

Hormonal Treatment after Cytotoxic Therapy Stimulates Recovery of Spermatogenesis¹

Marvin L. Meistrich,² Gene Wilson, and Ipo Huhtaniemi

Department of Experimental Radiation Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas 77030 [M. L. M., G. W.], and Department of Physiology, University of Turku, 20520 Turku, Finland [I. H.]

Abstract

Previous studies have shown that treatment of rats with gonadotropin-releasing hormone (GnRH) analogues or steroids either before exposure to procarbazine or radiation or after irradiation enhances subsequent levels of spermatogenesis. We demonstrate here that giving a GnRH agonist after procarbazine injection also enhances spermatogenesis and fertility. We also demonstrate that GnRH agonist stimulated recovery of spermatogenesis and fertility not only when the hormone was administered immediately after irradiation, but also at 20 weeks after irradiation, after the decline in spermatogenesis had occurred. These results suggest that GnRH agonist treatment given to azoospermic men after cytotoxic therapy for cancer may stimulate the recovery of spermatogenesis and fertility.

Introduction

Radiation and chemotherapeutic drugs, such as procarbazine and other alkylating agents, produce prolonged and sometimes irreversible azoospermia in humans (1). Because numerous patients are treated with radiation and/or chemotherapy before and during their reproductive years, and cure rates for some types of cancer are high, sterility caused by these treatments is a very significant concern. Single radiation doses of 2 Gy and scatter doses of 2.5 Gy that reach the testes from fractionated radiotherapy for lymphomas and sarcomas result in prolonged azoospermia lasting 1 year and at least 3 years, respectively (2). Doses of procarbazine of about 4 g/m² (body surface area in square meters) given as part of combination chemotherapy produce a similar effect (3). Certain strains of rats, such as LBNF₁, are also very sensitive to these agents (4, 5). Spermatogenesis in these rats does not recover within 60 weeks after irradiation with a single 3.5-Gy dose (6) or 10 weeks after 400 mg/kg procarbazine (equivalent to 3 g/m² in humans; Ref. 4). However, pretreatment of rats with hormones such as testosterone itself, testosterone plus 17 β -estradiol, or GnRH³ analogues that suppress gonadotropins and/or testosterone production results in enhanced levels of spermatogenesis after irradiation or procarbazine treatment (4, 5, 7). It was previously believed that the hormone treatment protected stem spermatogonia from the damaging effects of radiation or chemotherapy. However, all tests of possible mechanisms for stem cell protection failed to support any of the proposed mechanisms (8–10). Furthermore, we observed that some stem cells survived irradiation, although they failed to differentiate (6). Moreover, we recently showed that treating the rats with the same hormones after irradiation could stimulate spermatogenic recovery (11). In the present study, we wished to determine whether GnRH agonist treatment

would also stimulate recovery of spermatogenesis in rats after procarbazine administration. We also extended the results with GnRH agonist treatment after irradiation to examine the effects of the timing of the treatment and to test whether fertility could be restored.

Materials and Methods

Most procedures were identical to those described in previous studies (4, 8, 9, 11) to which the reader is referred for additional experimental details.

Adult LBNF₁ male and Sprague Dawley female rats were obtained from Harlan Sprague Dawley (Indianapolis, IN). Procarbazine [*N*-(1-methylethyl)-4-(2-methylhydrazine)methyl]benzamide monohydrochloride] was a gift from Hoffmann-La Roche (Nutley, NJ). It was dissolved in normal saline and injected i.p. at 250 mg/kg into five groups of six male rats each. The lower part of the body of anesthetized male rats was given a single 3.5-Gy dose of ⁶⁰Co γ -rays. Six groups of four rats each plus two groups of two rats each were irradiated.

Starting 1 day after procarbazine injection, rats were given GnRH agonist treatment with a depot formulation of Lupron (leuprolide acetate; D-Leu⁶, des-Gly-NH₂¹⁰-pro-ethylamide⁹-GnRH), which was kindly supplied by TAP Pharmaceuticals, Inc. (Deerfield, IL). Lupron was suspended in a carboxymethyl cellulose vehicle provided and given as three 1.83-mg injections (currently considered the optimal dose for rats⁴) 23–24 days apart, starting 1 day after procarbazine injection. In the case of irradiation, four groups of rats were also treated with three Lupron injections each, except that the dose/injection was 1.25 mg/rat (based on earlier estimates of the appropriate dose). Injections were initiated immediately after irradiation in two groups and at 20 weeks after irradiation in two groups.

Spermatogenesis was evaluated by testis weight, histology, and testicular sperm head counts (12). For histological analysis, the RI, which is the percentage of tubules containing three or more differentiating germ cells derived from surviving stem cells, was determined by scoring 200 seminiferous tubule cross-sections from each animal. Interphase and mitotic A spermatogonia in nonrepopulating tubules after procarbazine treatment were also counted (6). The weight of the right testis was measured after removing the tunica albuginea, the tissue was homogenized, an aliquot was sonicated, and sperm heads were counted.

To assess the fertility of the male rats, they were mated for 2 weeks before they were killed. Each male was caged with two females for 1 week and then caged with two different females for the second week. Females were killed 14 days after the end of mating, and the numbers of embryos were counted. A male was considered fertile if he produced one or more offspring by any of the four females.

LH and FSH were assayed by an immunofluorometric assay and a double-antibody RIA, respectively (11). Serum testosterone and ITT concentrations were measured using coated-tube RIA kits (11).

Algebraic means and SEs of the means were calculated for RI, testis weights, and FSH levels. For sperm head counts, LH, serum testosterone, and ITT, averages and SEs were calculated from log-transformed data. The effects of hormone treatment on hormone levels and on spermatogenic parameters were analyzed using the Mann-Whitney test. The fertility data were analyzed by the χ^2 method.

Received 4/7/99; accepted 6/16/99.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ Supported by Research Grant ES-08075 and Core Grant CA-16672 from the NIH.

² To whom requests for reprints should be addressed, at Department of Experimental Radiation Oncology, Box 66, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030. Phone: (713) 792-3424; Fax: (713) 794-5369; E-mail: meistrich@mdanderson.org.

³ The abbreviations used are: GnRH, gonadotropin-releasing hormone; FSH, follicle stimulating hormone; ITT, intratesticular testosterone; LH, luteinizing hormone; RI, repopulation index.

⁴ Dr. Eugene Bush (Abbott Laboratories), personal communication.

Results

Spermatogenesis after Procarbazine Treatment. A 250-mg/kg dose of procarbazine resulted in the failure of most spermatogenic tubules to repopulate from surviving stem cells (Fig. 1). Furthermore, the percentage of tubules with differentiated germinal cells declined with time, as was observed previously for irradiation (6). The numbers of A spermatogonia in nonrepopulating tubules were counted in three rats killed at 10 weeks after procarbazine treatment and in three rats killed at 20 weeks after procarbazine treatment, and because the results were the same, the values were pooled. There were numerous A spermatogonia in the tubules, amounting to 2.8 ± 0.2 A spermatogonia/100 Sertoli cells (12.3 ± 0.4 Sertoli cells/tubule cross-section). These numbers were not significantly different from those in concurrently scored rats 10 weeks after 3.5 Gy of irradiation, namely, 2.6 ± 0.7 A spermatogonia/100 Sertoli cells and 9.1 ± 1.6 Sertoli cells/tubule cross-section. The mitotic index in the procarbazine-treated rats was $11.4 \pm 1.4\%$, which is significantly ($P = 0.02$) lower than the value of $21.4 \pm 1.7\%$ obtained in irradiated rats. Together, these results indicate that proliferating A spermatogonia are present in nonrepopulating tubules after procarbazine treatment, suggesting that the potential to stimulate spermatogenesis exists.

Maintenance of Spermatogenesis after Procarbazine Treatment. Treatment with GnRH agonist for the first 10 weeks after procarbazine injection prevented the loss of spermatogenic cells. The

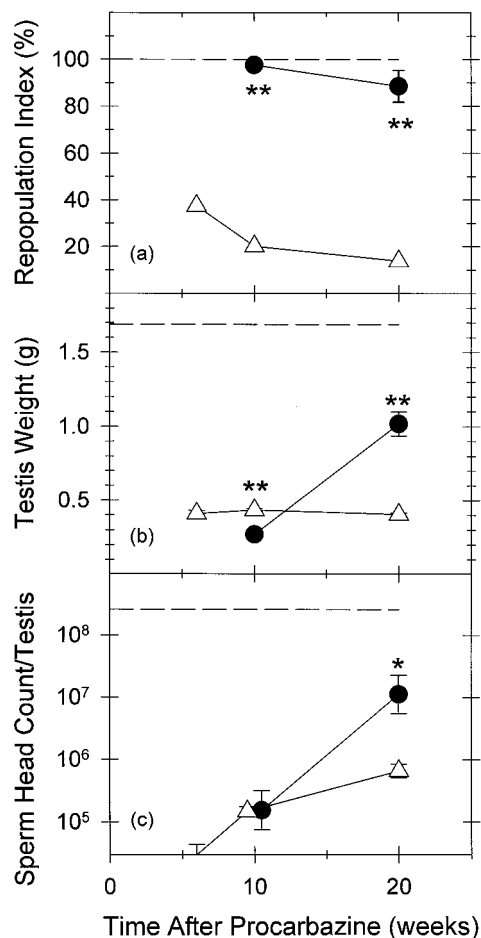


Fig. 1. Time course of testicular response to procarbazine treatment at a dose of 250 mg/kg without (Δ) and with (\bullet) GnRH agonist administration from weeks 0–10 assayed by seminiferous tubule RI (a), testis weight (b), and sperm head count (c). Bars, SE. The dashed line indicates the values in control rats receiving neither procarbazine nor hormone. Significance of difference between procarbazine/GnRH-treated rats and rats treated with procarbazine alone: *, $P < 0.05$; **, $P < 0.005$.

percentage of tubules with differentiating cells was 98% compared to only 20% in rats not treated with the hormone (Fig. 1). Most of the tubules in the GnRH-treated rats contained cells up to the pachytene spermatocyte or round spermatid stage, but only about 6% of the tubules contained elongated spermatids. This was expected because the GnRH agonist suppresses the intratesticular level of testosterone, which is required for maturation of the spermatids. Testis weight was also significantly suppressed at the 10 week time point, despite the increase in germ cells. This could have been a result of the atrophy of Leydig cells as well as a loss of testosterone-dependent functions, such as fluid secretion by Sertoli cells.

At 20 weeks after procarbazine treatment, the percentage of repopulating tubules was maintained at 89%, a value that was not significantly different from the 98% observed at 10 weeks, despite the cessation of the GnRH agonist treatment. However, because the GnRH agonist treatment was stopped, and spermatids were able to mature, testis weight and sperm counts showed increased values that were significantly above those observed in the rats not treated with the hormone. Fertility was likewise increased; whereas none of the six rats treated with procarbazine alone were fertile during weeks 18 and 19, a significantly ($P < 0.02$) greater fraction (four of six) of rats treated with procarbazine and the GnRH agonist were fertile.

Hormone Levels in Procarbazine-treated Rats. As observed previously with irradiation (11), the ITT concentration was elevated in the procarbazine-treated rats, tripling in value from 33 ng/g testis in untreated controls to 110 ng/g testis by 20 weeks after procarbazine treatment (Table 1). However, the rise after procarbazine treatment was neither as rapid nor as large as that which followed irradiation. Consistent with this delayed rise of ITT, LH was not elevated above control levels, and serum testosterone was reduced at 6 weeks after procarbazine treatment. However, LH did eventually rise at weeks 10 and 20. In contrast, FSH was already elevated at 6 weeks.

GnRH agonist treatment for 10 weeks decreased the ITT concentration to 11 ng/g testis. Serum testosterone levels fell to less than 0.05 ng/ml, compared to 1.2 ng/ml in the untreated rat and 1.0 ng/ml at 10 weeks after procarbazine treatment. Given that the GnRH agonist did not significantly decrease LH levels (Table 1), these results indicate that it must reduce testosterone production in the rat by acting directly on Leydig cells.

Stimulation of Spermatogenic Recovery after Irradiation. In a previous study, we showed that GnRH agonist treatment given during weeks 0–10 after 3.5 Gy of irradiation prevented the decline of spermatogenesis and resulted in fertility in two of four rats by week 16.5, whereas none of the four rats given radiation alone were fertile (11). In this study, we treated additional rats with a GnRH agonist during weeks 0–10 after irradiation. At 10 weeks after irradiation, spermatogenesis was evident in 93% of the tubules of the irradiated, GnRH agonist-treated rats but in only 54% of the tubules in rats treated with irradiation alone (Fig. 2). When the hormone treatment was stopped at week 10, and spermatogenesis was assessed at week 20, the irradiated, GnRH agonist-treated rats showed a RI of 100%, compared with a RI of 30% in rats treated with irradiation alone. Furthermore at this time, testis weights and sperm counts were dramatically increased in GnRH agonist-treated rats and were 58% and 78%, respectively, of the levels seen in control unirradiated rats. All four of the irradiated, GnRH agonist-treated rats were fertile, whereas only two of the four rats receiving irradiation alone were fertile. Although the increase in fertility based on this experiment alone was not statistically significant ($P = 0.10$), when the data were combined with those of the previous study using similar conditions (11), the results became significant ($P < 0.05$).

To determine whether recovery of spermatogenesis could be stimulated after regression of spermatogenesis had occurred, rats were

Table 1 Effect of procarbazine and irradiation and subsequent GnRH agonist treatment on hormone levels

Cytotoxic agent	Hormone treatment	Time after cytotoxic treatment (wk)	No. of rats	LH ^a (ng/ml)	FSH ^b (ng/ml)	Serum testosterone (ng/ml) ^a	ITT (ng/g testis) ^a
None	None		6–27	0.14 (0.12–0.17)	22 ± 1	1.2 (0.9–1.5)	33 (26–41)
Procarbazine ^c	None	6	6	0.15 (0.10–0.22)	29 ± 1 ^d	0.25 ^d (0.15–0.43)	54 (42–67)
	None	10	6	0.57 ^d (0.33–0.98)	34 ± 1 ^d	1.0 (0.9–1.2)	67 (53–86)
	None	20	6	0.63 ^d (0.54–0.72)	28 ± 1 ^d	1.3 (1.2–1.4)	110 ^d (99–122)
	Lupron ^e	10	6	0.40 (0.36–0.44)	14 ± 0 ^f	0.05 ^f (0.04–0.06)	10.7 ^f (9.7–12.1)
Irradiation ^g	None	10	2	0.23 (0.20–0.26)	32 ± 1 ^d	1.2 (0.9–1.6)	108 ^d (87–134)
	None	20	4	0.57 ^d (0.47–0.69)	29 ± 3 ^d	1.0 (0.7–1.4)	121 ^d (92–159)
	None	30	2	0.18 (0.18–0.19)	27 ± 1 ^d	0.7 (0.6–0.8)	101 ^d (72–140)
	None	40	4	0.31 (0.30–0.33)	29 ± 0 ^d	0.8 (0.4–1.4)	196 ^d (178–217)
	Lupron ^e	10	4	0.66 (0.61–0.72)	14 ± 0 ^f	0.16 ^f (0.08–0.32)	2.4 ^f (2.1–2.7)
	Lupron ^e	30	4	0.53 (0.43–0.64)	18 ± 1 ^f	0.07 ^f (0.06–0.07)	11.4 ^f (9.7–13.4)

^a Mean (–1 SE, +1 SE).^b Mean ± SE.^c Procarbazine dose, 250 mg/kg.^d Significantly different from value in untreated rats, $P < 0.05$.^e GnRH agonist treatment for 10 weeks before killing.^f Significantly different from value in rats with same cytotoxic treatment but without Lupron treatment, $P < 0.05$ (for this analysis, values for the rats irradiated with 3.5 Gy and killed during weeks 10 and 20 were pooled, as were those for rats killed on weeks 30 and 40, because of the small numbers on weeks 10 and 30).^g Radiation dose, 3.5 Gy.

treated with GnRH agonist beginning at 20 weeks after 3.5 Gy of irradiation, when only 30% of tubules contained germ cells past the A spermatogonial stage. Treatment with GnRH agonist for 10 weeks (until week 30 after irradiation) appeared to increase the RI to 54%

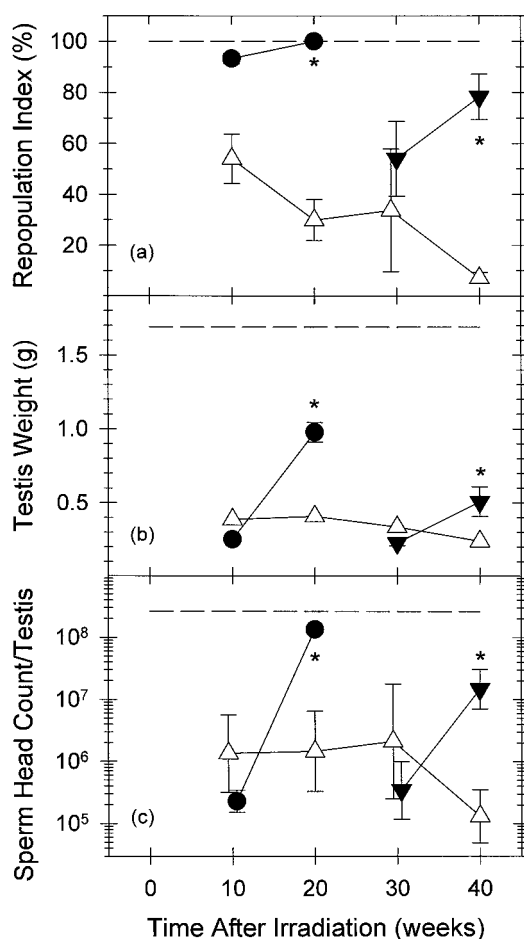


Fig. 2. Time course of testicular response to 3.5 Gy of irradiation without (Δ) and with GnRH agonist administration from weeks 0–10 (\bullet) or weeks 20–30 (\blacktriangledown) assayed by seminiferous tubule RI (a), testis weight (b), and sperm head count (c). Bars, SE. The dashed line indicates the values in control unirradiated rats. Significance of difference between irradiated/GnRH-treated rats and rats treated with irradiation alone: *, $P < 0.05$.

(Fig. 2). Testis weights and sperm counts were depressed at this time because of the suppression of testosterone by the GnRH agonist. At week 40, 10 weeks after the cessation of GnRH agonist treatment, RI, testis weights, and sperm head counts were significantly increased. Furthermore one of four GnRH agonist-treated rats was fertile (at week 39), whereas none of the rats treated with irradiation alone were fertile at that time. Comparing the level of spermatogenesis observed at week 40 with that observed at week 20, all three end points showed higher values when the hormone treatment was initiated immediately after irradiation than when it was started 20 weeks later (Fig. 2).

Hormone Levels in Irradiated Rats. The measurements (Table 1) confirmed that the GnRH agonist Lupron effectively reduced serum testosterone and ITT levels after irradiation without decreasing LH. The reduction in ITT concentrations was significantly greater when the rats were treated with GnRH agonist during weeks 0–10 than during weeks 20–30, suggesting less suppression of ITT levels as a cause of the lower level of repopulation observed after the latter treatment.

Discussion

The reason spermatogenesis fails to recover after radiation and chemotherapy and the mechanism by which GnRH agonist treatment results in its restoration are not fully understood. It was previously shown in irradiated rats that spermatogonial stem cells survive radiation treatment but fail to undergo differentiation (6). The presence of stem cells is a prerequisite for being able to stimulate recovery. In this report, we show that spermatogonia also survive after procarbazine treatment but fail to differentiate. In humans too, stem cells survive but fail to differentiate into spermatozoa for an extended period of time, as evidenced by the occasional recovery of spermatogenesis after several years of radiation- or chemotherapy-induced azoospermia (2, 13). The failure of recovery of spermatogenesis to occur naturally in the rat appears to be related to the high ITT concentrations and the stimulation of recovery by GnRH agonists to suppression of ITT and serum T levels (11).⁵

Nearly all previous attempts to improve fertility after cancer therapy have focused on hormonal treatment before and during treatment (7). Here we demonstrate that comparable recovery of spermatogenesis can be achieved by hormonal treatment of rats after cytotoxic therapy. The RI, testis weights, and sperm counts observed here at 20

⁵ G. Shetty and M. L. Meistrich. Exogenous testosterone inhibits GnRH analogue-induced stimulation of spermatogenesis in irradiated rats, submitted for publication.

weeks after 250 mg/kg procarbazine in rats given GnRH treatment for the first 10 weeks were 89%, 1.1 g, and 1.1×10^7 . These values are comparable to the average values of 93%, 0.8 g, and 3.6×10^7 in various studies from our laboratory using treatment with steroids or GnRH analogues before the same dose of procarbazine (14). Similarly the RI, testis weights, and sperm counts in rats at 20 weeks after 3.5 Gy of irradiation followed by a 10-week GnRH agonist treatment were 100%, 1.0 g, and 1.4×10^8 . Again, these values are similar to the 99%, 0.8 g, and 0.5×10^8 observed 10 weeks after 3.5 Gy of irradiation in rats pretreated with testosterone and estradiol. Thus, giving the hormone treatment after cytotoxic therapy is as effective as administering it before cytotoxic therapy.

Preliminary results showed that GnRH agonist treatment of rats after spermatogenesis had already declined due to irradiation was also slightly effective at stimulating repopulation (11). The present study confirms those results and shows a moderate level of repopulation and recovery of sperm count and fertility even when the GnRH agonist was given 20 weeks after 3.5 Gy of irradiation. It is not known whether even more recovery might have been achieved if we had taken measurements later, beyond 10 weeks after the completion of GnRH treatment, or, on the other hand, if further delays in treatment would have resulted in further reductions of recovery. Nevertheless, the recovery observed when GnRH agonist treatment was initiated at 20 weeks was less than when it was given immediately after irradiation.

We believe that the results obtained here with single doses of radiation or procarbazine will apply to fractionated regimens, which are given clinically. This is based on the similar effects of single and fractionated treatment with procarbazine on the testis and the ability of hormonal pretreatment to enhance recovery after both types of regimens (4).

Based on the current information, the development of clinical protocols to stimulate the recovery of spermatogenesis in men treated with cytotoxic agents for cancer should be considered. The best patient groups would be those treated with highly sterilizing regimens such as those containing procarbazine for Hodgkin's disease, pelvic irradiation for Hodgkin's and non-Hodgkin's lymphoma, and high-dose chemotherapy with busulfan and cyclophosphamide or total body irradiation before stem cell transplantation for leukemia and other diseases (1).

The use of hormone treatment after cytotoxic therapy rather than before and during cytotoxic therapy should be more acceptable to patients because they can better focus on quality of life issues such as future fertility after therapy. The possibility that hormone treatments before, during, and after cytotoxic therapy would be even better was not explored here. However, treatment with a GnRH antagonist before and during cytotoxic therapy and with testosterone after cytotoxic therapy has been reported to enhance the recovery of rat spermatogenesis from procarbazine-induced damage (15).

Based on the results in the rat, it would be advisable to institute the hormone treatment of patients as soon as possible, even during irradiation or chemotherapy. However, hormone treatment should still be considered as an option for patients who present with azoospermia several years after cytotoxic therapy.

GnRH agonists should be considered for such a protocol because they are effective in suppressing testosterone production in humans (16), although they act via a different mechanism in humans than in rats. In rats, GnRH agonists act directly on Leydig cells, whereas in humans, who lack functional Leydig cell GnRH receptors (17), GnRH agonists act by LH suppression (16). However, because the important factor for stimulation of spermatogenesis is the suppression of testosterone levels, and levels of LH do not seem to matter (11),⁴ GnRH

may be effective in stimulating spermatogenesis in humans. Because 10-week treatments were successful in rodents, one should consider treating at least twice as long in humans, because the decline in testosterone and sperm production during GnRH agonist treatment is generally slower (16, 18). Even if there is some uncertainty about applicability to humans because the mechanism of spermatogenic stimulation is not known, GnRH agonist treatment has such minimal side effects that patients would not be put at much risk (16). The major side effect is a temporary loss of libido in many men. Although this could be overcome by exogenous testosterone administration, testosterone supplementation should be avoided or at least delayed and given in as low a dose as possible because testosterone supplementation inhibits the GnRH-induced stimulation of spermatogenesis.⁴

Acknowledgments

We thank TAP Pharmaceuticals, Inc. for kindly providing Lupron, Kuria-kose Abraham for histological preparations, Walter Pagel for editorial advice, and Tarja Laiho for performing the LH and FSH measurements.

References

- Meistrich, M. L., Sellin, R. V., and Lipshultz, L. I. Gonadal dysfunction. *In*: V. T. DeVita, S. Hellman, and S. A. Rosenberg (eds.), *Cancer: Principles and Practice of Oncology*, pp. 2758–2773. Philadelphia: J. B. Lippincott Co., 1997.
- Clifton, D. K., and Bremner, W. J. The effect of testicular X-irradiation on spermatogenesis in man. A comparison with the mouse. *J. Androl.*, *4*: 387–392, 1983.
- da Cunha, M. F., Meistrich, M. L., Fuller, L. M., Cundiff, J. H., Hagemester, F. B., Velasquez, W. S., MacLaughlin, P., Riggs, S. A., Cabanillas, F. F., and Salvador, P. G. Recovery of spermatogenesis after treatment for Hodgkin's disease: limiting dose of MOPP chemotherapy. *J. Clin. Oncol.*, *2*: 571–577, 1984.
- Parchuri, N., Wilson, G., and Meistrich, M. L. Protection by gonadal steroid hormones against procarbazine-induced damage to spermatogenic function in LBNF₁ hybrid rats. *J. Androl.*, *14*: 257–266, 1993.
- Kurdoglu, B., Wilson, G., Parchuri, N., Ye, W-S., and Meistrich, M. L. Protection from radiation-induced damage to spermatogenesis by hormone treatment. *Radiat. Res.*, *139*: 97–102, 1994.
- Kangasniemi, M., Huhtaniemi, I., and Meistrich, M. L. Failure of spermatogenesis to recover despite the presence of A spermatogonia in the irradiated LBNF₁ rat. *Biol. Reprod.*, *54*: 1200–1208, 1996.
- Morris, I. D., and Shalet, S. M. Protection of gonadal function from cytotoxic chemotherapy and irradiation. *Baillieres Clin. Endocrinol. Metab.*, *4*: 97–118, 1990.
- Meistrich, M. L., Wilson, G., Ye, W-S., Kurdoglu, B., Parchuri, N., and Terry, N. H. A. Hormonal protection from procarbazine-induced testicular damage is selective for survival and recovery of stem spermatogonia. *Cancer Res.*, *54*: 1027–1034, 1994.
- Meistrich, M. L., Wilson, G., Zhang, Y., Kurdoglu, B., and Terry, N. H. A. Protection from procarbazine-induced testicular damage by hormonal pretreatment does not involve arrest of spermatogonial proliferation. *Cancer Res.*, *57*: 1091–1097, 1997.
- Wilson, G., Kangasniemi, M., and Meistrich, M. L. Hormone pretreatment enhances recovery of spermatogenesis in rats after neutron irradiation. *Radiat. Res.*, *152*: 51–56, 1999.
- Meistrich, M. L., and Kangasniemi, M. Hormone treatment after irradiation stimulates recovery of rat spermatogenesis from surviving spermatogonia. *J. Androl.*, *18*: 80–87, 1997.
- Meistrich, M. L., and van Beek, M. E. A. B. Spermatogonial stem cells: assessing their survival and ability to produce differentiated cells. *In*: R. E. Chapin and J. Heindel (eds.), *Methods in Toxicology*, Vol. 3A, pp. 106–123. New York: Academic Press, 1993.
- Meistrich, M. L., Wilson, G., Brown, B. W., da Cunha, M. F., and Lipshultz, L. I. Impact of cyclophosphamide on long-term reduction in sperm count in men treated with combination chemotherapy for Ewing's and soft tissue sarcomas. *Cancer (Phila.)*, *70*: 2703–2712, 1992.
- Meistrich, M. L., Wilson, G., Ye, W-S., Thrash, C., and Huhtaniemi, I. Relationship among hormonal treatments, suppression of spermatogenesis, and testicular protection from chemotherapy-induced damage. *Endocrinology*, *137*: 3823–3831, 1996.
- Pogach, L. M., Lee, Y., Gould, S., Giglio, W., and Huang, H. F. S. Partial prevention of procarbazine induced germinal cell aplasia in rats by sequential GnRH antagonist and testosterone administration. *Cancer Res.*, *48*: 4354–4360, 1988.
- Schürmeyer, T., Knuth, U. A., Freischem, C. W., Sandow, J., Akhtar, F. B., and Nieschlag, E. Suppression of pituitary and testicular function in normal men by constant gonadotropin-releasing hormone agonist infusion. *J. Clin. Endocrinol. Metab.*, *59*: 19–24, 1984.
- Clayton, R. N., and Huhtaniemi, I. T. Absence of gonadotropin-releasing hormone receptors in human gonadal tissue. *Nature (Lond.)*, *299*: 56–59, 1982.
- Ward, J. A., Furr, B. J. A., Valcaccia, B., Curry, B., Bardin, C. W., Gunsalus, G. L., and Morris, I. D. Prolonged suppression of rat testis function by a depot formulation of Zoladex, a GnRH agonist. *J. Androl.*, *10*: 478–486, 1989.