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Restoration of Spermatogenesis and Male Fertility by Transplantation of Dispersed Testicular Cells in the Chicken[1]

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ABSTRACT

Transplantation of male germ cells into sterilized recipients has been widely used in mammals for conventional breeding and transgenesis purposes. This study presents a workable approach for germ cell transplantation between male chickens. Testicular cells from adult and prepubertal donors were dispersed and transplanted by injection directly into the testes of recipient males sterilized by repeated gamma irradiation. We describe the repopulation of the recipient seminiferous epithelium up to the production of heterologous sperm in about 50% of transplanted males. In comparison to males transplanted with testicular cell preparations from adult donors, in which the first ejaculates with sperm were recovered about 5 wk after transfer, a substantial interval (about 10 wk) was necessary to obtain ejaculates after the transfer of testicular cells from prepubertal donors. However, in both cases, recipient males produced ejaculates capable of fertilizing ova and producing progeny expressing donor genes.

developmental biology, gametogenesis, implantation, spermatogenesis, testis

INTRODUCTION

The transplantation of spermatogonial stem cells provides a means to study germ cell proliferation and differentiation, but it can also be utilized to transfer exogenous genetic material into recipient males. This has given rise to extensive research in mammals, but remains, in part, unexplored in birds due to the difficulty in accessing the gonads in the abdominal cavity and in unequivocally differentiating spermatogonial stem cells from the other types of spermatogonia in the seminiferous epithelium. Indeed, a morphological description of the various cell categories involved in the processes of avian spermatogenesis has already been proposed in several species [1–11], but the identification of stem spermatogonia on the basis of cell surface markers has been achieved only recently [12].

In mammals, germ cell transplantation was first performed with dispersed testicular cell suspensions containing unknown numbers of stem spermatogonia [13–14]. With subsequent characterization of the spermatogonial stem cells recovered from the embryo, prepubertal, and adult stages, partial purification of the spermatogonial stem cells was accomplished. This was an important step towards further understanding their biology [15] and, more practically, to optimizing the chances of colonization of recipient testes by exogenous germ line populations within the same species [16–18] or between different species [19–22]. In the case of autologous and homologous transfer, the regeneration of functional though quantitatively depleted [23] spermatogenesis, leading to functional sperm, fertilization, and, ultimately, progeny bearing and expressing the transplanted haplotype was occasionally observed [24].

In avian species, germ line chimeras have been successfully produced by transferring primordial germ cells (PGCs) in quail and chicken embryos [25–28], but the transplantation of germ cells in prepubertal or adult recipients has not yet been achieved. Successful colonization of recipient testes requires their partial, if not entire, sterilization prior to transfer of spermatogonial stem cells. Several approaches have been proposed, including in ovo drug injection during the early stages of embryogenesis [29–30], surgical treatment, and exposure of embryos or posthatch birds to ionizing radiation [31–34]. Busulfan (1,4-butanediol dimethanesulfonate), an alkylating agent with a sterilizing effect on male mammals, was found to be harmful to developing chick embryos [35]. However, more recently it was reported that, when administered to eggs after 24 h of incubation, busulfan eliminated testicular germ cells with no consequential effect on embryonic or posthatching chick development [29]. As an alternative to busulfan, the sterilization of male mice [36–39] and rats [40] was successfully achieved by repeated exposure to high doses of gamma radiation. Likewise, in the chicken, complete sterilization was achieved following repeated exposure of the testes to five doses of 8 gray units (Gy) each distributed at 3- to 4-day intervals [41].

In this article, we demonstrate that the transfer of dispersed testicular cells from donor male chickens results in the recolonization of recipient testes previously sterilized by repeated radiation treatments. These recipient males ultimately produced viable and functional sperm capable of fertilization and, ultimately, several hatched chicks derived from transplanted donor male lineage.

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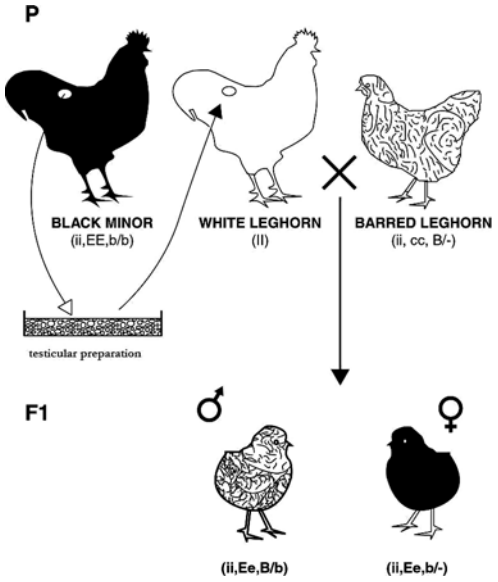

FIG. 1. Diagram of the germ cell transplantation model used in the experiment. Dispersed testicular cells from BM donor testes were transplanted to restore spermatogenesis in recipient testes from WL males sterilized by gamma irradiation (P = parental birds). BL females were inseminated with semen obtained from WL recipient males to test the origin and functional states of spermatozoa. Black or barred plumage in the progeny (F1 generation) revealed successful germ cell transfer, whereas white plumage would have indicated incomplete sterilization of the recipient male chicken.

MATERIALS AND METHODS

Experimental Animals

A total of 26 inbred black minor (BM; genotype *ii*, *EE*, *b*/*b*) male chickens, including 17 at adult (25–40 wk of age) and 9 at prepubertal (14 wk of age) stages, were used as donor males. Recipient males included 26 white leghorn chickens (WL; genotype *II*) at an adult stage (25–45 wk of age). An additional group of five males having the same genetic origin as recipients were retained as positive controls, whereas three other males, also of the same origin as recipient males, were subjected to the same initial treatment as recipients, and then kept as negative controls. All birds used in these experiments were obtained from the Experimental Animal Farm of the Institute of Molecular Genetics (Prague, Czech Republic). All experiments were performed in accordance with Czech and French legal requirements for animal handling and welfare.

Birds were kept in individual cages (4 200 cm^2) fitted with perches under standard husbandry conditions (photoperiod: 12L:12D). Feed and water were provided ad libitum. Egg incubation was performed in a forced-air incubator (Curfew Scientific Incubator Ltd, Curfew, UK).

Irradiation Treatment

A Theratron T1000 radiation treatment unit using ^{60}Co as source of gamma radiation (Theratronics International Ltd., Kanata, Ontario, Canada) was used to irradiate WL testes according to a previously described protocol [41]. Briefly, each of the 24 recipients along with the 3 negative control males was subjected to a series of 5 irradiations (8 Gy each) repeated at 3- to 4-day intervals. During the phase of irradiation, males were maintained in an upright position. Each testis was subjected to isocentric irradiation with the source axis maintained at a distance of 1 000 mm from the targeted area. The last irradiation treatment was taken as reference time for further measurements.

Preparation and Transplantation of Donor Germ Cells

Transplantations were performed at least 1 mo after the last irradiation treatment. All donor males were killed by decapitation, and their testes were quickly removed and washed in phosphate-buffered saline (PBS). The dispersed testicular cells were prepared from small, 3 × 3-mm pieces of testicular tissue, devoid of tunica albuginea made by repeated sieving through a grid (Screen, 50 mesh; Sigma-Aldrich, St. Louis, MO). Dispersed cells were collected in a Petri dish, suspended in 3 ml M199 medium (Sigma-Aldrich), transferred into 5-ml tubes, and centrifuged (300 × *g* for 3 min). The resulting pellet was resuspended in 1 ml M199 medium, and centrifuged a second time (300 × *g* for 3 min); the supernatant was discarded and replaced by 500 μl M199 medium. Approximately 200 μl of the dispersed testicular cell preparation containing a total of approximately 10^4–10^5 cells was injected through the tunica albuginea at 4 or 5 different, randomly selected locations into each testis of the 26 anesthetized WL male recipients (1 donor for 1 recipient); the other 5 irradiated males were kept either as negative controls (3 sterilized but nontransplanted males), or as labeled cell recipients (2 males). Injections of donor cell preparations were performed following anesthesia by intramuscular injection of a mixture of 15 mg/kg ketamine per male (Narkamon, Spofa, Czech Republic) and 4 mg/kg xylazine (Rometar, Spofa, Czech Republic) and bilateral incision of the abdomen to access the testes.

Labeling of Donor Germ Cells

Green Fluorescent Cell Linker Kit (Sigma-Aldrich), containing the cell linker dye PKH-67 GL, was used to label the dispersed testicular cells for further histological analysis of recipient testes in 2 of the 26 transplanted males. Prior to injection in the testes of these 2 males, dispersed testicular cells were stained with 2 μM PKH-67 GL dye (room temperature, 10 min). The staining procedure was stopped by addition of an equal volume of heat-inactivated fetal bovine serum (Gibco BRL, Prague, Czech Republic). The stained, dispersed testicular cells were washed four times to remove all unincorporated dye, and then were suspended in M199 medium to a final volume of 800 μl prior to injection into recipient testes. Testes in each male were then injected with 200 μl of the cell suspension, as described in the previous paragraph. Both males were killed 1 wk after injection, and their testes were removed and immediately frozen in liquid N_2 for further histological analyses. Twenty-four 10-μm-thick cryostat sections (Leica 1850) were inspected from randomly chosen portions in each testis. The distribution of PKH-67-positive donor cells was assessed using an Olympus IX-50 fluorescence microscope (absorption = 490 nm; emission = 502 nm; Olympus, Prague, Czech Republic).

Assessment of Sperm Output

Ejaculates from the 24 remaining males, along with those of the 3 negative and 5 positive control males, were collected twice/wk during the 24 wk after the transplantation using the conventional abdominal massage technique [42]. Semen concentration was estimated with a prescaled centrifuge [43] to determine the number of sperm per ejaculate, and volume was measured with a scale (nearest mg). Weekly semen output (WSO [44]) per male was estimated by addition of the number of ejaculated sperm/wk.

Artificial Insemination with Semen from Transplanted Males

A total of 96 sexually mature, inbred barred leghorn hens (BL; genotype *ii, ee, B/)* were artificially inseminated with semen collected from WL recipient males transplanted with dispersed testicular cells obtained from the BM donor males. Hens were inseminated twice a week with one individual ejaculate/hen collected from transplanted males producing sperm. Inseminations were performed intravaginally with semen doses containing a minimum number of 2×10^6 sperm and adjusted to a final volume of 100 μl with a chicken semen diluent [45]. Eggs were collected from Day 2 to Day 4 after a given insemination, and then were stored and incubated under standard conditions. Percent hatchability (H%) of eggs was calculated as: H% = number of hatched chickens (n) × 100 / number of incubated eggs (N). The number of eggs produced from hens inseminated with semen from individual recipient males ranged from 285 to 380. Under standard breeding conditions, the hatchability of eggs from BL hens inseminated once a week with 200×10^6 sperm may vary from 70% to 85%, depending on the flock's age (data not shown).

Chicken Germ Cell Transplantation Model

Identification of day-old chicks from BM donors was based on their black phenotype, which is due to a homozygous recessive allele (ii) at the I (white) locus, whereas WL males, homozygous dominant allele (II) at the I (white) locus, were sterilized and used as recipient males (Fig. 1). The dominant I allele of this locus gives rise to the white phenotype of WL male chickens through the epistatic inhibition of the E allele encoding eumelanin. Successful transplantation of dispersed testicular cells and restoration of normal spermatogenesis were detected in the F1 progeny of transplanted male recipients and BL females (ii). The dominant allele bar (B) is incompletely dominant in BL hens and localized on the W chromosome. It is phenotypically expressed as striping with broad stripes in cockerels (BB) and narrow stripes in homozygous hens (B–).

TABLE 1. Frequency of sperm recovery and percentages of males that achieved fertility in chickens previously transplanted with dispersed testicular cells from prepubertal or adult donors.

Age of BM donors (wk)	No. of WL recipients ejaculating spermatozoa/total transplanted males	No. of WL recipients producing progeny carrying donor genes (%)
14	4/9[a]	2 (22.2)[b]
25–40	8/15[a]	3 (20.0)[b]

[a,b] Values with the same superscript are not significantly different at 0.05 level (χ^2 test).

Due to the sex linkage of the barred locus used in our genetic model, hatched male chicks (Bb) were barred, whereas females (b–) were black. The presence of white chicks in the F1 progeny would indicate incomplete sterilization and persistence of WL germ cells.

Histological Analysis

Males were killed 2 wk after the last semen collection (total: 26 wk after injection) by lethal injection of pentobarbital in the wing vein (1 ml/kg body weight). Testicular fragments (approximately 5 × 3 mm each) were placed in Bouin fixative solution for 24 h at room temperature, then were dehydrated and subsequently embedded in Paraplast (Sherwood Medical, Tullamore, Ireland). Histological sections (5-μm thick) were cut with a Leica 2000 microtome (Leica, Wien, Austria) and stained with PAS or Alcian-blue-Mayer Hematoxylin for further observations by brightfield microscopy (Olympus IX-50; Olympus, Prague, Czech Republic).

Statistical Analysis

Comparisons of percentages of males producing ejaculates relative to donor age and hatchability of eggs from hens inseminated with semen from males transplanted with cells from prepubertal or adult donors were performed by χ^2 test. The best regression equation for the adjustment of the time course of ejaculated sperm from recipient males transplanted with adult or juvenile dispersed testicular cells was achieved using a linear model with polynomial interpolation [46]. Comparison between the regression equations was performed using the Barlett and Chow test. A box and whisker plot adjustment was performed for graphic visualization of number of spermatozoa in the two groups of transplanted males (SYSTAT 10 [SPSS, Inc.]). Finally, comparisons of the mean number of sperm per ejaculate between groups of males were performed using a single classification analysis of variance and Fisher protected least significant difference test, where appropriate.

RESULTS

Restoration of Spermatogenesis in Recipient Testes

At 24 wk after transplantation, spermatozoa were present in the ejaculates of a total of 12 WL recipients, including 4 males transplanted with prepubertal donor cells and 8 transplanted with adult donor cells (Table 1). No statistical difference was observed between the percentages of males producing ejaculates with regard to donor age ($P > 0.05$). The time course of ejaculated spermatozoa in recipient WL male chickens was assessed over 24 wk following transplantation. Sperm output from males injected with a preparation of adult testicular cells started at Week 5 after transplantation, and then WSO gradually increased to 450×10^6 sperm (adjusted values: see Fig. 2A) at Week 24. In contrast, sperm output from males transplanted with prepubertal testicular cells started at Week 10 after transplantation and reached a WSO of 366×10^6 sperm at Week 24 following transplantation (adjusted values: see Fig. 2B). The time intervals at initial recovery of sperm output and WSO were significantly different between the two groups of males ($P < 0.05$; see also Figs. 2 and 3). A comparison of the mean number of sperm per ejaculate between WL control and recipient males transplanted with adult or juvenile donors from Week 10 to Week 24 confirmed 1) the low rate of sperm output from transplanted compared to control males, and 2) the lower level of sperm output from males transplanted with juvenile compared to adult testicular cells (Table 2). The age-matched group of irradiated but nontransplanted WL males (negative control) failed to produce any semen after sterilization.

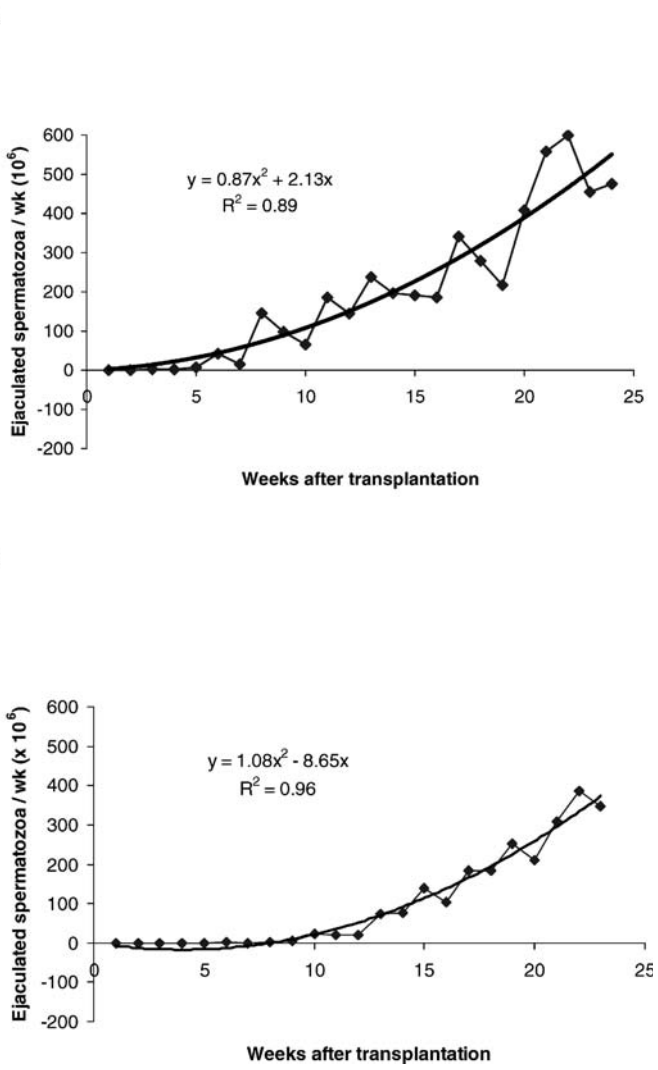

FIG. 2. Time course of the weekly sperm output in WL recipient chicken males transplanted with dispersed testicular cells from BM donors. **A**) Adult donors (n = 8) and (**B**) prepubertal donors (n = 4). Comparison of the polynomial regression equations revealed significant differences in the time interval necessary to obtain ejaculates ($P < 0.05$) and in the number of ejaculated spermatozoa produced 24 wk after transplantation ($P < 0.05$) with testicular cell preparations from adult (**A**) or prepubertal (**B**) donors.

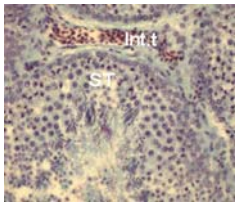

FIG. 3. Transversal section of a positive control WL chicken testis. All germinal cell categories of the seminiferous tubules (ST) are present. Interstitial tissue (Int.t.) represents about 10% of the entire testicular epithelium (data not shown). (Periodic acid-Schiff stain; original magnification ×400.)

TABLE 2. Mean number of spermatozoa per ejaculate in WL recipient chicken males transplanted with dispersed testicular cells from adult or juvenile BM donors.

Male category	No. of males	Sperm/ ejaculate ($x10^6$)*
WL positive control	5	4980 ± 287^a
WL transplanted males with adult BM donors	8	202 ± 72^b
WL males transplanted with juvenile BM donors	4	115 ± 54^c

* Data expressed as the mean number of spermatozoa per ejaculate ± SD.
[a,b,c] Values with different superscripts are significantly different ($P < 0.05$).

Hatching of Healthy BM × BL Progeny after Insemination with Semen from Recipient Males

Two of nine WL recipient males transplanted with dispersed testicular cells from prepubertal BM donors produced progeny expressing either the ii, Ee, B/b genotype (barred males) or the ii, Ee, b– genotype (black females), indicating that all hatched chicks issued from BM donors. Similarly, 3 of the 15 males transplanted with testicular cells from adult donors proved to be fertile. The percentage of WL recipients producing progeny carrying donor genes was not statistically different between those injected with adult and those injected with prepubertal dispersed testicular cells (Table 1).

The mean hatchability of eggs from hens inseminated with semen from recipient males transplanted with dispersed testicular cells from an adult donor was 10.7% (Table 3). This was significantly higher ($P < 0.01$) than hatchability in eggs fertilized with semen from recipient males transplanted with dispersed testicular cells from prepubertal donors (6.4%). The highest hatchability (23.2%) was observed in eggs fertilized with semen from one recipient transplanted with dispersed testicular cells from an adult donor.

Histology of the Testes in Control and Experimental Males

In contrast to seminiferous tubules of positive control males expressing active spermatogenesis (Fig. 4), seminiferous tubules in the testes of the three negative control males were entirely devoid of germ cells (Fig. 5). In these males, tightly apposed, columnar Sertoli cells, characterized by their basal nuclei possessing a prominent nucleolus, lined the luminal compartment of the seminiferous tubules. Interstitial tissue, including Leydig cells and capillaries, appeared unaffected by sterilization treatment. In the two males injected with dispersed testicular cells stained with the cell linker dye PKH-67 GL, labeled donor cells were distributed along the basal membrane of the seminiferous epithelium 7 days after injection (Fig. 6). Of the 24 cryostat sections from each of the 4 testes examined, 12% of the sections contained PKH-67-labeled cells and, within these sections, 22% of the seminiferous tubules had been colonized with labeled cells. With few exceptions, PKH-67-positive cells were not observed in the interstitial spaces.

TABLE 3. Hatchability of eggs following inseminations with semen from transplanted WL males.

Age of BM donors (wk)	No. of incubated eggs	No. of hatched chickens	Hatched eggs (%)
14	605	39^a	6.4^a
25–40	973	105^b	10.7^b

[a,b] Values with different superscripts are significantly different at 0.01 level (χ^2 test).

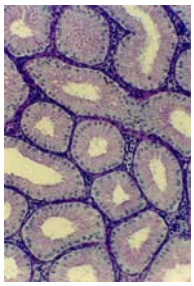

FIG. 4. Transversal section of a negative control WL chicken testis 3 mo after sterilization with repeated doses (5 × 8 Gy) of gamma radiation. The seminiferous epithelium is devoid of germinal cells but Sertoli cells are present. Leydig cells are morphologically normal (Haematoxylin stain; original magnification ×200.)

The recolonization of seminiferous tubules with unstained cell populations was found to be highly variable between individuals, regardless of age of the recipient at transplantation. Furthermore, the degree of recolonization was variable within males as some tubules possessed a fully functional seminiferous epithelium whereas adjacent seminiferous tubules remained devoid of any germinal cell population (Fig. 6). Finally, degenerating forms of spermatid nuclei was observable in a limited but nonquantified fraction of seminiferous tubule sections otherwise recolonized by germinal cell populations.

DISCUSSION

In the present study, dispersed cells from prepubertal and sexually mature chicken testes were successfully transferred into sterilized recipient chicken testes. As in mammals [13, 24, 47–50], this resulted in the recolonization of the seminiferous epithelium, a partial resumption of spermatogenesis, and production of viable and functional spermatozoa. We also confirmed that 1) repeated exposure (5 × 8 Gy) of mature chicken testes to gamma radiation results in the complete elimination of germ cell populations, and 2) seminiferous tubules made sterile by radiation remain functional [41], thus facilitating their recolonization by transplanted germ cell populations. Observations in the mouse also have demonstrated that two successive irradiations (1.5 + 12 Gy) induce marked depletion of germinal cell populations without side effects, other than local calcification of some individual seminiferous tubules [39, 51].

Spermatogenesis was observed only in about half of recipients, irrespective of donor age. This partial success observed 6 mo after transplantation may have originated, in part, from the use of dispersed testicular cells rather than a partially purified population of spermatogonial stem cells, which, in the chicken, have yet to be unequivocally identified. Consequently, the precise number of spermatogonial stem cells transferred into each recipient was unknown, increasing the chances for high interindividual variability in the process of recolonization. Studies in the mouse have revealed that the number of germinal cell colonies recovered from seminiferous tubules in transplanted males is indicative of the number of spermatogonial stem cells transferred to each recipient [23, 52, 53].

In the present study, treatment of donor cell preparations with the cell linker dye PKH-67 GL was used to trace donor-derived cells in the recipient testes. Among sections with PKH-67-positive cells, only 22% of the seminiferous tubule sections examined possessed stained cells presumably derived from transferred stem spermatogonia. Such a low rate of recolonization of recipient testes may have originated, in part, from the

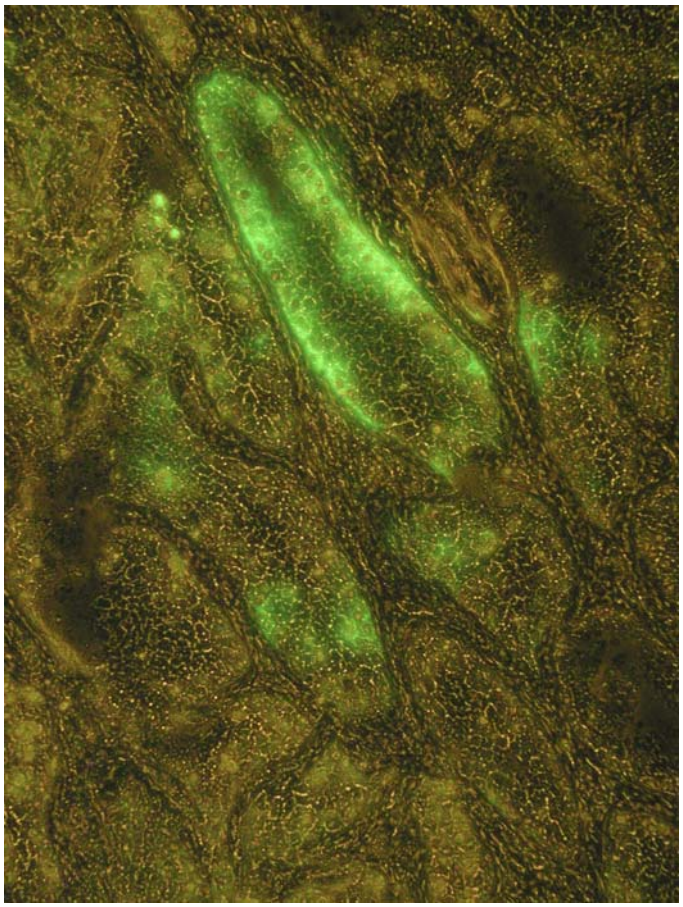

FIG. 5. Section of WL sterilized testis examined 7 days after transplantation with PKH-67-stained dispersed testicular cells from BM donors. (Original magnification ×800.)

limited number of donor stem spermatogonia injected in each testis. Furthermore, access of the donor cells to the seminiferous tubules was de facto limited to the number of seminiferous tubules punctured by the needle at the time of injection. The difficulty in precisely replicating the transfer of the donor cells was, at least in part, responsible for the high variability of recolonization between males and, also, between testes within the same male. In this study, intratesticular injections of donor cell preparations were chosen over cannulation of the rete testis because of the difficulties associated with accessing this region in the chicken, a species in which, as for other birds, testes are located in the anterior portion of the abdominal cavity. Interestingly, we found no visible evidence of punctured tubules among the 24 histological sections examined 1 wk after injection.

Despite the high intertubular variability of PKH-67-positive cell distribution among and within each of the histological sections examined, most labeled cells were detected inside the seminiferous epithelium rather than in the interstitial spaces. This may be due to the relatively larger surface area of the seminiferous tubules compared to the interstitial spaces. Previous observations in sexually mature chickens have indicated that seminiferous tubules occupy 85%–89% of the testicular parenchyma [54]. Meanwhile, in the present study, the absence of observations performed prior to 1 wk and from 1 to 24 wk after transfer was a major limiting factor in following the rate of recolonization in a time-course manner. From the present observations, we suggest that colonization began slightly before 7 days after injection and lasted up to several

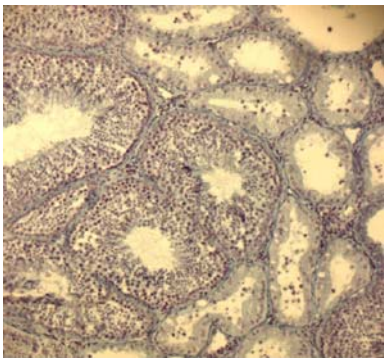

FIG. 6. Transversal section of adult WL chicken testis first exposed to repeated gamma irradiation and then transplanted with dispersed testicular cells from BM donors. Recipient male killed 26 wk after transplantation. The presence of contiguous functional and nonfunctional seminiferous tubules reveals the high variability of recolonization with donor-dispersed testicular cells. (Mayer Hemalun stain; original magnification ×200.)

weeks. Additional information is needed to obtain a more comprehensive view of the overall process of recolonization from donor-derived germ cell populations. Such information, obtainable only from observations performed over an extended period after cell transfer, was not gathered in this study due to the high cost of sterilization treatment required for each additional male.

Evaluation of the time interval necessary to recover the spermatozoa in ejaculates from transplanted males revealed an age-dependent effect of the male donor. We observed that males transplanted with dispersed testicular cell preparations from adult males began spermatogenesis earlier than males transplanted with dispersed testicular cell preparations from prepubertal males. Previous studies in the mouse have established that the population of heterologous stem spermatogonia increases with the age of the donor [23]. Moreover, in the mouse, the number of donor-derived colonies formed by stem spermatogonia originating from adult donors is limited by the accumulation of differentiating germ cells in recipient testes. This was unlikely to have occurred in the study reported here based on differences between the numbers of ejaculated spermatozoa collected from males transplanted with prepubertal compared to adult testicular cell preparations. Comparisons between the two groups of recipients revealed a persistent and significant tendency in the group transplanted with dispersed testicular cells from adult donors to produce more ejaculated spermatozoa than the group transplanted with dispersed testicular cells from prepubertal donors. Apart from previously mentioned differences between the two groups of males in the initial number of stem spermatogonia injected, stem spermatogonia originating from prepubertal donors may require some additional maturation prior to expressing full potential of recolonization when transplanted in adult seminiferous tubules. More precise identification of chicken stem spermatogonia in prepubertal and adult males will therefore be necessary to elucidate this point in the future.

In this study, we report for the first time the production of viable progeny from male chickens initially sterilized by gamma irradiation and then transplanted with juvenile or adult donor-derived dispersed testicular cells injected directly into their testes. When inseminated, semen from the recipient males fertilized ova, but egg hatchability was extremely low, irrespective of the origin of dispersed testicular cells (juvenile donors: 6.4%; adult donors: 10.7%; $P < 0.05$). Hatchability of eggs from routine artificial inseminations performed in our

facilities may vary from 70% to 85%, depending on flock age (inseminations performed once a week with 100–120 $\times$ 10^6 spermatozoa/insemination). In the present experiment, hatchability reached a maximum of 23.2% with semen collected from recipient males (donor dispersed testicular sperm was from adult males) and inseminated twice a week with a minimum of 2 $\times$ 10^6 sperm. We conclude that the low hatchability was a consequence of the low number of sperm being inseminated, which in turn was a consequence of the only partial recovery of functional recipient testes after transfer.

It is concluded that progeny (here, about 10% of eggs from inseminated hens) can be obtained from heterologous chicken germ cell populations transplanted into the testes of adult male recipients. This approach, validated for the first time in birds, not only offers a workable alternative to primordial germ cell manipulation and gene transfer in these species, but also provides a suitable model for transfer of rare genetic material, thus providing new pathways to preserve genetic resources in avian species.

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