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Mutational Insertion of a *ROSA26–EGFP* Transgene Leads to Defects in Spermiogenesis and Male Infertility in Mice

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Pronuclear injection has been a successful strategy for generating genetically engineered mouse models to better understand the functionality of genes. A characteristic of pronuclear injection is that random integration of the transgene into the genome can disturb a functional gene and result in a phenotype unrelated to the transgene itself. In this study, we have characterized a mouse model containing an insertional mutation that, in the homozygous state, severely affects spermatogenesis as characterized by lack of sperm motility and acrosomal aplasia. Whereas homozygous female mice had normal fertility, male mice homozygous for the insertional mutation were unable to produce pups by natural mating with either homozygous or wild-type female mice. No fertilized embryos were produced by matings to homozygous male mice, and no sperm were present in the reproductive tract of mated female mice. Spermatozoa isolated from homozygous male mice exhibited head and midpiece defects, but no major defects in the principal piece of these sperm. Histologic examination and immunohistochemical staining of the testes revealed vacuolar degeneration of Sertoli cells and loss of structural seminiferous tubule integrity and organization, indicating that spermatogenesis is severely affected in this mouse model. Although the males are always infertile, the severity of the histologic and sperm morphologic defects appeared to be age-related.

Abbreviations: EGFP, enhanced green fluorescent protein; GOPC, Golgi-associated protein.

The production of genetically engineered mice has enabled unprecedented advancements in understanding the functionality of genes. Genetically engineered mice can be produced in many ways, but pronuclear injection has been the standard method for many years.[2,4,7,23] Despite its success, pronuclear injection is associated with several problems. One important problem is that random integration of the transgene into the genome can disturb a functional gene and lead to associated problems. However, as we present here, random insertion of the transgene sometimes can result in interesting and important discoveries about gene function.

Numerous reports describe problems in spermatogenesis associated with transgene insertion,[10,16,18,24] and these mice have provided powerful research tools with which to study the complexities of male infertility and the production and maturation of spermatozoa. Here we characterize the male infertility phenotype of the FVB/NTac-Tg(Gt(ROSA)26Sor-EGFP)130910Eps/Mmmh strain[13] The original mouse strain was donated to the University of Missouri Mutant Mouse Regional Resource Center (http://www.mmrrc.org) and assigned designation MMRRC:000366. The founder animal for this strain was generated by random insertion of a transgene construct containing the enhanced green fluorescent protein (EGFP) under control of the mouse ROSA 26 promoter by pronuclear injection of FVB/NTac embryos. The strain has since been maintained for more than 20 generations by backcrossing to FVB/NTac mice. Efforts to cryopreserve the strain revealed that male mice homozygous for the transgene insertion were infertile, thereby leading to the studies presented here.

To determine the precise site of integration of the transgene, a chromosome-walking technique[3] was used and revealed a single integration site on chromosome 3 within the intronic region of a novel gene (ENSMUSG00000027939). Based on the predicted protein sequence for this gene, it represents a novel nucleoporin with shared similarity to members of the nuclear pore membrane glycoprotein 210 family. Nucleoporins are protein components of the nuclear pore complex and play a key role in nucleocytoplasmic transport. Importantly in the context of the infertility phenotype described for this mouse model, we speculate that the gene disrupted by the mutational insertion may be involved in nucleocytoplasmic trafficking, which is important for male germ cell differentiation.

In this report, we describe the infertility phenotype of the FVB/NTac-Tg(Gt(ROSA)26Sor-EGFP)130910Eps/Mmmh strain (*ROSA–EGFP*) and document age-related sperm defects and Sertoli cell abnormalities that underlie the male infertility seen in animals homozygous for the insertional mutation. These mice exhibited severe defects in spermatogenesis, as manifested by sperm head and midpiece abnormalities and a loss of sperm motility. In addition, we discuss the results of the histologic examinations, which revealed vacuolar degeneration of Sertoli cells, rare multinucleated cells, and reduced numbers of all stages of germinal cells in the seminiferous tubules as well as occasional vacuolation of the epididymal epithelium. The sperm head defects coupled with the Sertoli cell degeneration suggest possible disruption of

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normal Sertoli cell–spermatid interactions in homozygotes for this transgene insert. Furthermore matings with homozygous male mice failed to produce any pups, whereas heterozygous and homozygous female mice bred with wild-type male mice had normal litter sizes. Functional analysis of the homozygous male mice may provide additional insight into the molecular mechanisms of spermiogenesis, particularly those involving acrosomal biogenesis and the development of functional flagella.

Materials and Methods

Chemicals. All chemicals were purchased from Sigma (St Louis, MO) unless otherwise stated.

Animals. All animals were maintained in accordance with the policies of the University of Missouri Animal Care and Use Committee and the *Guide for the Care and Use of Laboratory Animals*.[21] Animals were housed in ventilated cages, fed a standard pelleted rodent chow, and housed in an environmentally controlled room with a 14:10-h light:dark cycle. FVB/NTac-Tg(Gt(ROSA)26Sor-EGFP)130910Eps/Mmmh (abbreviated as *ROSA–EGFP*) mice were imported into the University of Missouri's Mutant Mouse Regional Resource Center and rederived by embryo transfer. The rederived colony was maintained as specific pathogen-free for endoparasites, ectoparasites, *Helicobacter* spp., *Mycoplasma pulmonis*, *Citrobacter rodentium*, *Corynebacterium kutscheri*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Pasteurella multocida*, *Pasteurella pneumotropica*, *Salmonella* spp., *Streptococcus pneumoniae*, β-hemolytic *Streptococcus* spp., cilia-associated respiratory bacillus, ectromelia virus, mouse rotavirus, Theiler murine encephalomyelitis virus, mouse adenovirus types 1 and 2, lymphocytic choriomeningitis virus, lactate dehydrogenase-elevating virus, murine cytomegalovirus, mouse hepatitis virus, minute virus of mice, mouse norovirus, mouse parvovirus, murine polyoma virus, pneumonia virus of mice, reovirus 3, and Sendai virus.

All FVB/NTac mice (referred to as FVB throughout this paper) were purchased from Taconic (Germantown, NY). The *ROSA–EGFP* mouse breeding colony was maintained by mating hemizygous male mice to FVB female mice. To generate the homozygous male and female mice used in this study, hemizygous male and female mice were mated and offspring were genotyped to determine whether they were wild-type (0 copies of the transgene), hemizygous (1 copy), or homozygous (2 copies) for the transgene insertion.

Genotyping. DNA was extracted (Dneasy Tissue Kit, Qiagen, Valencia, CA) according to the manufacturer's instructions from tail samples collected at 3 wk of age, with a single elution volume of 200 µL. Genotyping was performed by PCR analysis by using primers synthesized by Integrated DNA Technology (Coralville, IA) with sequences as follows: ROSA26 s1 F, 5′ TCT CAG ACT AGC CAG AGC TAT ACA 3′; ROSA26 s1 R, 5′ CTG GCT TTC TTC CCT CAT TTC CCT 3′; and ROSA26 3F3, 5′ CAC TGC ATT CTA GTT GTG GTT TGT CC 3′. The PCR reaction mixture consisted of a total volume of 20 µL and contained 1 µL of the eluted DNA (approximately 25 ng), 2 µL 10× FastStart *Taq* Buffer with 20 mM $MgCl_2$ (Roche, Indianapolis, IN), 0.2 mM of each dNTP, 0.375 µM of each primer, and 1 U FastStart *Taq* DNA polymerase (Roche). Cycling conditions were: 94 °C for 5 min; 35 cycles of 94 °C for 30 s, 61 °C for 30 s, and 72 °C for 1 min; and 72 °C for 7 min. Amplification products underwent 3% agarose gel electrophoresis, ethidium bromide staining, and visualization by UV light. Animals lacking the transgene insertion produce 375-bp amplicons, animals homozygous for the transgene insertion produce 2 amplicons (200 and 280 bp), and hemizygous animals produce 3 amplicons (200, 280, and 375 bp).

Natural mating. Homozygous female mice (age, 8 to 10 wk) were cohoused with homozygous male mice. After 10 wk of cohousing, homozygous female and male mice were cohoused with wild-type male and female mice, respectively. The litters produced by either homozygous or wild-type mating were recorded. Homozygous animals producing no litters then were mated with wild-type animals to produce early-stage (day 1) embryos to determine whether the infertility was due to a lack of fertilization, implantation, or improper spermatogenesis.

Superovulation and embryo collection. FVB female mice (n = 24) were superovulated with intraperitioneal administration of 5 IU PMSG (National Hormone and Peptide Program, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD) followed 48 h later with 5 IU human chorionic gonadotropin (Calbiochem, San Diego, CA). Mice were cohoused (1 female per male mouse) with either a homozygous or FVB male mouse (4 male mice per strain were used). The morning after mating (E0.5), female mice were checked for the presence of a copulation plug as an indicator of mating. Pronuclear-stage embryos were collected in FHM medium[20] at 37 °C, and fertilization rates (that is, number of embryos with 2 pronuclei divided by the total number of embryos) were recorded.

Collection and processing of mouse spermatozoa. Male mice (age, 8 to 10 wk and 8 to 10 mo) were euthanized, the cauda epididymides and vas deferens were excised, and spermatozoa were collected by applying gentle pressure with forceps that were run lengthwise along the epididymis to expel the sperm into a 35-mm dish (Falcon 1008, Fisher Scientific, PA) containing 2 mL TL-HEPES medium[20] supplemented with 2 mg/mL bovine serum albumin (catalog no. A3311, Sigma). The motility of the sperm samples was analyzed by using a computer-assisted semen analysis system (Hamilton Thorne, Beverly MA). Samples then were fixed in 4% paraformaldehyde, and slides were prepared for morphologic analysis. For morphologic, a analysis a total of 100 sperm were counted per slide.

Group 1 male mice were 8- to 10-mo-old *ROSA–EGFP* homozygous male mice, which were compared with the 8- to 10-mo-old FVB male mice that were used for the breeding experiments. Group 2 male mice were 8- to 10-wk-old *ROSA–EGFP* homozygous male mice, which were compared with wild-type littermates for analysis.

Histologic examination. Two homozygous 8- to 10-mo-old male mice were euthanized, and the following tissues were fixed in neutral buffered 10% formalin: bulbourethral gland, coagulating gland, preputial gland, prostate, vesicular gland, pituitary, testes, epididymides, urethra, and bladder. Fixed tissues were embedded in paraffin, and 6-µm sections were stained with hematoxylin and eosin and examined. Testes and epididymides from an additional 4 homozygous male mice were collected subsequently and similarly examined. In addition, wild-type and homozygous 8- to 10-wk-old male mice (n = 12) were euthanized, and testes and epididymides were fixed in modified Davidson medium.[14]

Immunohistochemical staining for GATA4 and visualization of Sertoli cell nuclei. The GATA4 immunohistochemistry procedures used in this investigation were modified from previously described methods.[6,17] Paraffin sections (thickness, 5 µm) of testes were floated onto positively charged slides (Fisher Scientific, Pitts-

burgh, PA) and deparaffinized. A steam-induced epitope retrieval procedure was used, which involved immersion of slides in 0.01 M citrate buffer solution (pH 6.0), steaming for 20 min, and subsequent cooling at room temperature for 20 min. Testicular sections were rinsed with water and subsequently immersed for 5 min in a buffer containing 50 mM Tris-HCl, 150 mM NaCl, and 0.05% Tween 20 (pH 7.6). Endogenous peroxidase activity was blocked by incubating tissue sections in 3% H_2O_2 for 15 min at room temperature, followed by rinses with a buffer containing 50 mM Tris-HCl, 150 mM NaCl, and 0.05% Tween 20 (pH 7.6) and incubation for 5 min with Protein Block (Dako, Carpinteria, CA). The C terminus of GATA4 is highly conserved among several mammalian species, including humans, rats, mice, and pigs,[17] and the primary antibody used for immunolocalization of GATA4 in this study was a rabbit polyclonal antisera raised against an epitope of human origin corresponding to C-terminal amino acids 328 to 439 of GATA4 (H112, Santa Cruz Biotechnology, Santa Cruz, CA).[6] Testicular sections were incubated for 30 min at room temperature with a 1:100 dilution of this antibody. Negative controls for these procedures consisted of nonimmune rabbit IgG substituted for the primary antisera. After incubation with the GATA4 antisera and subsequent washing with Tris buffer, sections of testes were incubated (rabbit EnVision+ System, Dako, Carpinteria, CA) for 30 min to enhance the immunospecificity of the primary antibody and to reduce background staining. Visualization of the bound primary antibody for GATA4 was facilitated by incubation of the testicular sections for 10 min with NovaRED substrate (Vector Laboratories, Burlingame, CA). Subsequent counterstaining for 1 min with Mayer hematoxylin (Newcomer Supply, Middleton, WI) was followed by dehydration of the testicular sections and application of cover slips according to standard histology procedures. Sections of mouse testes stained for GATA4 were examined at ×200 and ×400 (BX41, Olympus America, Center Valley, PA). Images of hematoxylin-and-eosin- and GATA4-stained sections were captured by using a video camera and (SPOT Insight FireWire Color Mosaic camera, Diagnostic Systems, Sterling Heights, MI). Final images for this manuscript were generated by using Adobe Photoshop CS2 (Adobe Systems, San Jose, CA).

Results

Natural matings. In an effort to breed the FVB/NTac-Tg(Gt(ROSA)26Sor-EGFP)130910Eps/Mmmh colony to homozygosity to simplify colony maintenance, three 8- to 10-wk-old pairs of homozygous *ROSA–EGFP* mice were mated for 10 wk. During this time, no litters were produced. However, litters were produced from all 3 homozygous female mice within 3 to 4 wk of pairing them with wild-type FVB male mice and yielded an average litter size of 7 pups per female mouse. This litter size does not statistically ($P = 0.564$) differ from that of 8 pups per female mouse seen when hemizygous male mice are mated to FVB female mice. Even though the homozygous female mice produced litters, none of the 3 original homozygous *ROSA–EGFP* male mice placed into breeding with FVB female mice produced any offspring over a 12-wk time period. An additional 4 homozygous male mice were paired with FVB female mice for 12 wk, and no offspring where obtained from these matings. In contrast to the homozygous male mice, the hemizygous male mice were able to produce normal litters when mated to either FVB female mice (litter size [mean ± 1 SD], 8 ± 1.6 pups) or hemizygous female mice (9 ± 1.4 pups). With the inability of the homozygous male mice to produce a litter after several weeks in breeding, we still did not know the cause of the infertility seen in these male mice—the infertility could have been due to several factors, such as lack of fertilization, lack of implantation, and severely impaired spermatogenesis. We therefore placed homozygous *ROSA–EGFP* male mice into breeding for the collection of early (day 1) embryos, thereby allowing us to determine whether lack of fertilization was occurring.

Mating performance and embryo production. Zygotes were collected from superovulated female mice on E0.5 for evaluation of the fertilization capability of homozygous *ROSA–EGFP* male mice. In the FVB female mice mated to the FVB male mice (4 male mice evaluated; 1 female per male; 3 repetitions), all 12 females were positive for a copulation plug on the morning after mating. However, among the FVB female mice mated to the homozygous male mice (4 male mice; 1 female per male mouse; 3 repetitions), only 6 female mice were positive for the copulation plug. Three of the 4 homozygous males produced at least 1 copulation plug. Although the homozygous male mice mated as evidenced by the formation of copulatory plugs, none of the 169 embryos collected from these matings were fertilized. In contrast, 118 fertilized embryos were collected when mated to wild-type FVB males. In addition, uteri and oviducts were flushed, and there was no evidence of sperm in the female reproductive tract after mating to the homozygous males. These findings, coupled with the normal breeding of the homozygous female mice led us to believe that the infertility affected the male homozygous *ROSA–EGFP* mice.

Sperm morphology and concentration. We initially attempted to collect sperm from homozygous male *ROSA–EGFP* mice for motility and morphology analysis by using a swim-out technique. However, because this method was unsuccessful (no spermatozoa were recovered), we switched to manual collection. Sperm samples were collected from the four 8- to 10-mo-old homozygous male mice used in the breeding and superovulation experiments and 2 FVB males. The spermatozoa collected from 3 homozygous male mice had zero motility immediately after collection; spermatozoa were not recovered from the fourth male mouse. In addition, collected spermatozoa from the 3 male mice were analyzed for morphology and demonstrated similar defects. Approximately 80% of the sperm from the homozygous males were abnormal, exhibiting head defects and head-to-tail fusions (Figure 1 A, B). Many of the defects were head defects and included lack of the characteristic hook-like appearance and incomplete formation of the acrosome (Figure 1). In addition, many of the sperm also had midpiece defects, in which a small piece was missing (Figure 1 A).

To examine whether defects were present in younger animals, sperm analysis was performed on eight 8- to 10-wk-old male mice. There was no difference in sperm concentration between the wild-type and homozygous males (data not shown). However, the percentage of motile sperm from the homozygous mice was 14%, compared with 65% for wild-type littermates, and more than 80% of the sperm from these younger homozygous male mice demonstrated abnormal morphology, whereas wild-type littermates had less than 5% abnormal sperm (data not shown) .

Homozygous male mice showed an age-related increase in the severity of the sperm morphologic abnormalities. Whereas younger male mice had the same percentage of abnormal sperm as did older males, the severity of the abnormalities was greater in the 8- to 10-mo-old animals than in the 8- to 10-wk-old animals. The younger males had more normal-appearing sperm heads,

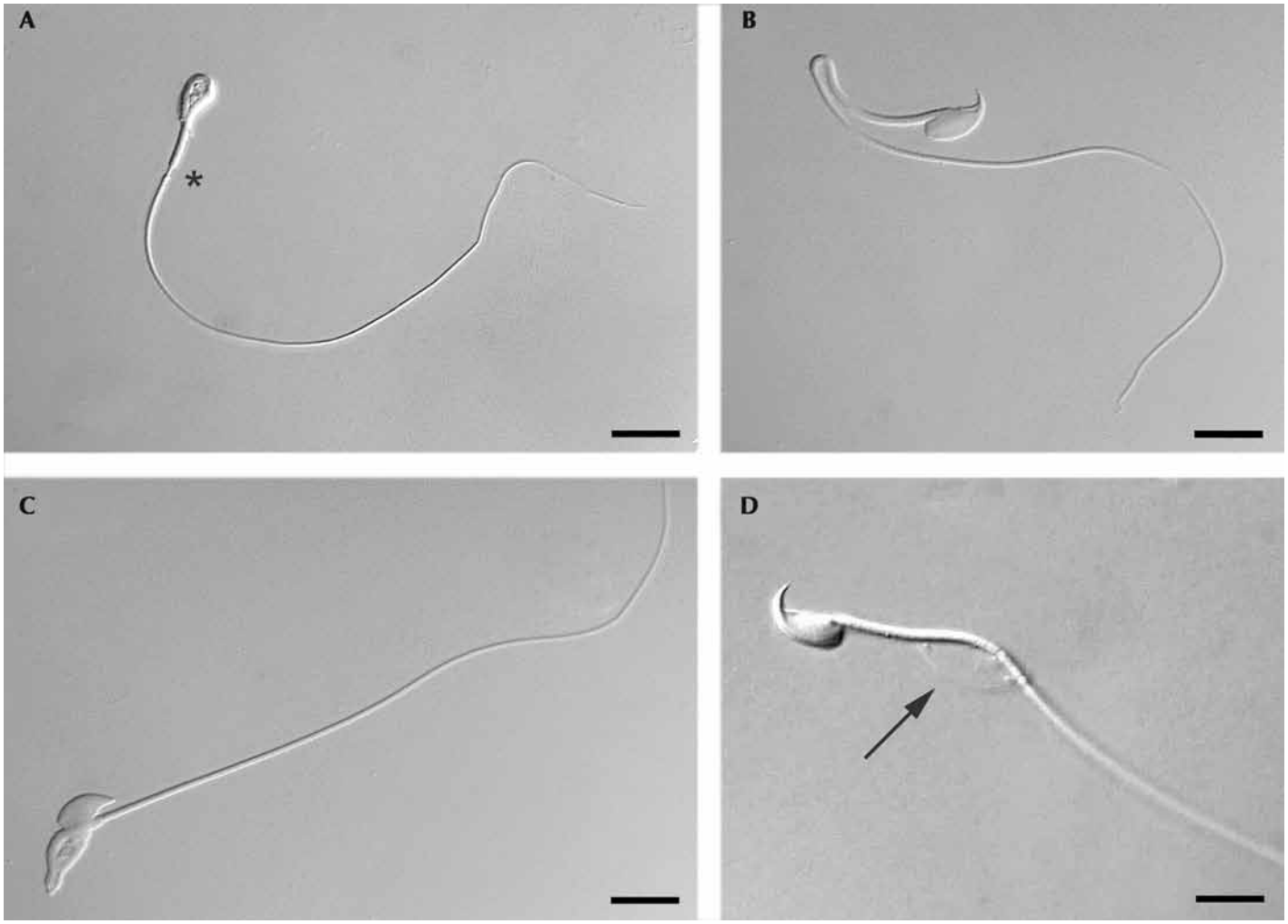

Figure 1. (A, B) Morphologic changes, head defects, and midpiece defects (asterisk) in spermatozoa from 8-to 10-mo-old homozygous *ROSA–EGFP* male mice. (C) Epididymal sperm that has not completed differentiation from 8- to 10-mo-old homozygous male mice. (D) Normal epididymal sperm with a cytoplasmic droplet (arrow) from 8- to 10-mo-old FVB male mice; magnification, ×400; bar, 10 µm.

compared with the older males (data not shown), but the 8 to 10-wk-old males exhibited more tail defects such as multiple tails or tails with no heads. Furthermore, whereas the sperm from 8- to 10-wk-old homozygous male mice exhibited some motility, albeit reduced as compared with wild type, that from 8- to 10-mo-old homozygous males exhibited little or no motility.

Histologic examination. The testes and epididymides of *ROSA-EGFP* mice displayed prominent histologic abnormalities that likely contributed to the impaired spermatogenesis observed in this mouse line. The seminiferous tubular epithelium contained large vacuoles, abundant cellular debris, and rare multinucleated giant cells, and the seminiferous epithelium was disorganized and contained decreased numbers of all of the stages of germ cells in 8- to 10-mo-old homozygous male mice (Figure 2). Furthermore, few mature spermatids were present. Immunohistochemical staining for GATA4 confirmed that the vacuolation in the seminiferous epithelium was localized in Sertoli cells (Figure 3). Similar to what was observed with regard to sperm motility, the degree of vacuolization was generally less in young 8- to 10-wk-old homozygous male mice (Figure 4) than in 8- to 10-mo-old homozygous males (Figure 2). Regarding the rest of the male reproductive tract, epithelial vacuolization, luminal debris, and lack of mature spermatozoa were evident in the epididymal epithelium (data not shown). No lesions were seen in accessory sex glands of the homozygous male mice or in the reproductive organs of homozygous female or hemizygous male mice (data not shown).

Discussion

Production of genetically engineered mouse models has increased dramatically over the past decade, but our understanding of problems associated with insertional mutagenesis has not increased proportionately. Unless gene targeting strategies are used in the production of transgenic animals, the site of integration of the transgene, as well as the copy number inserted into the genome, are random events. Although random insertion of the transgene can cause severe disruption of normal physiologic functions, it also can produce unique models for the study of gene function, as in the case of the *ROSA–EGFP* homozygous mouse model described here.

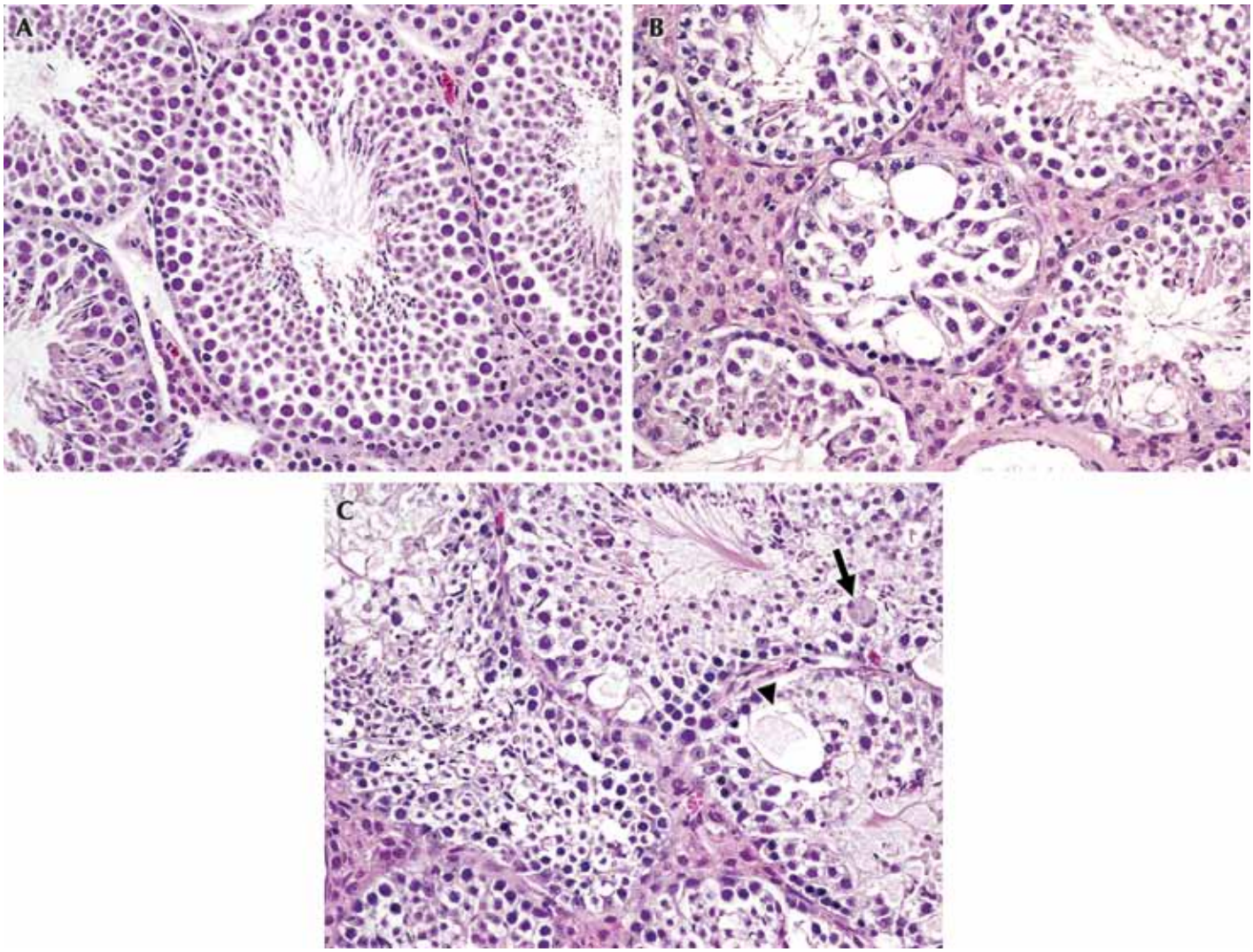

Figure 2. Photomicrographs of seminiferous tubules from 8- to 10-mo-old mice. (A) FVB mouse. (B, C) Homozygous *ROSA–EGFP* mice. The tubules of homozygous mice show degenerative changes of varying severity, including vacuolation (arrowhead), rare giant cells (arrow), and decreased numbers of germinal cells (all stages). Hematoxylin and eosin stain; magnification, ×200.

We characterized the *ROSA–EGFP* homozygous model and found a male-specific infertility, the severity of which is age-related. Hemizygous and wild-type mice showed no phenotypic abnormalities with regard to fertility. Whereas homozygous female mice were able to produce pups when mated to wild-type FVB male mice, homozygous *ROSA–EGFP* male mice were unable to produce pups when cohoused for 10 wk with either homozygous or wild-type FVB females.

To confirm that the observed male infertility was most likely the result of severely impaired spermatogenesis rather than an inability to mate or embryonic lethality after fertilization, homozygous male mice were mated to superovulated wild-type FVB female mice for the collection of pronuclear-stage embryos. Homozygous male mice were unable to produce any fertilized embryos, whereas control matings produced 9.8 embryos per female mouse. Flushing of the female reproductive tract failed to recover any spermatozoa, strongly suggesting severely impaired spermatogenesis rather than embryonic lethality as the cause of the apparent infertility. Furthermore, spermatozoa collected from homozygous male mice of different ages (8 to 10-wk-old and 8 to 10-mo-old) revealed an apparent age-related severity of the defects in the homozygous animals. Severe morphologic defects were present in the spermatozoa from both groups, but the older 8- to 10-mo-old mice had more head defects did the younger 8- to 10-wk-old animals. Furthermore, the older group had no motility at the time of collection, but a small percentage of sperm (14%) from the younger mice was motile.

The morphologic abnormalities in the spermatozoa collected from the homozygous *ROSA–EGFP* mice may reflect alterations in Sertoli cell specific genes. Some sperm from the homozygous 8- to 10-mo-old male mice had rounded heads and other head defects; *Hrb* mutants, in which the proacrosomal vesicles do not fuse, produce acrosome-deficient sperm with rounded heads.[12] However, sperm from *Hrb* mutants generally have multiple flagella, compared with the single relatively normal or slightly abnormal flagella typically present in the older homozygous *ROSA–EGFP* male mice (as assessed by light microscopy). However, the 8- to 10-wk-old male mice did produce some spermatozoa with mul-

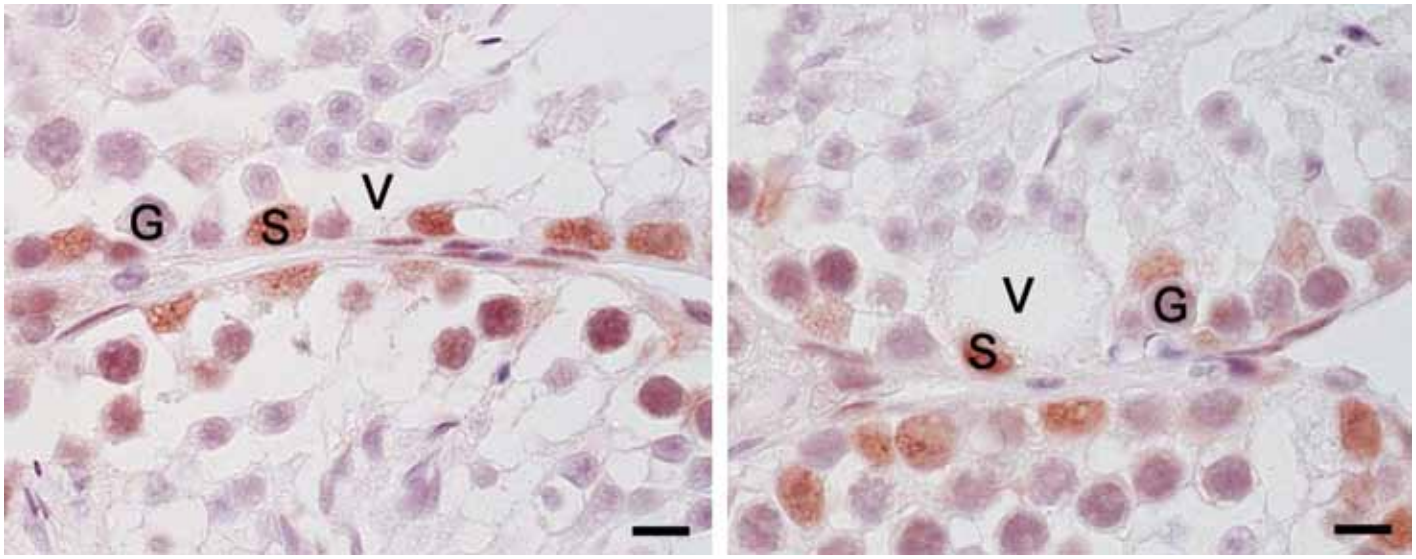

Figure 3. Photomicrographs of seminiferous tubules from a homozygous male stained with GATA4, which differentiates Sertoli cells (S) from germ cells (G) and reveals that the vacuolization (V) within the testes is associated with Sertoli cells and not germ cells. Magnification, ×400; bar, 10 µm.

tiple flagella like those of *Hrb* mutants. Similar to *Hrb* mutants, Golgi-associated protein (GOPC) mutants are also infertile with globozoospermia and, like the *ROSA–EGFP* model, generally have only 1 flagella.[25] Although the transgene insertional mutant in the present study is similar to the GOPC mutant with regard to infertility and frequent globozoospermia, homozygous *ROSA–EGFP* mice did not exhibit the 100% globozoospermia typical of GOPC mutants, and some spermatozoa with 'seminormal' (partial acrosome) heads were produced in this study (Figure 1), especially in the younger animals.

Unlike *Hrb* or GOPC-deficient male mice, which generally do not exhibit histologic abnormalities in the testes, the homozygous *ROSA–EGFP* mice had severe testicular degeneration (for example, vacuolation of Sertoli cells, rare multinucleated giant cells, and sloughing of cells within the tubules of the testes). In addition, vacuolation also was present in the epididymidal epithelium of the homozygous mice. Previous reports indicate that the presence of large vacuoles within the testis frequently is associated with what is thought to be alterations in Sertoli cell permeability.[5] GATA4 is a zinc finger transcription factor, which has been reported to play an important role in sex determination and differentiation and in gonadal steroidogenesis in several species, including humans, mice, rats, and pigs.[15] GATA4 is localized in the nuclei of Leydig and Sertoli cells, but not germ cells, in the testes of pre- and postnatal mice.[11,15] The indirect immunohistochemical staining for GATA4 performed in the present diagnostic investigation confirmed that the observed vacuolation in the seminiferous epithelium of our homozygous *ROSA–EGFP* male mice was indeed localized within Sertoli cells and not germ cells (Figure 3).

Changes in protein expression patterns in Sertoli cells alone are enough to cause morphologically aberrant spermatozoa. Nectin 2 is expressed only in Sertoli cells and is part of an adhesion unit of the Sertoli cells.[19] In a mouse knockout mutant of nectin 2, spermatozoa were morphologically abnormal with head defects and infertile.[19] In addition, others have reported that the Sertoli cell vacuolization and abnormal germ cell adhesion of the nectin 2 knockouts were due to the loss of inositol polyphosphate 5-phosphatase (Inpp5b).[8] These authors further reported that the vacuoles in the tubules contained N-cadherins, β-catenin, actin, and proteins that normally are found on the surface of Sertoli cells, suggesting that Inpp5b may be involved in plasma membrane recycling and endocytosis. In addition, the lack of Inpp5b may cause early release of germ cells from the epithelium of the tubules.[8]

We mapped the site of the insertion of the transgene in this strain by using a chromosome walking technique to chromosome 3,[3] and the transgene has inserted into an intronic region of a hypothetical gene sequence (ENSMUSG00000027939) based on the NCBI m37 mouse assembly (April 2007, strain C57BL/6J). The GenBank Accession number for the predicted mRNA is XM_982763. The predicted amino acid sequence of the protein encoded by this gene suggests it is a member of the nucleoporin glycoprotein family. The role of nucleoporins in the communication between Sertoli cells and the developing sperm is still unclear. However, nucleocytoplasmic trafficking is thought to be one of the driving forces behind differentiation in developing sperm.[9] We speculate that the insertional mutation disrupting this nucleoporin-like gene may adversely affect nucleocytoplasmic trafficking and consequently differentiation of the developing sperm.

In this study, we have identified a novel insertional mutation that causes a major defect during spermatogenesis and results in infertility in homozygous male mice. This insertional mutation causes abnormal sperm, loss of motility, and histologic changes in the male reproductive tract in an age-dependent manner. Homozygous male *ROSA–EGFP* mice were unable to produce pups or pronuclear-stage embryos by natural mating, but homozygous female mice were able to produce pups by natural mating. The *ROSA–EGFP* model likely will serve as an ideal model to further characterize the functional role of this gene in male fertility and spermatogenesis.

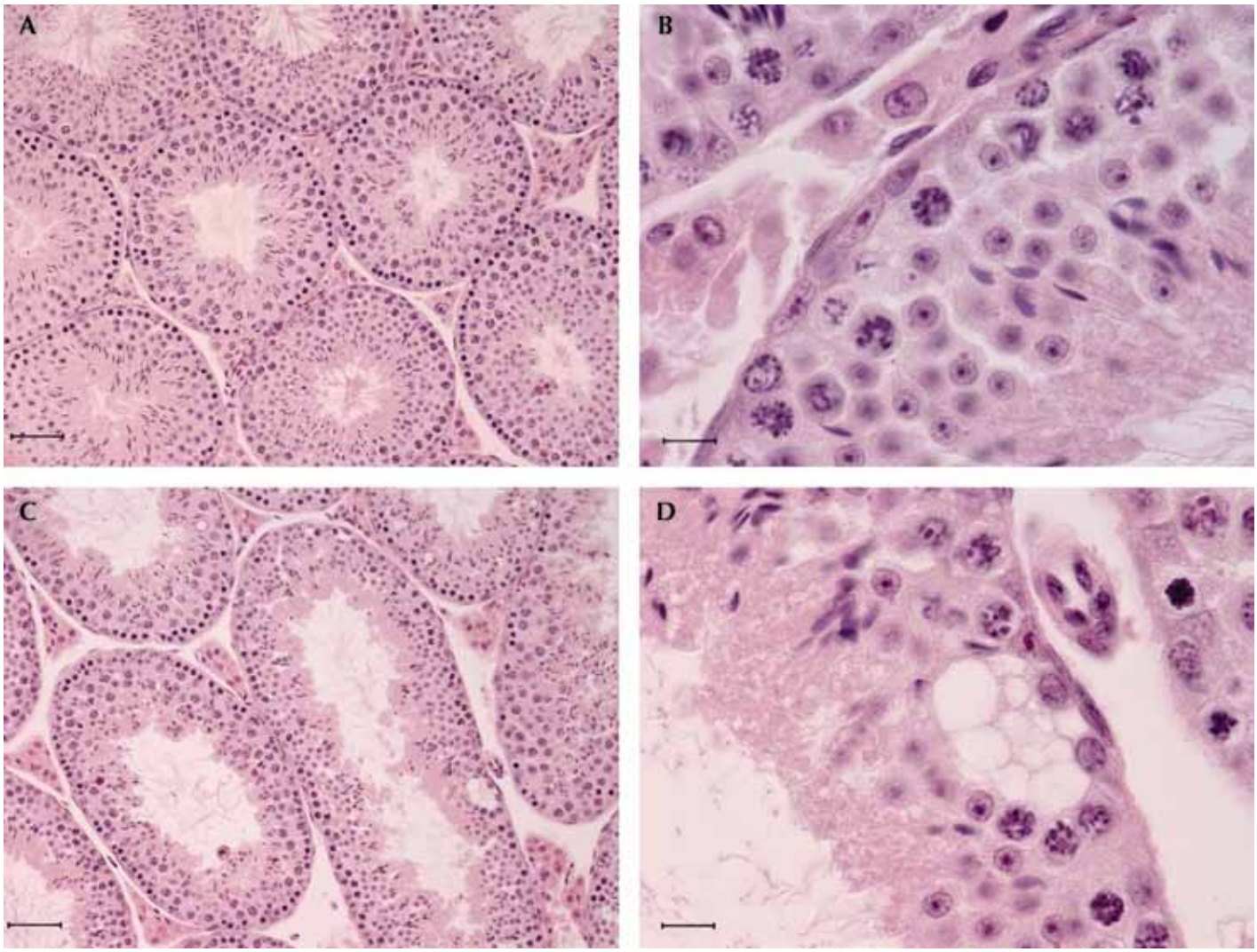

Figure 4. Photomicrographs of seminiferous tubules. (A, B) Wild-type littermates of (C, D) 8- to 10-wk-old homozygous *ROSA–EGFP* mice. The degenerative changes noted within the tubules of homozygous mice were more severe in 8- to 10-mo-old mice (Figure 2) than in 8- to 10-wk-old mice. Hematoxylin and eosin stain; magnification, ×200 (A, C); ×1000 (B, D).

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