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***In-vivo* delivery of FTY720 prevents radiation-induced ovarian failure and infertility in adult female non-human primates**

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Abstract

Objective—To determine if sphingosine-1-phosphate (S1P), or the S1P mimetic FTY720 shields ovaries of adult female rhesus monkeys from damage caused by 15 Gy of targeted radiotherapy, allowing for retention of long-term fertility; and, to evaluate if S1P protects human ovarian tissue xenografted into mice from radiation-induced damage.

Design—Research animal study.

Setting—Research laboratory and teaching hospital.

Animal(s)—Adult female rhesus macaques (8–14 years of age).

Patient(s)—Two women (24 and 27 years of age) undergoing gynecologic surgery for benign reasons, after informed consent and approval.

Intervention(s)—None.

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Main Outcome Measure(s)—Ovarian histology, ovarian reserve measurements and fertility in mating trials.

Result(s)—Rapid ovarian failure was induced in female macaques by ovarian application of 15 Gy of radiation. Females given S1P or FTY720 by direct intraovarian cannulation for 1 week prior to ovarian irradiation rapidly resumed menstrual cycles due to maintenance of follicles, with greater beneficial effects achieved using FTY720. Monkeys given the S1P mimetic prior to ovarian irradiation also became pregnant in mating trials. Offspring conceived and delivered by radioprotected females developed normally, and showed no evidence of genomic instability as measured by micronucleus frequency in reticulocytes. Adult human ovarian cortical tissue xenografted into mice also exhibited a reduction in radiation-induced primordial oocyte depletion when pre-exposed to S1P.

Conclusion(s)—S1P and its analogs hold clinical promise as therapeutic agents to preserve both ovarian function and fertility in female cancer patients exposed to cytotoxic treatments.

Keywords

Fertility preservation; cancer; ovary; oocyte; monkey; human; phingosine-1-phosphate; FTY720

INTRODUCTION

Premature ovarian failure and infertility are frequently observed in young girls and reproductive age women treated for cancer (1). Attempts to preserve ovarian function in these patients have met with little success, leaving assisted reproduction or cryopreservation of oocytes and ovarian tissue as the only currently-available clinical options other than egg donation (2,3). However, many patients cannot undergo hormonal stimulation protocols needed to retrieve oocytes due to time constraints or their type of cancer, and pregnancy success rates using frozen-thawed human eggs remain low. Although some of these issues can be circumvented by ovarian tissue banking, this procedure is experimental and presents the risk of re-introducing cancer cells back into the patient following transplantation (2–5).

Studies in mice have shown that anti-cancer treatments activate apoptosis in oocytes (6). Further, targeted deletion of genes encoding pro-apoptotic signaling factors, including acid sphingomyelinase (which generates ceramide), Bax (which disrupts mitochondrial membrane potential) or the protease caspase-2, prevents such oocyte death (7–10). The anti-apoptotic factor sphingosine-1-phosphate (S1P), which is produced through ceramide metabolism (11), recapitulates ovarian protective effects of acid sphingomyelinase deficiency (8). These data, and findings that ceramide is required for radiation-induced germ cell death in *Caenorhabditis elegans* (12), indicate that ceramide is a conserved mediator of germ cell death triggered by these insults. In fact, massive oocyte loss caused by irradiation of adult female mice can be prevented by prior treatment with S1P delivered into the bursal sac surrounding each ovary (7). Although the ovarian protective actions of S1P have been confirmed in several subsequent studies, all are confined to rodent models (13–15).

Importantly, S1P-protected oocytes do not show DNA damage and remain fully competent for maturation, fertilization and preimplantation embryonic development *in vitro* and *in vivo* (7,8). Follow-up studies showed that F₁ offspring conceived by female mice protected from radiation-induced ovarian damage with S1P, as well as F₂ offspring conceived by the F₁ animals, show no anatomical, histological, behavioral or cytogenetic evidence of transgenerational genomic damage or other abnormalities (8). Here we conducted a preclinical trial to evaluate the effects of S1P and its long-acting mimetic, FTY720 (16), on ovarian function and fertility in adult female rhesus macaques following irradiation.

MATERIALS AND METHODS

Animals

Adult female rhesus monkeys during prime reproductive life (8–14 years of age) and of proven fertility were used under research protocols approved by Oregon National Primate Research Center (ONPRC) institutional animal care and use committee. Each animal was implanted subcutaneously with osmotic minipumps attached to intraovarian catheters [17], which were designed to continuously deliver vehicle (60% polyethylene glycol, 30% ethanol, 10% Tween-20; PET), S1P (0.2 mM in PET, 10 μ l per hour; Biomol Research Laboratories, Plymouth Meeting, PA) or FTY720 (0.2 mM in PET, 10 μ l per hour; Cayman Chemical Company, Ann Arbor, MI) for 1 week prior to irradiation.

Ovarian X-Irradiation (OXI)

Ovaries were externalized from anesthetized animals and exposed to X-ray-based radiation for 13 min to generate 1,500 rads of absorbed dose (15 Gy), and then returned to the peritoneal cavity.

Steroid Hormone Measurements

Serum steroid levels were assayed in the ONPRC Endocrine Services Laboratory to monitor menstrual cyclicity.

Follicle Counts

Ovarian sections were analyzed using a Marianas™ digital imaging workstation and the Stereology module of Slidebook 4.2 (Intelligent Imaging Innovations).

Mating Trials

Females were paired with a single male. Pregnancies and vaginal delivery of offspring continued without intervention in all animals except for one FTY720-infused OXI-treated female who underwent Cesarean section 1 week after her expected due date.

Micronucleus Assay

Micronuclei frequency in blood reticulocytes was assessed by flow cytometry (18).

Human ovarian xenografting

Cortical biopsies obtained from ovaries of two patients (24 and 27 years of age) after ovariectomy under an IRB-approved protocol were grafted into NOD-SCID mice (19), treated as indicated, and analyzed for the incidence of oocyte degeneration (20).

Data Analysis

Compiled data from the replicate experiments were analyzed using ANOVA followed by the Neuman-Keuls multiple range test, unpaired *t*-tests or Fisher's exact test, with differences in mean values considered significant at $P < .05$.

RESULTS

Menstrual Cyclicity and Ovarian Reserve

One ovary was removed from each animal before manipulation to provide baseline information on ovarian architecture and follicular dynamics in each female. The remaining ovary was subjected to treatment and then removed 316 ± 7 days later for analysis. Sham-manipulated control females infused with vehicle ($n = 3$) resumed normal menstrual

cyclicity within 92 ± 21 days post-sham manipulation (Fig. 1). Acyclicity was observed throughout the post-irradiation interval in 2 of 3 vehicle-infused females subjected to OXI. The remaining female had a single menstrual cycle starting on day 139 post-OXI (Fig. 1), which was likely due to inadvertent slippage of her ovary out of the radiation field during treatment. No further cycles were observed in this animal. Five of 6 S1P-infused females resumed menstrual cyclicity within 163 ± 30 days post-OXI, whereas 1 animal remained acyclic (Fig. 1).

Ovaries from vehicle-infused control females appeared normal and resembled ovaries collected prior to manipulation (Fig. 2). Ovaries obtained post-OXI from vehicle-infused females were small and completely devoid of oocytes and follicles (Fig. 2). In contrast, ovaries collected from S1P-infused females that resumed menstrual cyclicity following OXI retained a small number of immature follicles with normal morphology (Fig. 2). Corpora lutea from the cycle prior to ovariectomy were noted in 3 of these females, indicating the occurrence of endogenous ovulatory cycles (data not shown).

In parallel we tested the long-acting S1P mimetic, FTY720, for its ability to protect ovaries from radiation-induced damage. In females whose ovaries were infused with FTY720 for 1 week prior to OXI ($n = 3$), all animals resumed menstrual cyclicity within 78 ± 5 days post-OXI (Fig. 1). Ovaries of females infused with FTY720 and collected 317 ± 10 days post-OXI contained significantly more resting (primordial) and growing (primary, secondary) follicles than ovaries of S1P-infused OXI females (Fig. 2), indicating that more pronounced protective effects are observed with FTY720 compared with S1P.

Mating Trials

Natural mating trials were then conducted with female monkeys given bilateral intraovarian vehicle infusion followed by sham manipulation ($n = 3$) or bilateral intraovarian FTY720 infusion followed by OXI ($n = 3$). Females receiving vehicle infusion followed by OXI were not included since female macaques lacking ovarian function exhibit decreased proceptivity, attractivity and receptivity to males (21). Although mounting can be observed in the absence of estrogen, bona fide mating with intromission and ejaculation is absent and pregnancy does not occur (22; M.B. Zelinski, unpublished observations). A resumption of menstrual cyclicity was observed within 65 ± 9 days in the vehicle-infused sham control group and 69 ± 5 days in the FTY720-infused OXI group (Supplementary Table 1). Each female was individually mated with a fertile male during the second ovarian cycle post-treatment for up to 5 consecutive cycles. Successful pregnancies, as confirmed by evidence of fetal cardiac activity during ultrasound at 33 days post-mating, were established in all vehicle-infused sham females within 104 ± 11 days post-treatment and in all FTY720-infused OXI-treated females within 123 ± 17 days post-treatment (Supplementary Table 1). With the exception of femur length at day 145 of gestation, fetal growth parameters and final birth weights were similar for both groups (Supplementary Tables 1 and 2).

Offspring Analysis

All offspring were delivered within 1 week of their expected due date, and developed past the first year of life without complications, abnormalities or behavioral differences (Fig. 3A–F). Although femur growth between gestational days 101–145 in offspring of radioprotected females was approximately 10 mm less than that of controls (Supplementary Table 2), femur length is now similar in the two groups (Supplementary Table 3). Cytogenetic instability measured by frequency of micronucleated reticulocytes revealed no differences in offspring conceived by radioprotected mothers compared with those delivered by non-irradiated sham controls (Fig. 3G). The 3 females whose ovaries were protected from radiotherapy-induced damage with FTY720 were mated again after weaning their first set of

infants. Two of the 3 females became pregnant and delivered a second set of offspring (Fig. 3H, I). Like the first set of offspring, the second set of offspring exhibited normal gestational parameters, birth weights and neonatal development, and no evidence of elevated micronuclei frequency (data not shown). The third radioprotected female has failed to conceive again after repeated mating attempts, despite continuation of menstrual cyclicity.

Human Ovarian Radioprotection by S1P

In a final set of experiments, we assessed if S1P exerts similar protective effects against radiation-induced destruction of human ovarian follicles using a mouse xenograft model. An approximate 4-fold increase in the incidence of degenerative (atretic) primordial follicles was detected in human ovarian grafts 6 days after exposure to 2 Gy of radiation; however, this response was completely absent in human ovaries pre-exposed to S1P for 1 hour prior to irradiation (Fig. 4). Radiation-induced damage to the early growing (primary) follicles in human ovaries was not inhibited by S1P pre-exposure (Fig. 4).

DISCUSSION

Approximately 1 of 49 human females in the United States between birth and age 40, which spans the pre-reproductive and reproductive period, will be diagnosed with cancer (23). Many of these patients will be treated with cytotoxic therapies known to cause significant side-effect damage to the ovaries. Despite the importance of this problem, little is available to female cancer patients seeking fertility preservation other than assisted reproduction or cryopreservation of embryos, eggs or ovary tissue. Even if these approaches prove successful for birth of a child, these patients are left with the very real possibility of developing significant health problems associated with premature ovarian failure. The only method proposed thus far for protecting the ovaries *in situ* involves gonadotropin-releasing hormone analog therapy during anti-cancer treatment; however, clinical data supporting the usefulness of this approach for fertility preservation in female cancer patients have been a subject of considerable debate (24–27).

More than a decade of work with rodent models has consistently demonstrated a remarkable efficacy of S1P in preserving ovarian function and fertility *in vivo* in females exposed to anti-cancer treatments (9–13, 15). Despite these encouraging results, possible human application has remained uncertain. Aside from traditional problems often associated with extrapolating drug efficacy studies in rodents to primates, the fact that the primate ovary is not enclosed within a bursal sac (as is the case with rodents) presents an anatomical hurdle to confining S1P to the ovaries. Through the use of an intraovarian catheter-osmotic mini-pump system, we showed that direct and controlled long-term delivery of S1P or FTY720 to the ovaries of adult female primates can be successfully achieved. In turn, this approach has provided important translational proof-of-concept that S1P, and in particular its mimetics, protect the primate ovary against radiation-induced damage.

It is important to mention that while radiotherapy was selected as the gonadotoxic agent for this study, many chemotherapeutic drugs cause equally concerning irreversible damage to the ovaries (1). However, the costs of running preclinical trials with monkeys did not permit us to conduct a parallel analysis of both treatments, and thus we focused on radiotherapy-induced ovarian failure for the following reasons. First, the extent of damage to the gonads caused by 15 Gy of direct radiation treatment is at least equivalent to, if not considerably worse than, that resulting from treatment with most chemotherapeutic regimens (1, 28; see also <http://www.fertilehope.org>). In fact, the LD₅₀ for radiation-induced oocyte destruction in humans is less than 2 Gy (29). Second, radiation can be directed specifically to the gonads, thus minimizing the potential for widespread systemic toxicity – and in particular, immunologic failure – resulting from intravenous chemotherapy. Third, irradiation provides

a means to achieve ovarian failure in a simple and consistent manner, whereas chemotherapy protocols vary widely, not only in terms of which particular drug to focus on but also of whether single agent or combination chemotherapy should be used. Finally, work with mice has shown that S1P appears equally effective at sparing the ovaries from damage caused by chemotherapy or radiotherapy (7,8,13,14). Still, it remains to be determined if S1P or FTY720 can protect primate ovaries from cytotoxic drug-induced damage *in vivo*.

Fertility preservation in adult female primates exposed to sterilizing doses of radiation through the use of the potent S1P mimetic, FTY720, offers many advantages over other experimental fertility preservation strategies being evaluated that rely on ovarian tissue cryopreservation or *in-vitro* follicle maturation (5,30–32). These latter methods are designed to provide a possible chance at conception through assisted reproduction, but do not alleviate the onset of premature ovarian failure and its associated health complications in cancer patients post-treatment. Further, for those patients with blood cell-borne cancers or metastatic disease, the risk of introducing cancer cells back into the patient post-therapy by ovarian tissue auto-transplantation is of concern (4,5). The present findings offer compelling evidence that natural conception and delivery of healthy offspring by adult female primates whose ovaries were exposed to very high levels of radiation can be achieved with a small molecule delivered directly to the gonads prior to treatment. Using a xenograft model, we further observed that radiation-induced degeneration of primordial follicles in adult human ovary tissue transplanted into immunodeficient mice is prevented by injection of S1P at the graft site 1 hour prior to irradiation. These findings collectively support the feasibility of ovarian protection *in situ* through the use of S1P or its mimetics as a promising option for improving the quality of life in female cancer patients in the future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Meiorow D, Nugent D. The effects of radiotherapy and chemotherapy on female reproduction. *Hum Reprod Update*. 2001; 7:535–543. [PubMed: 11727861]
2. Lee SJ, Schover LR, Partridge AH, Patrizio P, Wallace WH, Hagerty K, et al. American Society of Clinical Oncology recommendations on fertility preservation in cancer patients. *J Clin Oncol*. 2006; 24:2917–2931. [PubMed: 16651642]
3. Oktay K, Sönmezer M. Chemotherapy and amenorrhea: risks and treatment options. *Curr Opin Obstet Gynecol*. 2008; 20:408–415. [PubMed: 18660694]
4. Shaw JM, Bowles J, Koopman P, Wood EC, Trounson AO. Fresh and cryopreserved ovarian tissue samples from donors with lymphoma transmit the cancer to graft recipients. *Hum Reprod*. 1996; 11:1668–1673. [PubMed: 8921114]

5. Sönmezer M, Shamonki MI, Oktay K. Ovarian tissue cryopreservation: benefits and risks. *Cell Tissue Res.* 2005; 322:125–132. [PubMed: 15912406]
6. Tilly JL. Commuting the death sentence: how oocytes strive to survive. *Nat Rev Mol Cell Biol.* 2001; 2:838–848. [PubMed: 11715050]
7. Morita Y, Perez GI, Paris F, Miranda S, Ehleiter D, Haimovitz-Friedman A, et al. Oocyte apoptosis is suppressed by disruption of the *acid sphingomyelinase* gene or by sphingosine-1-phosphate therapy. *Nat Med.* 2000; 6:1109–1114. [PubMed: 11017141]
8. Paris F, Perez GI, Haimovitz-Friedman A, Nguyen H, Fuks Z, Bose M, et al. Sphingosine-1-phosphate preserves fertility in irradiated female mice without propagating genomic damage in offspring. *Nat Med.* 2002; 8:901–902. [PubMed: 12205432]
9. Perez GI, Knudson CM, Leykin L, Korsmeyer SJ, Tilly JL. Apoptosis-associated signaling pathways are required for chemotherapy-mediated female germ cell destruction. *Nat Med.* 1997; 3:1228–1232. [PubMed: 9359697]
10. Bergeron L, Perez GI, McDonald G, Shi L, Sun Y, Jurisicova A, et al. Defects in regulation of apoptosis in caspase-2-deficient mice. *Genes Dev.* 1998; 12:1304–1314. [PubMed: 9573047]
11. Cu villier O, Pirianov G, Kleuser B, Vanek PG, Coso OA, Gutkind S, et al. Suppression of ceramide-mediated programmed cell death by sphingosine-1-phosphate. *Nature.* 1996; 381:800–803. [PubMed: 8657285]
12. Deng X, Yin X, Allan R, Lu DD, Maurer CW, Haimovitz-Friedman A, et al. Ceramide biogenesis is required for radiation-induced apoptosis in the germ line of *C. elegans*. *Science.* 2008; 322:110–115. [PubMed: 18832646]
13. Jurisicova A, Lee H-J, D'Estaing SG, Tilly JL, Perez GI. Molecular requirements for doxorubicin-mediated death in murine oocytes. *Cell Death Differ.* 2006; 13:1466–1474. [PubMed: 16439991]
14. Hancke K, Strauch O, Kissel C, Göbel H, Schäfer W, Denschlag D. Sphingosine 1-phosphate protects ovaries from chemotherapy-induced damage *in vivo*. *Fertil Steril.* 2007; 87:172–177. [PubMed: 17081530]
15. Kaya H, Desdicioglu R, Sezik M, Ulukaya E, Ozkaya O, Yilmaztepe A, et al. Does sphingosine-1-phosphate have a protective effect on cyclophosphamide- and irradiation-induced ovarian damage in the rat model? *Fertil Steril.* 2008; 89:732–735. [PubMed: 17517398]
16. Adachi K, Chiba K. FTY720 story. Its discovery and the following accelerated development of sphingosine 1-phosphate receptor agonists as immunomodulators based on reverse pharmacology. *Perspect Med Chem.* 2007; 1:11–23.
17. Zelinski-Wooten MB, Stouffer RL. Intraluteal infusions of prostaglandins of the E, D, I, and A series prevent PGF_{2α}-induced, but not spontaneous, luteal regression in rhesus monkeys. *Biol Reprod.* 1990; 43:507–516.
18. Hotchkiss CE, Bishop ME, Dertinger SD, Slikker W Jr, Moore MM, Macgregor JT. Flow cytometric analysis of micronuclei in peripheral blood reticulocytes IV: an index of chromosomal damage in the rhesus monkey (*Macaca mulatta*). *Toxicol Sci.* 2008; 102:352–358. [PubMed: 18211907]
19. Weissman A, Gotlieb L, Colgan T, Jurisicova A, Greenblatt EM, Casper RF. Preliminary experience with subcutaneous human ovarian cortex transplantation in the NOD-SCID mouse. *Biol Reprod.* 1999; 60:1462–1467. [PubMed: 10330106]
20. Matikainen T, Perez GI, Jurisicova A, Schlezinger JJ, Ryu H-Y, Pru JK, et al. Aromatic hydrocarbon receptor-driven *Bax* gene expression is required for premature ovarian failure caused by biohazardous environmental chemicals. *Nat Genet.* 2001; 28:355–360. [PubMed: 11455387]
21. Dixson, AF. Primate sexuality: comparative studies of the prosimians, monkeys, apes and human beings. Oxford: Oxford University Press; 1998.
22. Borman SM, Schwinof KM, Niemeyer C, Chwalisz K, Stouffer RL, Zelinski-Wooten MB. Low-dose antiprogesterone treatment prevents pregnancy in rhesus monkeys and is reversible after 1 year of treatment. *Hum Reprod.* 2003; 18:69–76. [PubMed: 12525443]
23. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. *CA Cancer J Clin.* 2010
24. Blumenfeld Z, Avivi I, Eckman A, Epelbaum R, Rowe JM, Dann EJ. Gonadotropin-releasing hormone agonist decreases chemotherapy-induced gonadotoxicity and premature ovarian failure in

- young female patients with Hodgkin lymphoma. *Fertil Steril*. 2008; 89:166–173. [PubMed: 17601603]
25. Badawy A, Elnashar A, El-Ashry M, Shahat M. Gonadotropin-releasing hormone agonists for prevention of chemotherapy-induced ovarian damage: prospective randomized study. *Fertil Steril*. 2009; 91:694–697. [PubMed: 18675959]
26. Oktay K, Sönmezer M, Oktem O, Fox K, Emons G, Bang H. Absence of conclusive evidence for the safety and efficacy of gonadotropin-releasing hormone analogue treatment in protecting against chemotherapy-induced gonadal injury. *Oncologist*. 2007; 12:1055–1066. [PubMed: 17914075]
27. Beck-Fruchter R, Weiss A, Shalev E. GnRH agonist therapy as ovarian protectants in female patients undergoing chemotherapy: a review of the clinical data. *Hum Reprod Update*. 2008; 14:553–561. [PubMed: 18820006]
28. Garcia, JE. Fertility after cancer treatment. In: Trimble, EL.; Trimble, CL., editors. *Cancer – Obstetrics and Gynecology*. Philadelphia: Lippincott, Williams and Wilkins; 1999. p. 53-86.
29. Wallace W, Thomson A, Kelsey T. The radiosensitivity of the human oocyte. *Hum Reprod*. 2003; 18:117–121. [PubMed: 12525451]
30. Huser M, Crha I, Hudecek R, Ventruba P, Zakova J, Smardova L, et al. Ovarian tissue cryopreservation--new opportunity to preserve fertility in female cancer patients. *Eur J Gynaecol Oncol*. 2007; 28:249–255. [PubMed: 17713087]
31. Xu M, Kreeger PK, Shea LD, Woodruff TK. Tissue-engineered follicles produce live, fertile offspring. *Tissue Eng*. 2006; 12:2739–2746. [PubMed: 17518643]
32. West ER, Shea LD, Woodruff TK. *In vitro* grown human ovarian follicles from cancer patients support oocyte growth. *Hum Reprod*. 2009; 24:2531–2540. [PubMed: 19597190]

FIGURE 1.

In-vivo delivery of S1P or FTY720 leads to a rapid return of reproductive cyclicity in adult female primates after OXI. Menstrual cyclicity in unilaterally ovariectomized monkeys that received intraovarian infusion of vehicle (Veh), S1P or FTY720 (FTY) 1 week prior to sham manipulation or OXI (each horizontal line represents one animal). *Red* bars, ovarian cycles with normal follicular and luteal phases; *blue* bars, ovarian cycles with a normal follicular phase and no luteal phase (based on circulating estradiol and progesterone levels); M, menses; *, termination of study.

FIGURE 2.

In-vivo delivery of S1P or FTY720 attenuates radiation-induced ovarian damage in adult female primates. Representative histological appearance of ovaries from rhesus monkeys 9–10 months after intraovarian infusion of vehicle (**A**, **B**), S1P (**C**) or FTY720 (**D**) for 1 week prior to sham manipulation (**A**) or OXI (**B–D**). Percentage of ovarian follicles remaining in each animal (**E**), defined as the number of follicles counted in an ovary obtained 9–10 months after sham or OXI divided by the total number of follicles counted in the contralateral ovary from each animal prior to sham or OXI. Bars, represent the mean $\pm$ SE ($n = 3$ –5 animals per group) with P -values determined by one-way ANOVA followed by the Neuman-Keuls multiple range test (different superscript letters, $P < .05$); ND, none detected.

FIGURE 3.

Radioprotection of primate ovaries with FTY720 preserves natural fertility. Panels A–F show the first set of offspring conceived and delivered by adult female rhesus monkeys following FTY720-mediated ovarian protection from 15 Gy of radiation exposure (A and B, Hope at 7 and 453 days of age, respectively; C and D, Boneita at 7 and 432 days of age, respectively; E and F, Trinity at 7 and 416 days of age, respectively). Analysis of the frequency of micronucleated reticulocytes in blood of these infants over the first 70 days of age (G) shows no significant differences in offspring conceived by vehicle-infused non-irradiated controls (Veh+Sham) versus radioprotected females (FTY+OXI). Bars are the mean $\pm$ SE ($n = 3$ animals per group sampled at 1, 2, 3, 4 and 10 weeks of age; value range

for each group is provided on the x -axis). Birth of the first 3 offspring was followed by a second successful pregnancy in 2 of 3 radioprotected females, with delivery of Victor (**H**; Hope's brother) and Victoria (**I**; Boneita's sister). As of the 1st of October 2010, the first set of offspring conceived and delivered by the FTY720-radioprotected females were 777 (Hope), 756 (Boneita) and 740 (Trinity) days of age; the second set of offspring were 352 (Victor) and 331 (Victoria) days of age.

FIGURE 4.

In-vivo exposure to S1P for 1 hour prevents radiation-induced degeneration of primordial follicles in adult human ovary tissue xenografted into immunodeficient mice. Ovarian cortical biopsies (1–2 mm²) were grafted into NOD-SCID mice. One set of mice were euthanized 1 week after grafting to collect baseline information on oocyte numbers (Control, open bars; n = 6 mice), whereas the remaining mice were subjected to irradiation (2 Gy) 1 hour after injection of the graft sites with either vehicle (VEH+IR, gray bars; n = 6 mice) or S1P (S1P+IR, black bars; n = 6 mice). Grafts were collected from all mice 6 days later and assessed for the number of degenerative oocytes out of the total number of oocytes present by morphological criteria. *Bars*, represent the mean $\pm$ SE of combined data from 6–8 ovarian grafts per group (the total number of follicles scored in each group is provided over the respective bar).