The Effect of Testicular X-irradiation on Spermatogenesis in Man

A Comparison with the Mouse

DONALD K. CLIFTON AND WILLIAM J. BREMNER

From the Departments of Medicine and Obstetrics and Gynecology, and Population Center for Research in Reproduction, University of Washington School of Medicine and Veterans Administration Medical Center, Seattle, Washington

Existing data concerning the effects of x-irradiation on spermatogenesis in man were analyzed and the results were compared to published data on the mouse. Testicular x-irradiation produced a transient, but substantial, suppression of sperm counts in man, with an ED_{50} near 11 rad. The length of time to recovery was proportional to the irradiation dose. The ED_{50} for suppression of type A spermatogonia following radiation exposure in man was similar (9.7 rad), although the response curves for spermatogonia and sperm count were not parallel. The effect of irradiation on type A spermatogonia in the mouse was parallel to that found in man, but with an ED_{50} of 30.0 rad. These results suggest that, compared to the mouse, spermatogenesis in man is approximately 3.1 times more sensitive to ionizing irradiation.

Key words: irradiation, spermatogenesis, testicular toxins, human, mouse, sperm counts, spermatogonia.

It has become apparent over the last several years that environmental factors can exert profound effects on human reproductive capacity. The recent example of dibromochloropropane (DBCP) toxicity in factory and agricultural workers demonstrates the ability of environmental toxins to induce complete reproductive failure in men who are otherwise apparently well (Whorten et al, 1977; Glass et al, 1979).

Because of obvious ethical constraints, few experimental data are available relating the effects of potential toxins to human reproductive function. Therefore, most studies of the pathologic effects of toxin exposure must be conducted in experimental animals, and risk assessment for human beings must be based on inference and extrapolation. There is, however, a dearth of formalized information about how to translate animal data to man.

One factor known to have profound effects on reproduction is high energy irradiation. In the 1960s, the Atomic Energy Commission sponsored two independent studies of the effects of x-irradiation on testicular function in normal men. Although a large volume of data was collected in each of these studies, most of the information has never been published. The investigators who conducted these studies have kindly made their data available for our analysis. We have assembled and analyzed data concerning sperm counts and testicular histology from these studies. We have compared these results to published reports of similar work using the mouse (Oakberg, 1959) and have calculated an estimate of the relative sensitivity of the two species to testicular irradiation.

Materials and Methods

Data Sources

Raw data on the effects of x-irradiation on human testicular function were obtained from the principal investigators of two independent research projects sponsored

This work was supported by the Environmental Protection Agency, NIH grant P-50-HD-12629, and by the Veterans Administration.

Reprint requests: Dr. William J. Bremner, Veterans Administration Medical Center, 4435 Beacon Avenue South, Seattle, Washington 98108.

Submitted for publication December 20, 1982; revised version received May 19, 1983; accepted for publication June 22, 1983.

by the Atomic Energy Commission (AEC). These two projects will be referred to here as Study A (AEC contracts AT(45-1)-1781 and AT(45-1)-2225) and Study B (AEC contract AT(45-1)-1780).

Study A was conducted at the Washington State Penitentary (Paulsen, 1973). The subjects were healthy volunteers from the prison population, ranging in age from 22 to 50 years (mean = 33.7, SD = 7.4) at the time of exposure to radiation. Before volunteering for the study, each man had decided that he wanted a vasectomy. The vasectomies were performed at the end of the study. Before being allowed to participate in the project, each subject was fully informed and signed a detailed informed consent form. In the case of married volunteers, the wife's consent was also obtained.

The study consisted of a preirradiation control period followed by a postirradiation recovery period. The length of the control and recovery periods varied considerably from individual to individual, ranging from several weeks to several years. Each subject received exposures of 0 (n = 4), 7.5 (n = 5), 15 (n = 8), 30 (n = 5), 50 (n = 8), 100 (n = 23), or 400 (n = 7) rad of x-radiation to the testis from a 250 KVP source with a Thoreus II filter and a 10 × 10 cm cone. The half value layer was 2.5 mm copper, and the FSD was 75 cm.

Sperm counts were measured by hemocytometer and Coulter counter (Gordon et al, 1967) in seminal fluid samples that were collected at one-week intervals during both the pre- and postirradiation periods. Biopsies were performed (Tjioe et al, 1967) at various times throughout the course of the study. The number and timing of the biopsies differed for each subject. Most individuals had at least one biopsy before treatment and one at the completion of the study. Each biopsy specimen was processed histologically, and the number of spermatogonia per Sertoli cell was quantified (Barr et al, 1971).

Study B was conducted at the Oregon State Penintentary and was very similar to Study A, the major exception being the dosages used. In this study, exposures of 8 (n = 2), 10 (n = 2), 15 (n = 1), 20 (n = 7), or 25 (n = 1), 50 (n = 4), 77 (n = 6), 100 (n = 7), 200 (n = 8), 235 (n = 1), 300 (n = 2), 400 (n = 4), and 600 (n = 6) rad were given. Details concerning the subject population, recruitment procedures, and experimental design have been published previously (Rowley et al, 1974).

Mouse Studies. Data on the effects of radiation on mouse type A spermatogonia were obtained from the literature (Oakberg, 1959). The mice received either 20 R gamma-ray or 100, 300, or 600 R X-ray whole or partial body irradiation.

Data Analysis

To minimize the variability that is inherent in sperm count data, the results from each man were subjected to several processing steps. Because biopsies have been shown to depress sperm counts in some men (Gordon et al, 1965; Rowley et al, 1969), all of the sperm counts taken within 16 weeks after a biopsy procedure were deleted. All remaining sperm count values were log transformed (values less than 0.5×10^6 were treated as 0.5×10^6). The sperm count values for each individual were normalized with respect to the average of his preirradiation counts. A five-week moving average was used to represent the sperm count value at each time point (ie, the value for week 3 was set equal to the average sperm count for weeks 1 through 5, the value for week 4 was set equal to the average sperm count for weeks 2 through 6, and so forth). For each dose group, the average sperm count across all the individuals within the group was evaluated at each time point from one year before radiation to two years after. These procedures were performed independently for Study A and Study B. To maximize the number of subjects in the Study B dose groups, the 8- and 10-rad groups and the 20- and 25-rad groups were combined into 8- and 20-rad groups, respectively.

Information on type A spermatogonia (both A dark and A pale) from both human studies were pooled. The data from each man at various times after irradiation were normalized with respect to his average pre-exposure biopsy values. Normalization of the mouse data was done by dividing the number of type A spermatogonia at each time point by the control value.

Both the minimum sperm count and minimum number of type A spermatogonia following radiation exposure were calculated by first fitting a quadratic curve to the log value of the normalized data over a limited time segment for each dose group. The quadratic regression was a weighted least-squares fit that was forced through 100% at time t = 0. The time segments that were used were 0 to 50 weeks for human sperm counts, and 0 to 300 days for human spermatogonia, and 0 to seven days for mouse spermatogonia. The nadir of the quadratic curve was used as the minimum value for that dose.

Dose-response curves were constructed for minimum sperm counts and minimum number of spermatogonia as a function of radiation dose. There were not enough points on any of the curves to fit adequately a multiple exponential to the data, as required by the two-component model (Wideroe, 1971), and the fit to a single exponential curve was poor, expecially for the human data. However, we found that all of the data could be fit by a straight line following a log-logit transformation.

Results

The effects of radiation on human sperm counts are shown in Fig. 1 for Study A and Fig. 2 for Study B. There were clear dose-related effects on sperm production, in terms of both the magnitude and the duration of sperm count suppression. No clear effect on sperm count was noted at exposures of 8 rad or less. Transient suppression of sperm counts was noted following doses of 15 rad or greater. Sperm counts in all 11 individuals receiving 40 rad dropped below 1×10^6 for at least one week between 15 and 47 weeks after exposure. The overall length of the suppression was dose related, with the 400 rad dose causing a complete inhibition of sperm counts in four of five individuals for at least 40 weeks. Sperm counts returned to control levels in all individuals for whom adequate follow-up data were available. Following the

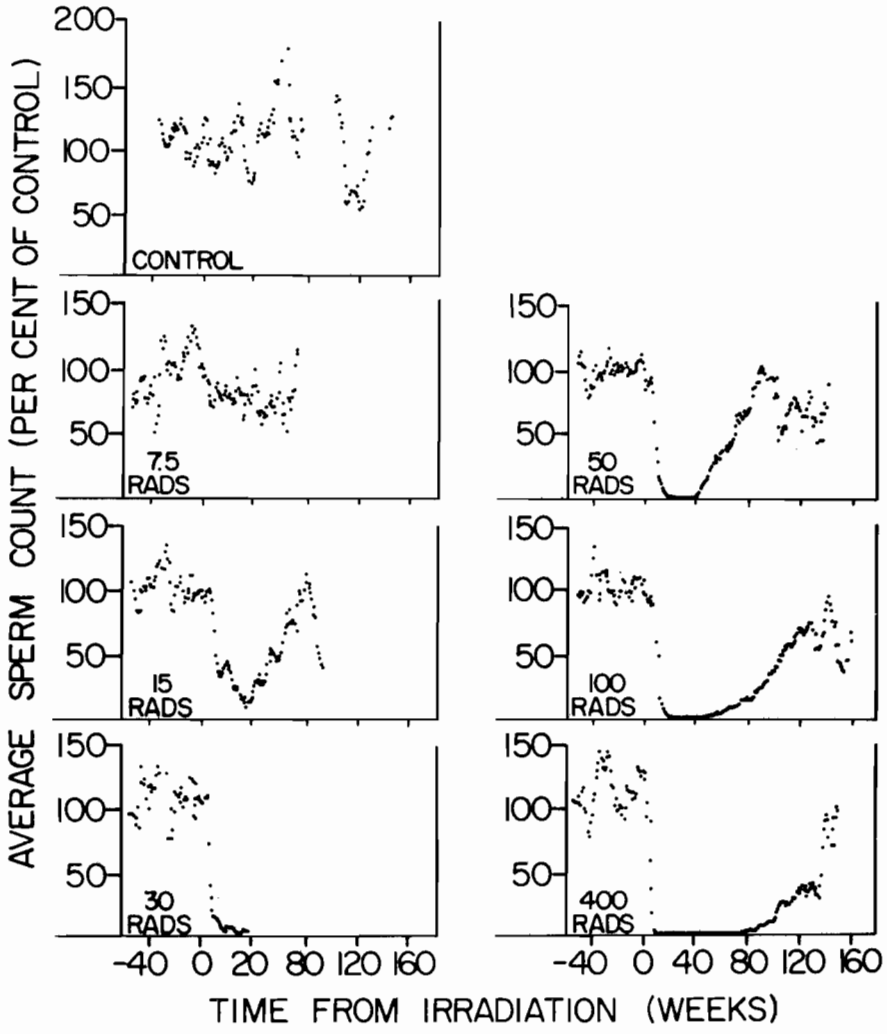

Fig. 1. The effects of various doses of testicular x-irradiation on sperm counts in men aged 22 to 50 (Study A). Each point represents the mean for two or more individuals. All sperm counts within 16 weeks of biopsy were deleted, and the rest were log transformed. For each individual, values were normalized in relation to pre-irradiation counts.

600 rad dose, however, one individual became azoospermic and did not recover within two years, after which he was lost to follow-up.

Dose-response curves for the minimum mean sperm counts produced by each radiation dose are shown in Fig. 3 for both human studies (A and B). The results for the two studies were very similar. The ED_{50}, as determined by the two studies, and 10.4 and 11.6 rad, respectively. The overall ED_{50}, based on a weighted regression was 10.6 rad.

Figure 4 illustrates the grouped biopsy results from both human studies. Type A spermatogonia were suppressed to about 20% of control values by 20 rad and less than 1% by 400 and 600 rad. The maximum suppression occurred between 100 and 250 days for all groups, with the lower dose groups recovering sooner than those with higher doses.

The dose-response relationships for Type A spermatogonia for both mouse and man are shown in Fig. 5. The two lines are parallel (slopes = -0.820 and -0.857 for human and mouse, respectively), however the ED_{50} is 30.0 rad for the mouse and 9.7 rad for the human. This suggests that human type A spermatogonia are about 3.1 times more sensitive to radiation than those of the mouse. Comparing Fig. 3 with Fig. 5, it is clear that, although the ED_{50} values for human sperm counts and human type A spermatogonia are similar (10.6 *vs* 9.7 rad), the slopes are quite different (-0.356 *vs* -0.820). Sperm counts appear to be less sensitive to low doses and more sensitive to high doses than type A spermatogonia.

No local adverse effects of irradiation were noted, including no harmful effects on the scrotal skin. There have been no known late adverse consequences of these studies.

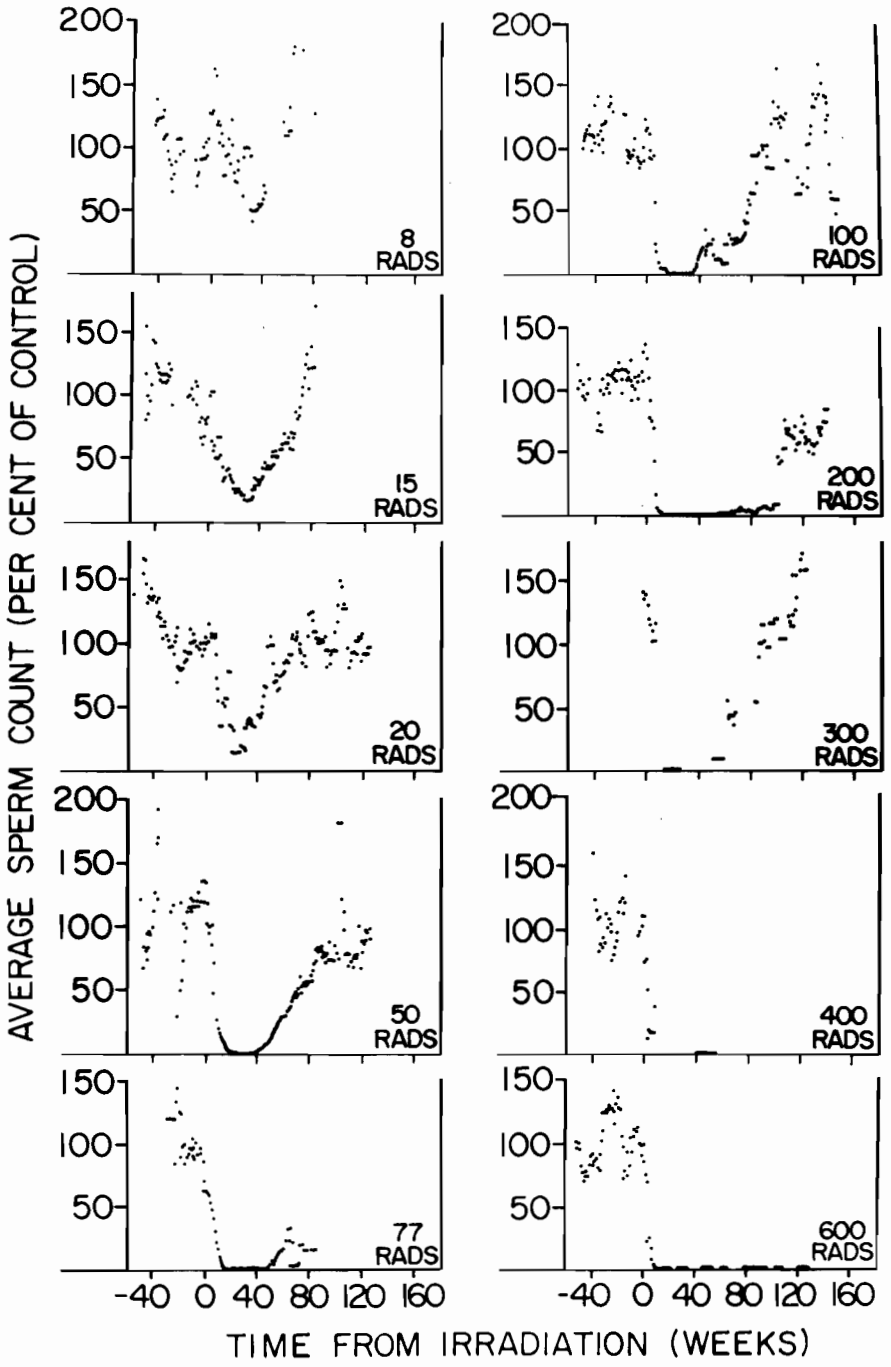

Fig. 2. The effects of various doses of testicular x-irradiation on sperm counts in men (Study B). All sperm counts within 16 weeks of biopsy were deleted and the rest log transformed. For each individual, sperm count values were normalized in relation to pre-irradiation counts.

Discussion

These results confirm the profound effect of x-irradiation on testicular function (Oakberg, 1975; Bardin and Paulsen, 1981). Although the two studies in men were performed somewhat differently, the results are remarkably consistent, both in terms of sperm production and of testicular histology. A marked suppression of sperm production was evident with dosages as low as 15 rad, and sperm production was transiently eliminated in most men following dosages of 50 rad. Higher dosages led to more prolonged periods of sperm count suppression, presumably due to having eliminated the rapidly dividing pool of germinal cells (Oakberg and Clark, 1963).

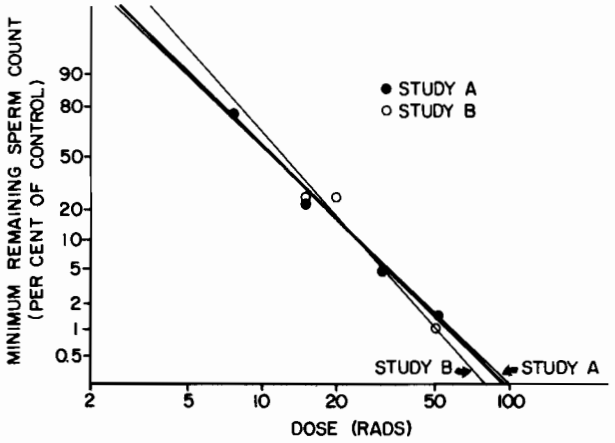

Fig. 3. Dose-response curves for minimum sperm counts following testicular x-irradiation as determined from Studies A and B (thin lines) and both studies combined (heavy line). Lines were fit using log-logit transformation.

When adequate follow-up data were available, significant return of sperm production was invariably seen, even following dosages as high as 400 rad. It is questionable whether sperm production returned fully to control levels following the higher radiation dosages. A longer period of follow-up would have been necessary to provide clear information in this regard, since sperm counts were frequently still rising at the end of the study.

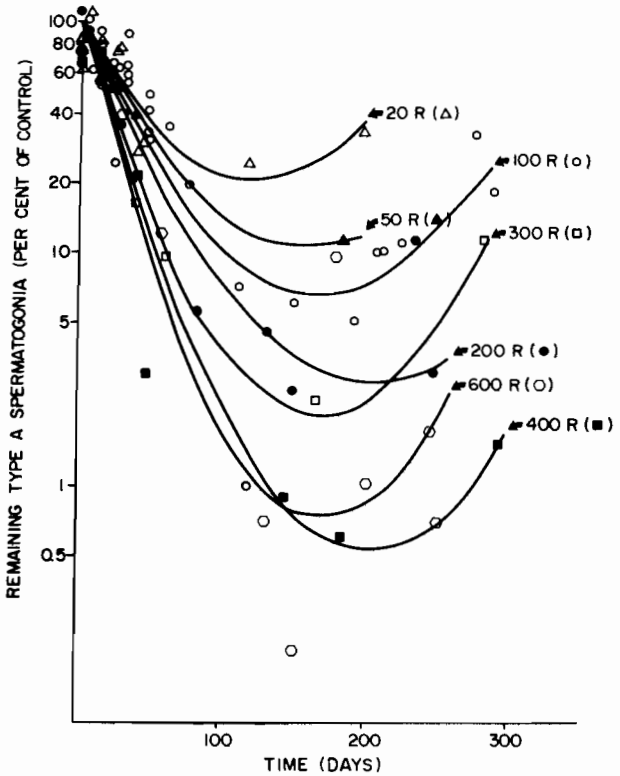

Fig. 4. The effects of various doses of testicular x-irradiation at day zero on Type A spermatogonia over time (Studies A and B combined). Lines represent least squares quadradic regression over the data points for each dose group.

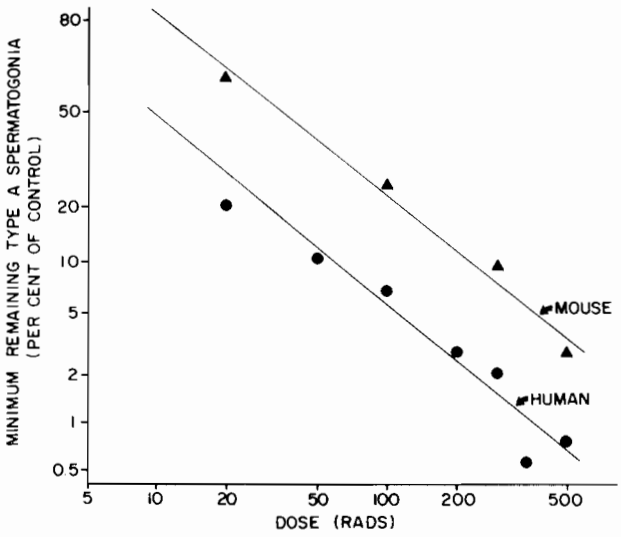

Fig. 5. Dose-response curves for minimum number of Type A spermatogonia in man and mouse following x-irradiation. Lines were fit using log-logit regression.

An extremely small proportion (less than 1%) of the originally present type A spermatogonia persisted at 20 to 30 weeks after the higher radiation dosages (Fig. 4). Despite the very small numbers of remaining stem cells, sperm production eventually returned to a remarkable degree. The slowness in return of sperm production may have been because the relatively radioresistant spermatogonia remaining after high dose irradiation have a very slow replication time (Oakberg and Clark, 1963).

There was striking parallelism of the dose-response curves for the effect of radiation on type A spermatogonia in the human and mouse studies. This parallelism was used to calculate the ED_{50} for each species and the relative sensitivity of mouse and human testicular tissue to irradiation. This parallelism occurred despite the lack of certainty that the cells called "type A spermatogonia" are precisely analogous in men and mice and the markedly shorter time required to reach minimum cell counts in mice compared to men. The fact that the dose-response curves for human spermatogonia and ejaculated sperm counts were not par-

allel underscores the importance of using the same measured variables when making cross-species comparisons.

In addition to the Oakberg data used here, other investigators have published dose- response data for radiation effects on mouse testicular function. Bateman and Bond (1964) reported ED_{50}s for mouse spermatogonia of 27.7 and 40.5 rad in two different experiments using ^{60}Co gamma radiation. They also presented a set of data from Oakberg (different from the one used here) showing an ED_{50} of 21.7 rad for mouse spermatogonia following ^{60}Co irradiation. The reason for so much variability in data from within the same laboratory is unclear, especially since the data from the two independent human studies presented here were in good agreement. Nevertheless, the range of values reported by Bateman and Bond (21.7 to 40.5 rad) brackets the value (30.0 rad) we have calculated from the original Oakberg (1959) paper.

Data on ejaculated spermatozoa following irradiation are not available for the mouse, so a direct inter-species comparison was not possible for that variable. Mian et al (1977) have measured testicular sperm-head counts 29 days after exposure to ^{137}Cs radiation and found an ED_{50} of 53 rads. These data also suggest that the mouse testis is less sensitive to radiation than the human testis. Complete dose-response studies, in which prolonged follow-up data were collected, are not available for other species, making more general comparisons impossible at this time.

Despite these deficiencies in available information, the present data demonstrate the feasibility of making quantitative comparisons of the sensitivity of various species to a testicular toxin. Other classes of toxins for which extensive human and animal data are available for comparison include hormonal steroids such as testosterone and chemotherapeutic agents. Potentially, the development of such quantitative estimates of sensitivity for a variety of toxins may allow more confidence to be placed in human risk assessments based on animal studies of new toxins as they appear in the environment or the workplace.

Acknowledgments

The authors would like to thank Dr. C. A. Paulsen and M. J. Rowley for supplying data used in this report. We would also like to express our appreciation to Elaine Rost, Anne Bartlett, Louise Parry, Patricia Jenkins, and Maxine Cormier for illustrations and manuscript preparation.

References

Bardin CW, Paulsen CA. The testes. In: Williams RH, ed. Textbook of endocrinology, 6th ed. Philadelphia: WB Saunders, 1981; 326.

Barr AB, Moore DJ, Paulsen CA. Germinal cell loss during human spermatogenesis. J Reprod Fertil 1971; 25:75–80.

Bateman JL, Bond VP. The effects of radiations of different LET on early responses in the mammal. Ann NY Acad Sci 1964; 114:32–47.

Glass RI, Hyness RN, Mengle DC, Powell KE, Kahn E. Sperm count depression in pesticide applicators exposed to dibromochloropropane. Am J Epidemiol 1979; 109:346–351.

Gordon DL, Barr AB, Herrigel JE, Paulsen CA. Testicular biopsy in man. I. Effect upon sperm concentration. Fertil Steril 1965; 16:522–530.

Gordon DL, Herrigel JE, Moore DJ, Paulsen CA. Efficacy of Coulter counter in determining low sperm concentrations. Am J Clin Pathol 1967; 47:226–227.

Mian TA, Suzuki N, Glenn HJ, Haynie TP, Meistrich ML. Radiation damage to mouse testis cells from [^{99m}Tc] pertechnetate. J Nucl Med 1977; 1116–1122.

Oakberg EF. Initial depletion and subsequent recovery of spermatogonia of the mouse after 20r of gamma-rays and 100, 300, and 600r of x-rays. Radiat Res 1959; 11:700–719.

Oakberg EF, Clark E. Species comparisons of radiation response of the gonads. Proc Int Symp on effects of ionizing radiation in the reproductive system. Oxford: Pergamon, 1963; 11–24.

Oakberg EF. Effects of radiation on the testis. In: Hamilton DW, Greep RO, eds. handbook of physiology, Section 7, Vol. V, male reproductive system. Washington, DC: Am Physiol Soc, 1975; 233–243.

Paulsen CA. The study of radiation effects on the human testis: including histologic, chromosomal and hormonal aspects. Final progress report of AEC contract AT(45-1)-2225, Task Agreement 6. RLO-2225-2, 1973.

Rowley MJ, O'Keefe KB, Heller CG. Decreases in sperm concentration due to testicular biopsy in man. J Urol 1969; 101:347–349.

Rowley MJ, Leach DR, Warner GA, Heller CG. Effect of graded doses of ionizing radiation on the human testis. Radiat Res 1974; 59:665–677.

Tjioe DY, Steinberger E, Paulsen CA. A simple method for quantitative analysis of seminiferous epithelium in human testicular biopsies. J Albert Einstein Medical Center 1967; 15:56–62.

Whorton D, Krauss RM, Marshall S, Milby TH. Infertility in male pesticide workers. Lancet 1977; 2:1259–1261.

Wideroe R. Quantitative and qualitative aspects of radiobiology and their significance in radiation therapy. Acta Radiol Ther Phys Biol 1971; 10:605–624.