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IN 1940 CHARNY described a method for performing a testicular biopsy and emphasized its innocuous nature. Since then it has been readily accepted as a valuable tool in studying human testicular structure. Furthermore, serial testicular biopsies and seminal fluid examinations have been performed by many investigators to document the effects of various agents upon spermatogenesis.

In spite of the evidence that testicular biopsy has a damaging effect on the germinal epithelium in the bull,^{3, 6, 15} the influence of this procedure on human spermatogenesis has not been reported.

During a study of the effects of radiation on the human testis, we noted a decrease in sperm concentration* in some of our research subjects following the control testis biopsy. This report documents our observations with respect to the effect of testicular biopsy on sperm concentration in 20 adult males.

MATERIALS AND METHODS

Serial seminal-fluid specimens were collected from 20 healthy inmate volunteers at the Washington State Penitentiary by masturbation into clean glass containers. Examination of each specimen was carried out as previously described.⁸ For this study, particular attention was paid to observations of sperm concentration and seminal fluid volume. The former was

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*The terms "sperm concentration" and "sperm count" will be used interchangeably and represent the number of sperm per milliliter of seminal fluid. This should be differentiated from total sperm count, which represents the total number of sperm in the ejaculate.

determined by either the hemacytometer and/or electronic methods as described by Gordon *et al.*⁸

During the control period, at least 9 seminal-fluid specimens were obtained from each subject. A specimen was examined only if the subject had not had an ejaculation for at least 2 days prior to collection. To insure the accuracy of this interval, each subject was specifically questioned as to extracurricular masturbation or nocturnal emissions.

When the control observations had been completed, an open testicular biopsy was performed by a modification of Charny's technic.¹³ Bilateral testicular biopsy specimens were obtained in 19 and a unilateral biopsy specimen was obtained in 1 inmate (RV-14). Following the biopsy, dry, sterile 4- X 4-in. dressings were held in place by a Lister scrotal suspensory.* An ice pack was placed over the scrotum and left in place for 12-18 hr. The patient's biopsy sites were examined periodically during the first 24 hr. for hematoma formation by one of us (C.A.P.). Thereafter, patients were examined and dressings changed daily by the inmate nursing staff. Complete bed rest was maintained for about 18 hr., and the patients were discharged to light activity 3 days after biopsy. Full activity was resumed on the fifth postoperative day, and sutures were removed on the seventh day. Follow-up examination was made by us 1 week later. The scrotal suspensories were usually discarded by the fourteenth postoperative day.

Seminal fluid collections were resumed when testicular tenderness had disappeared, which was usually 7-21 days after the biopsy.

Any patients with local complications or febrile episodes within 40 days of the biopsy were not included in this study.

For analysis, the sperm counts following biopsy were arranged into chronologic groups. Each group (consisting of all seminal fluid specimens collected in four 21-day periods after biopsy) of sperm counts was compared separately with the control counts by the Mann-Whitney statistical technic.⁷

RESULTS

In those subjects demonstrating a drop in sperm concentration below the lowest control sperm count, the timing of the initial decrease in concentration occurred between the eighteenth and thirty-eighth days after biopsy, with one exception (Table 1). In this instance the initial drop was observed 74 days following the biopsy. However, since no seminal fluid specimens were submitted by this subject (RV-32) during the 22- to 42-day period, an earlier dip may have been missed.

*Johnson & Johnson, New Brunswick, N. J.

TABLE 1. Sperm Count Changes Following Testicular Biopsy

Subject No.	Control sperm concent.		Postbiopsy sperm concent.		Significance of dropt (days after biopsy)			
	No. of counts	Range (million/ml.)	Lowest concent. (million/ml.)	Day of dropt*	1-21	22-42	43-63	64-14
RV-3	12	81-343	42	30	(1) N.S.	(2) <0.05	(2) N.S.	(3) N.S.
RV-4	11	16.1-229	15.0	18	(1) <0.1	—	(2) N.S.	(2) N.S.
RV-8	12	4.5-52	5.8	—	—	(2) N.S.	(3) N.S.	(4) N.S.
RV-9	11	52-118	8.9	30	—	(3) <0.05	(3) <0.05	(3) <0.05
RV-10	13	9.1-55	4.9	27	(1) N.S.	(2) <0.1	(4) N.S.	(3) N.S.
RV-11	10	37-83	4.1	29	—	(2) <0.05	(2) <0.05	(2) <0.05
RV-14	9	33-79	26	21	(3) N.S.	(1) N.S.	(1) N.S.	—
RV-15	11	135-366	146	—	(1) N.S.	(3) N.S.	(3) N.S.	(2) N.S.
RV-16	11	32-84	40	—	(1) N.S.	(2) N.S.	(2) N.S.	(2) N.S.
RV-17	16	11.8-186	26	—	(1) N.S.	(2) N.S.	(3) <0.1	(2) N.S.
RV-18	10	82-286	148	—	(1) N.S.	(3) N.S.	(2) N.S.	(2) N.S.
RV-20	12	95-358	55	35	(1) N.S.	(2) <0.05	(4) <0.05	(3) <0.05
RV-26	11	13.6-159	50	—	(1) N.S.	(1) N.S.	(2) N.S.	(2) N.S.
RV-28	15	8.2-89	2.4	28	(2) N.S.	(2) <0.05	(2) <0.05	(3) N.S.
RV-29	10	3.9-51	1.4	31	(1) N.S.	(2) N.S.	(1) <0.1	(3) N.S.
RV-31	16	80-416	55	31	(1) N.S.	(2) <0.05	(3) N.S.	(2) <0.05
RV-32	11	39-128	34	74	(1) N.S.	—	(1) N.S.	(1) <0.1
RV-33	12	95-206	37	31	(2) N.S.	(3) <0.05	(3) <0.05	(3) <0.05
RV-34	12	24-79	11.3	37	(2) N.S.	(3) <0.05	(2) N.S.	(3) <0.1
RV-41	10	199-652	130	34	(2) <0.1	(2) <0.05	(1) <0.1	(1) N.S.

*Day when concentration first dropped below lowest control value.

†Determined by Mann-Whitney statistical technique. Number of determinations given in parentheses; N.S., not significant.

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Nine of 20 patients (45%) demonstrated a significant drop in sperm count ($p < 0.05$) at some period within 84 days of the testicular biopsy. While none experienced a depression in sperm concentration in the first 21 days following biopsy, all of the patients who showed a significant drop in sperm count did so during the 22- to 42-day period. Furthermore, this drop persisted throughout Days 43-63 and 64-84 in 5 of our subjects in each period.

It should be pointed out that sometimes a postbiopsy sperm count falls below each of the control counts and is still not statistically significant ($p < 0.05$). Although this may be important as a single observation, adequate analysis is not possible unless one has at least 20 control sperm counts available or 2 or more postbiopsy sperm counts in that particular period being tested statistically. Results in 3 of our patients (RV-4, RV-29, and RV-32) illustrate this.

Also, when the "ranking" technic of analysis (Mann-Whitney) is used, a postbiopsy sperm count may be lower than any of the control counts but may not be statistically significant because "high" sperm counts are noted during the same period being tested. The counts in 1 patient (RV-14) demonstrate this point.

Seminal fluid volume was not influenced by the biopsy procedure. Therefore, the changes following biopsy are of a similar nature whether expressed as sperm concentration or total sperm count (Table 2). Representative examples of different seminal fluid responses to testicular biopsy are depicted in Fig. 1-4.

DISCUSSION

Ordinarily, when we have performed a testicular biopsy in man, changes in sperm count were not anticipated or appreciated unless the procedure was complicated by bilateral epididymitis. Therefore, when we examined our data with respect to the effect of testicular biopsy on sperm counts as part of another study, the decrease in sperm concentration in 45% of those patients who did not develop local complications was unexpected. In retrospect, there are several reasons that this phenomenon was previously overlooked.

First, when sperm counts were performed by the hemacytometer method, a single determination on each seminal fluid specimen was considered to be sufficient for our purposes. Now we recognize that two or more counts on each specimen are necessary to obtain an accurate estimate of sperm concentration because of the inherent variability in this counting procedure. Alternatively, the newer technic for electronically counting

TABLE 2. Analysis of Results on Changes in Sperm Concentration and Total Sperm Count following Testicular Biopsy

	Sperm concentration (million/ml.)		Total sperm count (million/ejaculate)	
	No.*	%	No.	%
Days after biopsy				
1-21	0/17	0	1/17	6
22-42	9/18	50	8/18	44
43-63	5/20	25	4/20	20
64-84	5/19	26	4/19	21
Total No. of patients	9/20	45	9/20	45
p value	0.05		0.05	

*Numerator indicates number of patients showing decreased sperm from control values; denominator indicates total number of patients evaluated during each postbiopsy period.

sperm is at least as reliable as two determinations on the same specimen by the hemacytometer method.⁸

Second, the possible effects of testicular biopsy have frequently been obscured in past studies by initiating drug therapy immediately after the procedure. Since 26% of our patients experienced either a persistent or secondary drop during the 64- to 84-day period after biopsy, it is evident that any evaluation of a drug upon spermatogenesis during this period might yield erroneous results. As yet, we do not know how long the depression in sperm count may last.

In bulls, an open testicular biopsy is usually followed by a depression in sperm count which occurs at about the same time interval noted in our study on men.^{3,9} Germinal cell destruction is the main histologic finding in bulls after biopsy.^{3,6,15} While recovery usually occurs, the period of cellular regeneration is variable, and frequently many months elapse before the sperm concentration returns to its prebiopsy level.^{5,9}

At least 3 reasons can be put forth to explain the depression in sperm count in man.

Traumatic subclinical orchitis might produce a depression in spermatogenesis by germinal cell degeneration or obstruction of the tubuli recti due to testicular swelling. Minimal intratesticular edema might not be clinically noticeable because of the encapsulation of the testis by a relatively rigid structure, the tunica albuginea. Since a depression in sperm concentration was noted in the bull after a unilateral biopsy,⁹ a bilateral local effect would have to be postulated to explain this phenomenon. Therefore, the theory of a traumatic orchitis resulting from the biopsy's causing a drop in sperm count seems unlikely.

A thermogenic or pressure effect due to the insulation resulting from sterile dressings and a suspensory might inhibit spermatogenesis. It is well known that increased temperature is deleterious to the germinal epithelium. In the few studies carried out in human beings, temperatures greater than 40° C. have been employed.^{11, 16} What is not known, though, is the

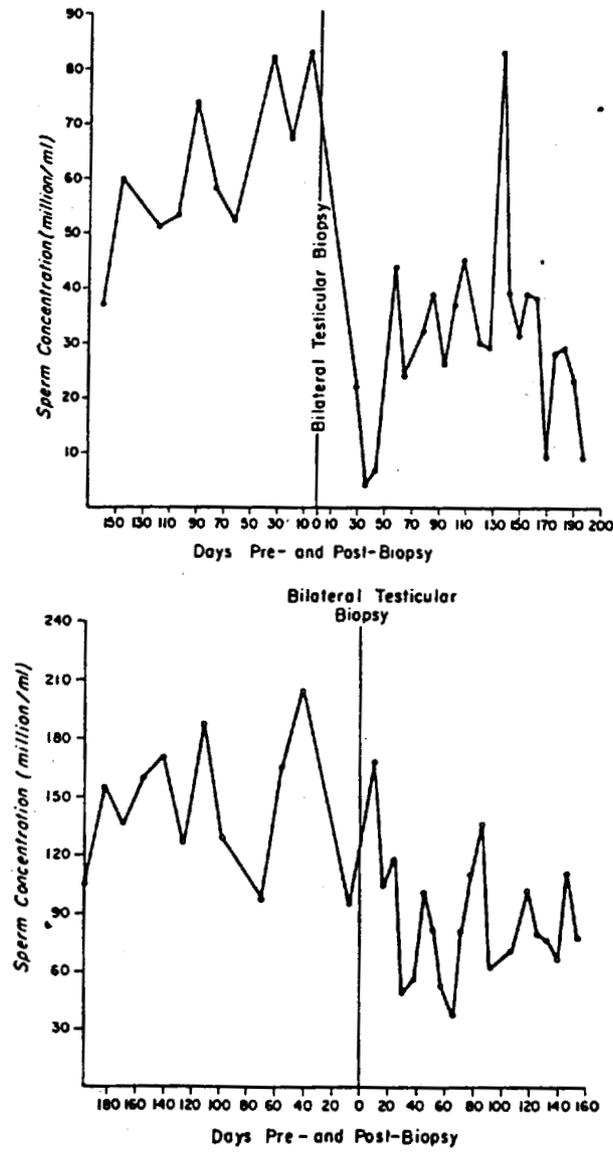


Fig. 1 (top). Persistent sperm concentration depression for over 190 days after testicular biopsy (Subject RV-11). Fig. 2 (bottom). Persistent sperm concentration depression for over 150 days after biopsy (Subject RV-33).

minimum rise in temperature which will cause damage. Marberger and Marberger observed testicular damage after the application of scrotal pressure dressings. Unfortunately, they performed testicular biopsies before and after the dressings were applied, so that the influence of increased temperature *alone* was not tested. However, if the seminal fluid changes

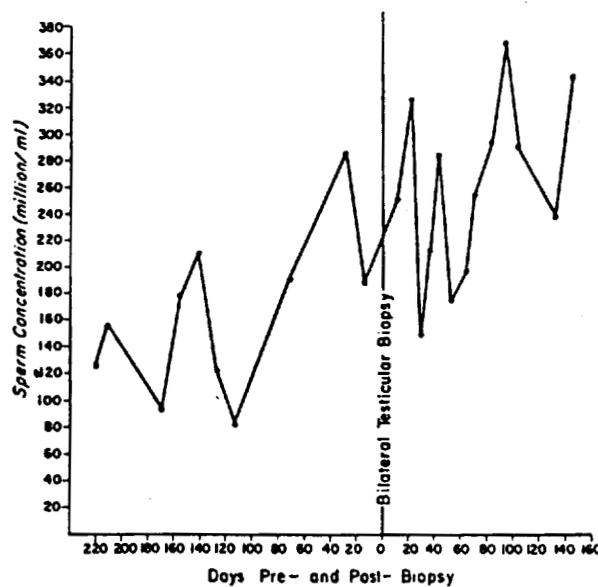
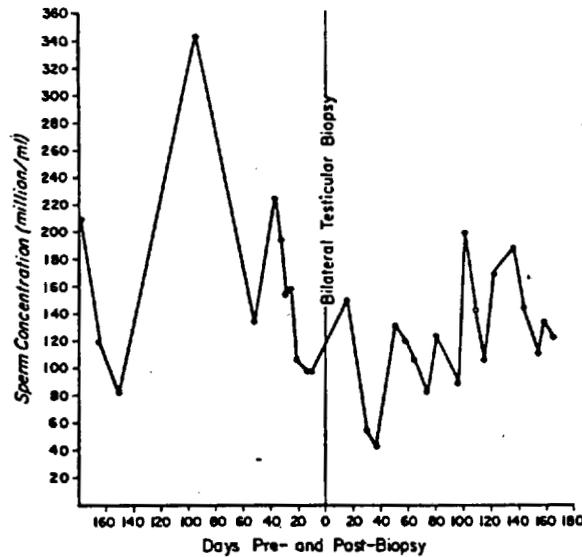


Fig. 3 (*top*). Note the drop in sperm concentration which returned completely to pre-biopsy levels by 100 days (Subject RV-3). Fig. 4 (*bottom*). No decrease in sperm concentration (Subject RV-18).

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we observed in man are analogous to what happens in the bull following testicular biopsy, then increased testicular temperature, per se, is not the responsible mechanism, since scrotal suspensories or dressings were not employed in these animals.

Finally, an antigen-antibody reaction may have been initiated by the biopsy procedure which then resulted in destruction of spermatozoa and/or more immature cells in the germinal epithelium. There is ample evidence that testicular tissue and spermatozoa are antigenic.^{1, 10, 14} Indeed, testicular lesions have been produced in the contralateral testis of guinea pigs following unilateral traumatic insult to the testis and stimulation of the reticuloendothelial system.² While this appears to be the most promising explanation at present, further study is needed before the pathogenesis of the decreased sperm counts following testicular biopsy in man is fully understood.

SUMMARY

Twenty healthy adult males each underwent an open testicular biopsy. Nine to 16 sperm counts were determined prior to the procedure, and serial sperm counts were performed after the biopsy. Local or systemic complications were not experienced in these patients.

Nine of the 20 subjects (45%) experienced a significant depression ($p < 0.05$) in sperm concentration. This drop was first observed 22-42 days following the biopsy in all 9 patients. A persistent or secondary drop in count occurred in 26% of the patients 64-84 days after the procedure. The results of sperm concentration and total sperm count determinations were compared and found to yield approximately similar results.

The possible mechanisms involved in causing a depression in sperm count due to the biopsy procedure are discussed. It is suggested that a testicular biopsy may initiate an antigen-antibody reaction directed against spermatozoa or more immature elements of the germinal epithelium.

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