42	34 (81%)	252	102 (40%)
Unsuccessful rederivation attempts					
C57BL/6J	3	16	7 (44%)	41	0 (0%)

Table 2. Mouse parvovirus elimination by mouse embryo transfer

Egg donor strain	Strains rederived	Embryo transfer recipients	Number of pregnancies (%)	Number of pups born	Pups with desired genotype (%)
C57BL/6J	46	72	61 (85%)	610	225 (37%)
Mixed	1	5	4 (80%)	55	36 (65%)
Combined Total	47	77	65 (84%)	665	261 (39%)
Unsuccessful rederivation attempts					
C57BL/6J	9	17	12 (71%)	93	0 (0%)

One strain was a transgenic line on a mixed genetic background and the other was a spontaneous mutation in C57BL/6. In order to maintain their unique genetic backgrounds, infected embryo donors were superovulated and mated with infected stud males to obtain fertilized eggs for embryo transfer for both strains. Fertilized eggs were transferred to 12 recipients resulting in six pregnancies. A total of 48 pups were born. Since egg donors and male studs were from the same genetic background, all of the pups born carried the desired genotype. Thus, embryo transfer succeeded in the rederivation of both mouse strains that were positive for *Helicobacter* species.

Elimination of pinworm

We rederived three mouse strains with pinworm (*Aspicularis tetraptera*) infections, as diagnosed by flotation of cecal contents. One was a gene-targeted strain backcrossed to 129S1/SvImJ: the second was a gene-targeted strain backcrossed to C57BL/6 and the third was a transgenic strain backcrossed to C57BL/6. Fertilized eggs were transferred to 19 recipients resulting in sixteen pregnancies (84%) (Table 3). A total of 127 pups were born for an average of eight pups per dam; 51 of the pups had the desired genotype. Thus, embryo transfer successfully eliminated pinworm infections in all three lines.

Combined MHV, EDIM, and mite infections

We performed 154 embryo transfers to rederive 32 mouse strains that were infected with two viruses and an ectoparasite (Table 4). The founder colonies were seropositive for MHV and EDIM. In addition, inspection of pelage demonstrated the

Table 3. Mouse pinworm elimination by mouse embryo transfer

Egg donor strain	Strains rederived	Embryo transfer recipients	Number of pregnancies (%)	Number of pups born	Pups with desired genotype (%)
C57BL/6J	2	12	11 (92%)	80	32 (40%)
129S1/SvlmJ	1	7	5 (71%)	47	19 (40%)
Combined total	3	19	16 (84%)	127	51 (40%)

Table 4. Multiple pathogen elimination by mouse embryo transfer

Egg donor strain	Strains rederived	Embryo transfer recipients	Number of pregnancies (%)	Number of pups born	Pups with desired genotype (%)
C57BL/6J	28	138	98 (71%)	865	403 (47%)
C3H	1	6	3 (50%)	18	10 (56%)
CD-1	1	3	2 (67%)	11	5 (45%)
Mixed	2	7	6 (86%)	58	25 (43%)
Combined total	32	154	109 (71%)	952	443 (46%)
Unsuccessful rederivation attempts					
DBA/2	2	8	1 (12%)	1	0 (0%)
Mixed	1	3	0 (0%)	0	0 (0%)

presence of fur mites. Twenty-eight strains were backcrossed to C57BL/6, 15 were transgenic, nine were spontaneous mutations, and four were gene-targeted strains. One strain was a spontaneous mutant backcrossed to C3H/HeJ, one was a transgenic on the CD-1 background and two were a spontaneous mutants on mixed genetic backgrounds. In order to maintain their unique genetic backgrounds, infected embryo donors from mixed genetic backgrounds were superovulated and mated with infected stud males to obtain fertilized eggs for embryo transfer. In the successful rederivations, 71% of the embryo recipients became pregnant and delivered an average of nine pups (Table 4). All three pathogens were eliminated in each case; however, no pups of the desired genotype were obtained from three strains.

Combined MHV, MPV, EDIM, MAD, GDVII, mycoplasma and pinworm infection

We rederived three mouse strains that were infected with multiple pathogens: MHV, MPV, EDIM, MAD, GDVII, *Mycoplasma pulmonis*, and pinworm. These three transgenic lines were on unique mixed genetic backgrounds so that infected embryo donors were superovulated and mated with infected stud males to obtain fertilized eggs for embryo transfer. We collected the eggs and cultured them overnight to eliminate infectious mycoplasma (Hill & Stalley ,1991) prior to embryo transfer. Cleavage eggs that developed normally were transferred to five recipients. All five recipients became pregnant and 27 pups were born. Since the egg donors and male studs were both from the same mixed genetic background, all of the pups born carried the desired genotype. Because of the complex pathogen load in these mice, dams were sent to Charles River Laboratory for health assessment plus tests that showed the animals were pathogen free.

Discussion

We established a mouse pathogen rederivation program based on embryo transfer and show that it is effective for the elimination of mouse viral, bacterial, and parasitic infections. A total of 96 independent mouse strains were successfully rederived. Our embryo transfer procedures eliminated every mouse pathogen that we encountered and attempted to eradicate: MHV, MPV, EDIM, TMEV (GDVII), MAD, *Helicobacter* spp., *Mycoplasma pulmonis*, pinworm and fur mites. Although this is not a comprehensive list of all mouse pathogens, we eliminated three categories of organisms: viruses, bacteria, and parasites. It is likely that the application of embryo transfer to the rederivation of other mouse pathogens, such as protozoa, will be equally successful. Embryo transfer eliminates organisms from the environment of the preimplantation embryo by extensive washing in sterile media. Consequently, the pups that are born have the same health status as their surrogate mothers. In agreement with this, it was demonstrated that germ free mouse lines can be successfully derived by embryo transfer to germ free surrogate mothers (Okamoto & Matsumoto, 1999). We conclude that embryo transfer is an efficient method for the pathogen free rederivation of infected mouse lines.

We observed that in all embryo transfer recipients, pathogens were eliminated whether egg donors were obtained from an enzootically infected founder colony or imported from a commercial vendor. When we mated heterozygous transgenic or gene-targeted stud males to wild type egg donors, we expected half the pups to have the desired genotype. In the successful rederivation procedures, we observed that 857 (43%) of the 1996 pups born carried the desired genotype. This is fewer than expected ($p < 0.005$, χ^2 Test). The reasons for this lower than expected frequency of pups with the desired genotype is unclear. It may the result of technical problems (insensitive genotyping assays) or the reduced viability of genetically engineered pups.

In addition to the 96 successful rederivations, we were unable to rederive 15 mouse lines. These were characterized by the failure to identify rederived pups of the desired genotype and not by the failure to eliminate pathogens. Factors that reduced the efficiency of embryo transfer rederivation in the 15 unsuccessful attempts can be divided into logistical problems: e.g. only one stud male available for embryo production and biological problems, e.g. inefficient production of fertilized eggs for embryo transfer. The principle difference between successful and unsuccessful rederivation procedures was the availability of four

or more stud males with good reproductive performance. On several occasions, replacement of stud males older than 6 months with young animals changed unsuccessful attempts to successful ones. Initial compliance by laboratories to provide 4–6 stud males in prime reproductive condition minimized the time to complete successful rederivations. Of the 15 rederivations that did not produce pups with the desired genotype, nine depended on a single stud male, three depended on old males (>8 months) and three others depended on the use of strains that did not produced fertilized eggs in response to superovulation and stud mating (one mixed background and DBA/2). Another possible factor is the sensitivity and accuracy of genotyping assays, which we did not perform and thus are unable to assess. Similarly we are unable to directly assess the effects of genetic manipulations on prenatal viability of the transferred embryos. Additional studies are necessary to address this issue.

Factors that affect the success of embryo transfer can be divided into three areas: (1) technical competency in surgery, (2) production of fertilized eggs for transfer, (3) selection of surrogate mothers and appropriate husbandry, and (4) the sensitivity of genotyping assays to detect the desired genotype. The first, third, and fourth factors are easily controlled by providing training to staff and by selecting outbred or vigorous hybrid female recipients. The single most critical factor in post-surgery husbandry to maintain pregnancy and establish lactation is provision of appropriate rodent diet, containing 6.0–6.5% fat. Pregnancy and post-partum survival is severely reduced in surrogate dams fed diet containing 4.5% fat (T. Saunders, unpublished observations). The second factor, production of fertilized eggs, depends on biological factors that are not always under control of the investigator. For example, some inbred strains respond poorly to superovulation treatments (Spearow, 1988) and produce few eggs for fertilization. Other inbred strains, such as PL/J have poor sperm quality that affect numbers of fertilized eggs (Pyle & Handel, 2003; T. Saunders, unpublished observations). A less quantifiable factor is embryo sensitivity to experimental manipulation. In particular, DBA/2 rederivation is consistently difficult due to low birth rates after embryo transfer (Table 4 and unpublished observations). Transgenic offspring are generally identified by PCR reactions that detect unique DNA sequences in the transgene. Our experience with numerous research laboratories indicates that genotype assignations are conservative, designed to exclude borderline results and to accept only robust positive reactions. Routine fluctuations in PCR assays can affect genotyping accuracy, particularly in the absence of rigorous quality control procedures. Factors that affect genotyping reproducibility include: (1) extraction of pure DNA from tail tip biopsies, (2) determination of the limit of PCR sensitivity (does the assay detect transgene DNA with single copy gene equivalency?), (3) appropriate positive and negative controls to demonstrate that mouse DNA is present in the assay, and (4) the absence of transgene DNA contamination. The majority of mouse rederivations proceed smoothly and successfully when all of these variables are controlled.

In the few cases, where mice are not rederived by embryo transfer the most common problem is obtaining fertilized eggs for embryo transfer. Often this is resolved by increasing the number of stud males to four and/or replacing old stud males with animals in their reproductive prime (2–6 months old). If this does not result in fertilized eggs then alternative methods to obtain fertilized eggs include: *in vitro* fertilization (IVF), natural mating, and intracytoplasmic sperm injection (ICSI). IVF procedures can yield hundreds of fertilized eggs for embryo transfer from a single procedure with sperm pooled from a few males (Thornton et al., 1999). Suzuki et al. (1996) successfully eliminated MHV and *Pasteurella pneumotropica* with IVF followed by embryo transfer. Fewer eggs are produced by natural mating than by superovulation. The need for several females to ovulate spontaneously at the same time increases the number of animals required for fertilized egg production by natural mating. If natural mating is not effective due to low sperm counts then ICSI can be used because few individual sperm are needed to produce fertilized eggs by this method. This approach may not be suitable when specific genetic backgrounds must be rederived because ICSI is significantly less efficient in inbred strains than in hybrid strains (Kawase et al., 2001). The alternative to rederivation by embryo transfer is rederivation by hysterectomy. This approach can be used with strains of very low male and female fertility or for

mouse embryos that do not survive well in culture. Hysterectomy relies on the production of synchronized pregnancies between the strain undergoing rederivation and an SPF mouse colony that is used for fostering pups. The disadvantages include the loss of viable pups during hysterectomy, the increased probability of infecting pups or surrogate mothers during the procedure, the elimination of pups from the SPF litter, and the loss of pups ignored by SPF surrogates. Although animal-intensive hysterectomy may be indicated when other rederivation procedures are ineffective.

In the context of animal welfare principles (Flecknell, 2002) pathogen rederivation offers refinement of experimental design by reducing pain and distress. It can be anticipated that healthy, SPF mice will more faithfully reflect physiological changes due to genetic alterations than diseased mice. This, in turn, can reduce variability in experimental groups and the number of animals needed to draw statistically meaningful conclusions. In comparison to other methods, embryo transfer rederivation reduces the number of animals required for rederivation. In the average rederivation procedure, we used four superovulated egg donors. IVF and ICSI procedures often require 10–20 females per procedure. Hysterectomy requires multiple synchronized litters of infected and SPF mice. Some pups from infected litters do not survive the procedure and pups are lost from SPF litters when they are replaced by rederivation pups. As a consequence, hysterectomy is the most animal intensive rederivation procedure.

Embryo transfer is an effective method to eliminate pathogens from genetically engineered mouse strains. The study of pathogen free animals eliminates the confounding effects of pathogen infection from research results. Further studies are needed to determine if lower than expected transmission ratios of transgenes and mutant alleles are due to inaccurate genotyping assays or reduced embryo survival. Future directions include the wider application of this method to additional pathogens and to the establishment of germ free mouse strains.

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