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QUANTITATIVE ANALYSIS OF TESTICLE BIOPSY: DETERMINATION OF PARTIAL OBSTRUCTION AND PREDICTION OF SPERM COUNT AFTER SURGERY FOR OBSTRUCTION*

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Twenty-one male patients without ductal obstruction were studied prospectively by a simplified method of quantitative testicle biopsy. The mean number of mature spermatids per tubule in the testicle biopsy correlated closely with the sperm count in all 21 cases. Patients with less than 10,000,000 sperm/ml always had less than 20 mature spermatids per seminiferous tubule. Patients with more than 10,000,000 sperm/ml always had more than 20 mature spermatids per tubule.

In azoospermic patients with epididymal obstruction, the sperm count after vasoepididymostomy could be predicted correctly by testicle biopsy. In severely oligospermic patients (less than 10,000,000 sperm/ml) the presence of more than 20 mature spermatids per tubule indicated partial epididymal obstruction.
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Testicle biopsy has been used by most clinicians in a nonquantitative fashion only. This has severely limited its usefulness and has led to many errors in its interpretation.[1-3] For that reason testicle biopsy has not found much use in men with oligospermia.[4] Yet a precise study of the seminiferous epithelium in oligospermia is urgently needed.

Heller and Clermont first described the histologic characteristics and kinetics of spermatogenesis in the human.[5] They determined through radioactive tracer studies that the rate of spermatogenesis in humans is always constant even when sperm output is reduced. Therefore the amount of sperm being produced by the testicle should be reflected by what is seen in a fixed specimen of testicle biopsy.

Steinberger, Tjioe, and Paulsen then developed a method of quantitative interpretation of the testicle biopsy.[6, 7] Unfortunately they had a small number of patients and were therefore limited in trying to make a precise correlation with sperm count. Furthermore, their technique was elaborate and time-consuming.

In 1978 Zukerman et al., working with Steinberger, counted all components of spermatogenesis and found a superb correlation with sperm count.[8] But the difficulty still remained that each quantitative testicle biopsy using the method they described was time-consuming, and very few fertility specialists had any inclination to put that much effort into photographing and analyzing the biopsy of each of their patients.

The purpose of this paper is to describe a simplified modification of Steinberger's quantitative method that any clinician should be able to utilize in his oligospermic patients with an expenditure of only 10 to 15 minutes of his time. We analyzed patients with oligospermia, as well as patients with normal sperm counts, to see how closely the testicle biopsy could predict a patient's mean

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sperm count. We then attempted to determine whether testicle biopsy at the time of microsurgery to correct epididymal obstruction would be able to predict the patient's postoperative sperm count and whether the anastomosis was partially obstructed. Finally, we wished to see whether a discrepancy between the quantitative testicle biopsy and sperm count could diagnose those cases of oligospermia caused by partial obstruction.

In previous studies we showed that patients who were severely oligospermic after strictured vasovasostomy became fertile after reanastomosis. It is now established that obstruction in the ductal system can cause oligospermia and poor motility.[9] Schoysman in Belgium has performed a thorough scrotal exploration of several hundred severely oligospermic males and found epididymal dilatation suggesting possible obstruction in 20% of the cases.[10] A quantitative method of evaluating testicle biopsy could document such a diagnosis and make surgical judgment more reliable.

MATERIALS AND METHODS

Patients

Twenty-one male patients with no evidence of ductal obstruction after scrotal exploration underwent quantitative evaluation of testicle biopsy. An additional two oligospermic male patients whose scrotal exploration revealed epididymal obstruction underwent quantitative evaluation of testicle biopsy. Three azoospermic male patients with "normal spermatogenesis" determined by preoperative testicle biopsy underwent microsurgical vasoepididymostomy, and the biopsy was studied quantitatively in an effort to predict postoperative sperm count. The technique for vasoepididymostomy in these three patients was a specific anastomosis to the epididymal tubule (Fig. 1, A and B).

Of the 21 patients without ductal obstruction, 12 had severe oligospermia (less than 10,000,000/ml). Eight of these 12 patients had sperm counts under 3,000,000/ml. Nine patients had counts greater than 10,000,000/ml.

All sperm counts represented an average of four to six separate semen analyses performed on separate occasions over a 3-month period on each of the patients. Three days of abstinence were standardly required. Semen volume varied from 2 to 5 ml and averaged 3.2 ml. There were no patients in this group with either extremely high or extremely low semen volumes. Sperm counts were performed on a Makler Counting Chamber with phase contrast microscopy. Each semen specimen was counted twice, and the variation in numerical count in each specimen was always within 10%. Thus the sperm count value for each patient was usually the result of eight to twelve separate counts. The patients were all cared for in St. Louis, and biopsy evaluations were performed in St. Louis.

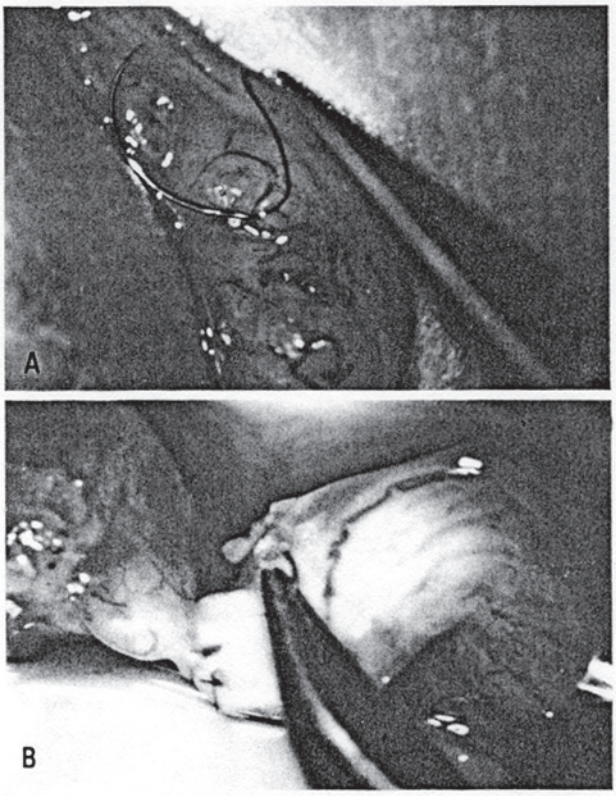

FIG. 1. A, Microscopic vasoepididymostomy: placement of first mucosal stitch. B, Microscopic vasoepididymostomy: placement of final mucosal stitch.

Method for Performing Testicle Biopsy

Each biopsy was performed with a careful "no touch," atraumatic technique under general anesthesia. The procedure usually lasted no more than 10 minutes. A tiny 1-cm transverse incision was made through all layers of the scrotum down to the tunica albuginea. The tunic albuginea was sharply incised with a scalpel. The protruding seminiferous tubules were then excised with wet, extremely sharp, microiris scissors and then allowed to fall into Zenker's solution without being

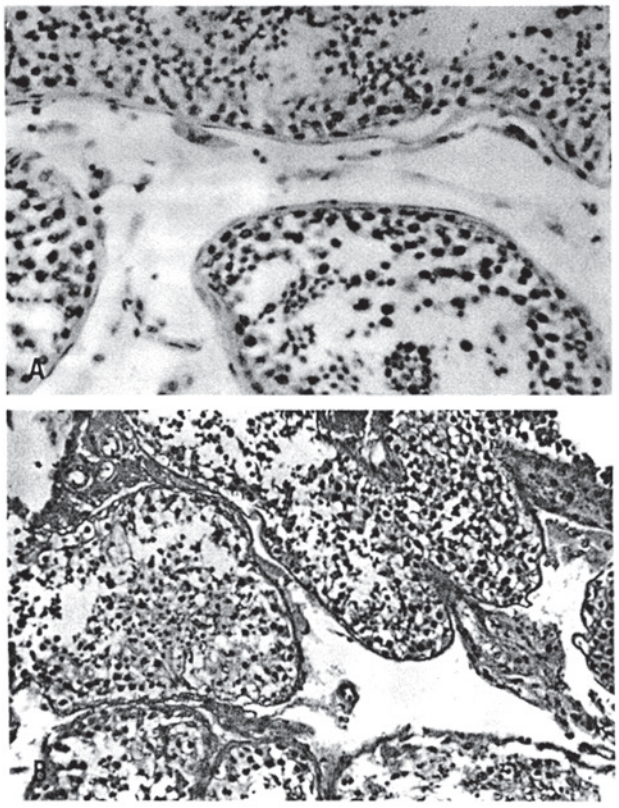

FIG. 2. A, Formalin-fixed testicle biopsy specimens are unreadable. B, Roughness in handling the testicle biopsy specimen produces crush artifacts such as "sloughing and disorganization."

handled. Specimens were carefully fixed, cut in thin sections, and stained with hematoxylin and eosin. The patients were discharged with no more pain than they would have experienced from a simple vasectomy. The technique of biopsy is important. If the specimen is handled roughly, or fixed in formalin, it is impossible to identify accurately the cellular components of the seminiferous tubule (Fig. 2, A and B).

Method of Quantitative Analysis of Spermatogenesis

At least 10 seminiferous tubules were included in the count on each side. The testicle biopsy was performed bilaterally. Thus, a total of 20 or more tubules were included in the biopsy evaluation on each patient. Only the mature spermatids (stages I, II, V, and VI) were counted. Put in more simple terms, only the oval spermatids with dark, densely stained chromatin were counted (Figs. 3-6). All of the steps of spermatogenesis from spermatogonia through resting spermatocyte, leptotene spermatocyte, zygotene spermatocyte, pachytene spermatocyte, and early spermatids (S_A, S_{B1}) were excluded from consideration. Only the easily recognized mature spermatids were included in the count. The reason for this choice is that these are the cells that are most easily characterized. In the interest of modifying quantitative analysis of testicle biopsy for routine clinical use, we wanted to count only those cells that previous studies had shown would have the greatest correlation with sperm count and that would be most easily recognized.

Sertoli cells were not counted, because previous studies have demonstrated so much variability in Sertoli cell population that it does not serve as a useful guide. We did not photograph the specimens or compute the circumference of the tubular walls (as Steinberger did in his original quantitative studies), but simply totaled the number of mature spermatids in a minimum of 20 tubules and divided that by the number of tubules.

RESULTS

The results are summarized in Figure 7. In unobstructed patients with less than 10,000,000 sperm/ml there were always less than 20 mature spermatids per tubule in the testicle biopsy. In patients with over 10,000,000 sperm/ml, there were always greater than 20 mature spermatids per tubule. The number of mature spermatids per tubule correlated very closely to the sperm count per milliliter. Using an exponential curve, summarized in the graph, we could use the number of mature spermatids per tubule to predict what the sperm count would be.

In the absence of obstruction there was a remarkably close correlation between the number

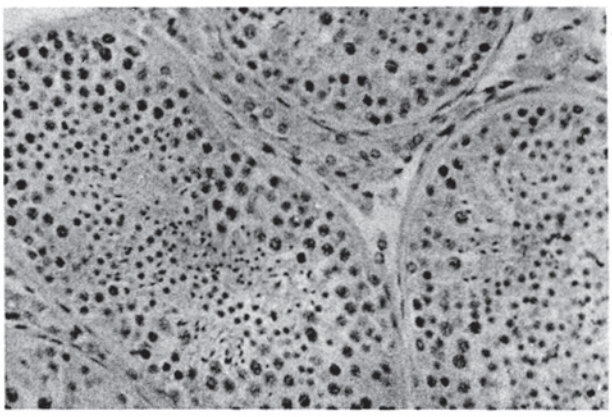

FIG. 3. Histologic specimen (×160) of seminiferous tubules. Left lower tubule contains 102 mature spermatids. Right lower tubule contains 21 mature spermatids.

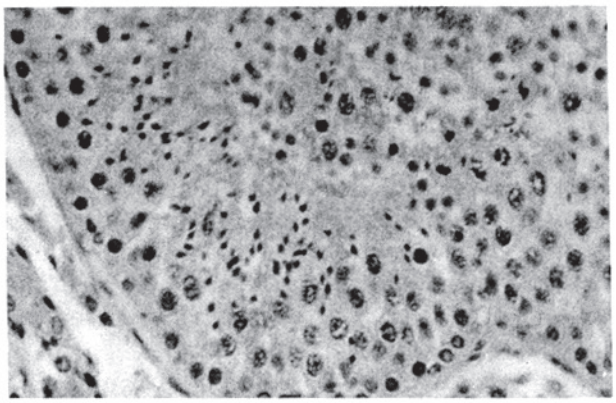

FIG. 4. Histologic specimen (×250) of a seminiferous tubule that contains 83 mature spermatids.

of mature spermatids per tubule and the actual sperm count in the semen. If the patient had 40 mature spermatids per tubule, the sperm count would be just under 60,000,000/ml. If there were 45 mature spermatids per tubule, the sperm count would be just over 85,000,000/ml. If the patient had a sperm count of only 3,000,000/ml, one would expect him to have only 6 to 10 mature spermatids per tubule.

The postoperative sperm count of the three patients who underwent microscopic vasoepididymostomy correlated very closely with their quantitative testicle biopsy. One patient's postoperative sperm count was 42,000,000/ml, another's was 76,000,000/ml, and a third was 108,000,000/ml. Each of these ranges of postoperative results was predicted correctly by simple counting of the number of mature spermatids per tubule in the preoperative testicle biopsy specimen.

Two patients not included on the graph were oligospermic men who had evidence of epididymal obstruction on scrotal exploration. One of the patients had a mean sperm count of 4,000,000/ml, and his testicle biopsy showed he had 42 mature spermatids per tubule. According to the previous graph, this patient's sperm count should have been approximately 72,000,000/ml. The other such patient had a sperm count of 5,600,000/ml, and his testicle biopsy revealed 50 mature spermatids per tubule, indicating that his sperm count should have been approximately 105,000,000/ml. The obstruction in these two oligospermic patients was diagnosable by comparison of the large number of mature spermatids in their testicle biopsy specimens and the relatively severe degree of oligospermia observed in the sperm count. Similarly, in cases of surgery to correct obstructive azoospermia, a low sperm count postoperatively may be caused by continuing partial obstruction.

We found no "sloughing," "disorganization," or any other type of qualitative abnormality in oligospermic patients with varicocele, as compared with other oligospermic patients. Like all other oligospermic patients, the number of mature spermatids per tubule in the testes of patients who had varicocele correlated accurately with sperm count.

DISCUSSION

We now have an extremely simple and immensely useful tool, not only for helping to manage azoospermic obstructed patients, but also for evaluating oligospermic patients, a significant number of whom may have partial obstruction as the cause of their infertility. Ten percent of our oligospermic patients with sperm counts under 10,000,000/ml have obstruction as the cause. Schoysman has reported that 20% of patients with sperm counts under 5,000,000/ml have obstruction as the cause.[10] Quantitative testicle biopsy can accurately confirm this diagnosis prior to an involved scrotal exploration, which could otherwise require a great deal of guesswork about whether or not there truly is epididymal blockage. With comparison of the number of mature spermatids per tubule with the patient's sperm count, unwarranted medical therapy will not be haphazardly administered to patients who have obstruction as the cause of their problem.

Quantitative testicle biopsy is also useful in predicting what the postoperative sperm count should be in patients who undergo surgery to correct obstruction. The most common cause of persistent oligospermia and poor motility after sur-

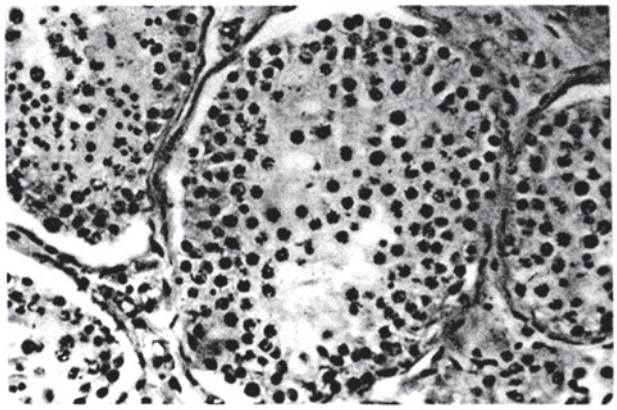

FIG. 5. Histologic specimen (×160) of seminiferous tubules. The central tubule contains 1 mature spermatid.

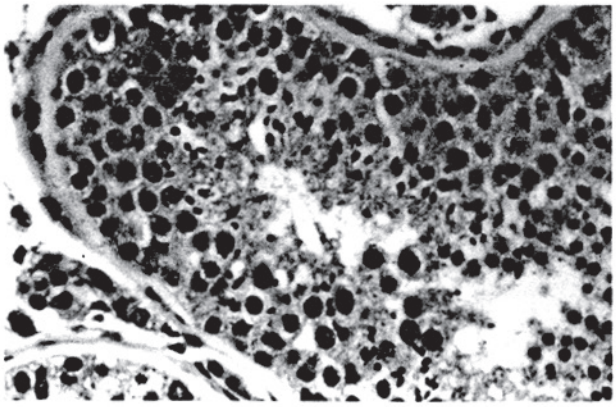

FIG. 6. Histologic specimen (×250) of a seminiferous tubule that contains 81 mature spermatids.

gery to correct ductal obstruction in the male is persistent obstruction, whether partial or complete.[11-14] Persistent oligospermia after such surgery can now be diagnosed reliably, by quantitative analysis of the testicle biopsy, giving sufficient documentation to reoperate on such failures. But caution should be exercised so that conclusions about the final sperm count are not drawn until 1½ years following vasoepididymostomy, because it sometimes takes such a period of time for epididymal transport mechanisms to completely recover.

Frequently patients undergo vasoepididymostomy inappropriately because the pathologist qualitatively reports "normal spermatogenesis." The pathologist's readings are almost universally not quantitative, but rather a vague impression he has that the tubules are filled with spermatocytes and some mature sperm. This "impression" of "normal spermatogenesis" has led many nonobstructed patients into an unwarranted vasoepididymostomy. If the biopsy shows thick tubules with large numbers of spermatocytes but only two or three mature spermatids per tubule, such a patient does not have obstruction as a cause of his "azoospermia." A quantitative appraisal of the testicle biopsy is required for a determination of whether the patient can be managed surgically.

Despite the brilliant work of Steinberger, Tjioe, and Paulsen, quantitative analysis of testicle biopsy has unfortunately never been applied clinically, simply because it was extremely laborious. In the original approach, spermatids were not counted. Yet the spermatid is the simplest cell to identify and has the closest correlation to sperm count. Therefore, at Steinberger's suggestion, we limited the count strictly to mature spermatids. Another simplification is the discovery that one need not photograph the specimen and measure the perimeter of each seminiferous tubule. As long as a total of 20 seminiferous tubules were counted, the number of mature spermatids per tubule correlated just as well with the sperm count as the number of mature spermatids per unit of circumference. These two modifications reduced the amount of time required for quantitative reading of the testicle biopsy from several days to 15 minutes.

The reason that a mere count of the number of mature spermatids per tubule on a histologic section can give an accurate indication of the number of sperm in the semen is that the rate of spermatogenesis is constant in any species, including man. When men have low sperm counts or high sperm counts, it is not because they are making sperm at a slower or faster rate; there are simply fewer cells. Thus by counting the number of spermatids in a fixed tissue specimen, we can obtain accurate kinetic information that correlates with the patient's sperm count.

Some clinicians have attempted to use the serum follicle-stimulating hormone (FSH) level to monitor spermatogenesis. If the FSH level is in a normal range and the patient has azoospermia, supposedly obstruction is indicated. Unfortunately, the FSH level correlates very poorly with spermiogenesis.[15] FSH level correlates best with the number of spermatogonia, but very poorly with the number of sperm. Most patients with maturation arrest and azoospermia have normal FSH levels. The feedback mechanism is simply not tuned finely enough for the serum FSH level to give any indication of sperm count.[16]

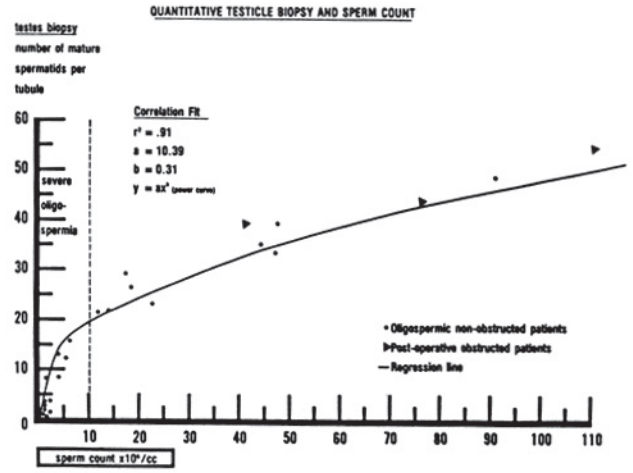

FIG. 7. Correlation between the number of mature spermatids per tubule and the sperm count.

Ironically, it is the scattered mosaic arrangement of the various stages of spermatogenesis in the human seminiferous tubule (as opposed to the orderly wave of spermatogenesis moving across the seminiferous tubule in most other species) that allows quantitation of the human testicular biopsy to be so simple. In rats a cut through any particular seminiferous tubule will show only one particular stage of spermatogenesis. In the human, however, a single cut through one seminiferous tubule will demonstrate several different stages of spermatogenesis. Thus in humans very few tubules are required for one to get a good statistical sample of the total range of spermatogenesis in the entire testicle.

In summary, the quantitative reading of testicle biopsy is now simple to perform and correlates closely with sperm count in the absence of obstruction. It can be of great use, therefore, in the evaluation and follow-up of males with oligospermia or obstruction.

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