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Motile Sperm in Human Testis Biopsy Specimens

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ABSTRACT: We prospectively studied 62 consecutive infertile men who underwent 100 intraoperative wet prep cytological examinations of testis biopsy material obtained simultaneously with permanently fixed specimens. Wet preps were performed by placing a small sample of fresh testicular tissue on a slide, adding a drop of Ringer's lactate, and compressing the specimen under a glass coverslip. Among these 100 wet preps, complete sperm with tails were identified in 62 specimens, of which 44 contained nonmotile sperm and 18 contained motile sperm.

Reproductive tract obstruction was documented in 65 testes (65%) on subsequent reconstructive surgery and/or inferred from histological evaluation, including mean mature spermatid counts on the permanent sections fixed in Bouin's solution. Obstruction was absent in the remaining testes (35%).

All 18 testes with motile sperm found on wet prep were obstructed. These testes were also found to have complete spermatogenesis, a category selected to include normal spermatogenesis and slight hypospermatogenesis, determined by examination of the permanently fixed sections. The finding of motile vs. nonmotile sperm on a wet prep has positive predictive values of 100% vs. 81% for the presence of reproductive tract obstruction and 94% vs. 86% for complete spermatogenesis, respectively.

The presence of motile sperm in human testis biopsy specimens is a novel finding. When any complete sperm with tail is found in a testis biopsy wet prep, obstruction is likely. When motile sperm are present, obstruction is almost certain, and immediate exploration and reconstructive surgery can be justified. Furthermore, the finding of motile sperm on wet prep suggests that testicular sperm might be used for *in vitro* fertilization in cases where the entire epididymis and efferent ductules have been ablated or destroyed.

Key words: Wet prep, motility, reproductive tract obstruction, spermatogenesis.

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Testis biopsy for evaluation of male infertility was first reported over 50 years ago by Hotchkiss (1942) and Charny (1940). It is most useful in distinguishing between reproductive tract obstruction vs. primary testicular failure in azoospermic or severely oligozoospermic men. Because sperm motility is generally considered to be acquired during passage through the epididymis, after sperm have left the testis, we were surprised to find motile sperm on testis biopsy wet prep cytological specimens. Over the past few years, we and others have observed the presence of motile sperm in the efferent ductules of men with chronic obstruction of the reproductive tract or congenital absence of the vas (Silber, 1988). The above observations prompted us to conduct a prospective study to investigate the frequency and clinical implications of finding motile and nonmotile sperm on wet prep cytological preparations of testis biopsy specimens.

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Methods

Between January 1989 and October 1991, 100 consecutive testis biopsies with wet preps were performed on 62 patients at the New York Hospital–Cornell Medical Center. All patients were either azoospermic or severely oligozoospermic and had serum follicle-stimulating hormone levels less than three times normal.

During open testis biopsy, extruded testicular tissue was obtained from a single incision in the tunica albuginea and placed directly into Bouin's solution using the no-touch technique (Magid et al, 1990). An adjacent piece of extruded testicular tissue, weighing approximately 50 mg, was excised through the same incision in the tunica and placed on a slide. A drop of Ringer's lactate was added, and the specimen was compressed under a glass coverslip. The wet prep slide was immediately examined microscopically by a pathologist under a high-dry (40×) objective. The use of phase-contrast microscopy is preferred but not imperative. The presence of sperm and determination of motility was best evaluated just outside the margins of the compressed tissue (Fig. 1).

The testicular tissue was fixed in Bouin's solution and processed routinely in a Fisher tissue-processor Histomatic, embedded in paraffin, sectioned at a thickness of 3–4 μm, and stained with hematoxylin and eosin. Permanent sections were evaluated by one pathologist (M.S.M.), and classified as demonstrating normal spermatogenesis, hypospermatogenesis (graded slight, moderate, or severe), maturation arrest (complete or

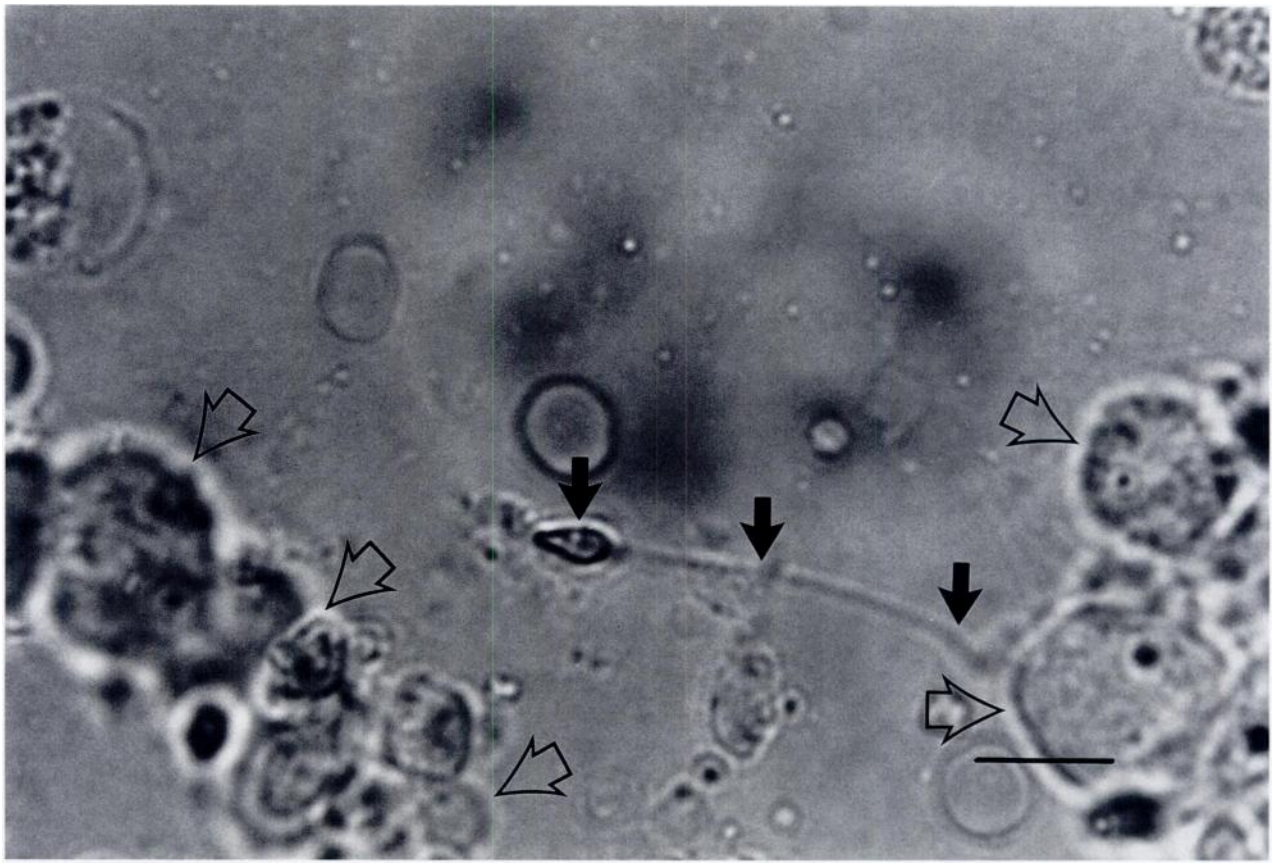

FIG. 1. High-dry (1,800×) photomicrograph demonstrating a mature sperm (➧) just outside the margins of the compressed testis biopsy tissue (⇨) using the wet prep technique (bar = 10 μm).

partial), Sertoli cell-only pattern, or tubular and peritubular sclerosis. In this study, we elected to employ the term complete spermatogenesis to encompass normal spermatogenesis and slight hypospermatogenesis, both of which contained all stages of sperm maturation to mature spermatids. The latter included cases with a slight decrease in the number of mature spermatids, which was felt to have an insignificant effect on fertility. The mean mature spermatid count was evaluated using the technique of Silber and Rodriguez-Rigau (1981) as the number of mature spermatids per seminiferous tubule averaged over 10 circular tubules.

The presence or absence of reproductive tract obstruction was determined for each testis. Obstruction was categorized into anatomical vs. functional. Anatomical obstruction was determined on subsequent surgical exploration or confirmed by physical examination in cases of congenital absence of the vas. In other cases, anatomical obstruction was assessed by the histological appearance of normal spermatogenesis or slight hypospermatogenesis together with the presence of mean mature spermatid counts of greater than 15. Functional obstruction was determined by the presence of complete spermatogenesis in the testis, with an azoospermic ejaculate, no evidence of anatomical obstruction on vasography, but sperm in the semen after electroejaculations, if performed.

Results

Our patients ranged in age from 21 to 84, with a median age of 34. Azoospermia was present in 77% of the patients while the rest had severe oligozoospermia. In the latter category, four patients had sperm density between 2 to 10 million per ml; all others had less than 1 million sperm per ml of semen.

Among the 100 testes that underwent wet prep cytological examination, obstruction was present in 65 and absent in the rest. Anatomical obstruction, confirmed either on physical examination for congenital absence of the vas, or on subsequent surgical exploration, was present in 49 of the obstructed testes (75%). Nine testes (14%) were presumed to be obstructed based on normal histological appearance of the testicular biopsy with normal mean mature spermatid counts on the permanent sections in the face of azoospermia. Functional obstruction from ejaculatory failure (such as those resulting from retroperitoneal lymphadenectomy) was present in seven testes (11%).

Table 1 reveals that all 18 testes with motile sperm

Table 1. *Intratesticular sperm motility and reproductive tract obstruction*

	Obstruction present	Obstruction absent	Total
Motile sperm	18	0	18
Nonmotile sperm	32	12	44
No sperm	15	23	38
Total	65	35	100

found on wet prep were obstructed. Therefore, the presence of motile sperm on our wet prep specimens had a 100% positive predictive value for reproductive tract obstruction. In addition, it was found to be an excellent indicator of complete spermatogenesis, which was observed in 94% of the specimens with motile intratesticular sperm (Table 2). The only patient who had moderate hypospermatogenesis on the permanent section underwent microsurgical vasovasostomy of a solitary testis and had return of normal semen quality postoperatively, with a sperm concentration of 108 million per ml and 68% motility.

From these data, it was also apparent that the presence of any complete sperm with tails, regardless of motility, in a wet prep specimen, would predict obstruction and complete spermatogenesis 81% and 86% of the time, respectively. Conversely, in the setting of reproductive tract obstruction or complete spermatogenesis, the probabilities of finding motile sperm in a wet prep study were only 28% and 25%, while the corresponding probabilities of finding any sperm regardless of motility were 77% and 78%, respectively. The absence of motile sperm, on the other hand, had negative predictive values of only 44% and 38% for absence of obstruction and complete spermatogenesis, respectively. It was noteworthy that two of the nine testes categorized as maturation arrest contained nonmotile sperm on the wet prep. More interestingly, in both instances, maturation arrest was only partially present upon examination of the permanent histological sections.

Table 3. *Intratesticular sperm motility and levels of anatomical reproductive tract obstruction*

	Obstruction of vas deferens or ejaculatory ducts	Obstruction of epididymis	Total
Motile sperm	11 (27%)	2 (25%)	13
Nonmotile sperm	23 (56%)	5 (62%)	28
No sperm	7 (17%)	1 (13%)	8
Total	41 (100%)	8 (100%)	49

The levels of anatomical obstruction confirmed surgically or on physical examination in 49 testes are presented in Table 3. Vasal and epididymal obstructions were present in 84% and 16%, respectively. Although the presence of motile sperm on wet prep was apparently unrelated to the level of obstruction, i.e., 27% for vasal vs. 25% for epididymal obstruction, statistically meaningful correlations could not be made due to the small numbers of patients involved in each category.

Discussion

Testis biopsy is a useful tool in the andrologist's armamentarium for the evaluation of azoospermia. However, a definitive pathological diagnosis rests upon evaluation of the permanent histological sections. This often translates into staged testis biopsy and surgical exploration or unwarranted vasotomy and vasograms performed at the time of biopsy. In an attempt to offer a rapid intraoperative diagnosis, Coburn et al (1986) described the techniques of touch imprint and cytospin analysis of testis tissue during testis biopsy. They demonstrated the presence of mature sperm in all testis specimens with obstructive lesions. Oates et al (1989) later affirmed that the touch imprint technique was useful for distinguishing between late maturation arrest and obstruction. These methods, however, required intraoperative fixation and staining procedures; thus motility could not be assessed. The wet prep studies described here are easy to perform and allow

Table 2. *Wet prep findings and permanent histologic examination*

	Complete spermatogenesis*	Moderate/marked hyposperm-atogenesis	Maturation arrest	Sertoli cell-only	Tubular and peritubular sclerosis	Total
Motile sperm	17 (95%)	1 (5%)†	0	0	0	18
Nonmotile sperm	36 (82%)	6 (14%)	2 (4%)‡	0	0	44
No sperm	15 (40%)	4 (10%)	7 (18%)	9 (24%)§	3 (8%)‖	38
Total	68	11	9	9	3	100

* Includes normal spermatogenesis and slight hypospermatogenesis.
† Moderate hypospermatogenesis. This patient underwent vasovasostomy and had return of normal semen quality.
‡ Both cases demonstrated partial maturation arrest.
§ Includes three cases with focal spermatogenesis.
‖ Includes one case with focal spermatogenesis.

assessment of both the presence and motility of sperm, an advantage not offered by either of the above methods.

We found that 18% of testes biopsied in an azoospermic or severely oligozoospermic population contained motile sperm. The presence of motility is an excellent indicator of reproductive tract obstruction and complete spermatogenesis. On the other hand, the absence of sperm on wet prep did not predict the absence of obstruction. As an extension of our earlier experience, we now find that the presence of sperm motility can predict complete spermatogenesis better than the mere presence of any sperm at all (94% vs. 86%) (Steckel and Goldstein, 1991). The same holds true for prediction of reproductive tract obstruction (100% vs. 81%).

The presence of motile sperm on a wet prep may also be useful to exclude maturation arrest, as none of the nine testes with maturation arrest demonstrated any sperm motility. We observed, however, the presence of nonmotile sperm in two (22%) of these specimens. In these two cases, only a partial pattern of maturation arrest was noted, suggesting that the nonmotile sperm seen on wet prep might have originated from the rare tubules that contained small numbers of mature spermatids. Indeed, even with the conventional methods of testis biopsy, controversies regarding the exact pathological diagnosis and the subsequent clinical intervention are more likely to arise in cases where the histologic picture is heterogeneous.

Recent advances in microsurgical techniques and the expanding role of urologists in assisted reproduction have prompted a quest for a better understanding of the process of sperm maturation and motility at cellular and molecular levels. From the classic experiments of epididymal ligation performed by Young (1931), to recent observations on the fertilizing capacity of sperm that have not traversed the complete epididymis by Silber (1988) and Silber et al. (1990), the exact role of the epididymis in sperm maturation is again under reevaluation. Young inferred from his epididymal ligation experiments that epididymal factors were unimportant for the maturation of sperm, because sperm retrieved from the proximal region of guinea pig epididymides had higher fertilizing potential than those retrieved more distally (Young, 1931). In defense of a role for epididymal functions, Cooper (1990) cautioned against overzealous interpretation of these data before the pathological state of the tissue involved in an obstructed reproductive system could be better defined. As he pointed out, Young's data did not exclude the possibility of intermixing of luminal contents within the epididymal tubules. Furthermore, Orgebin-Crist et al (1976) observed from segmental ligation of rabbit epididymides that only sperm confined to regions distal to the proximal caput had fertilizing potential. Taken altogether, we concur with Cooper that current data neither support the view that intratesticular sperm are inherently fertile nor do they indicate that simple aging would allow full development of fertilizing capacity.

Based on our current understanding of sperm maturation, in an obstructed system, sperm may acquire motility through: (1) prolonged confinement within the reproductive tract, (2) direct contact with refluxed epididymal factors, or (3) retrograde migration of sperm after contact with epididymal environment. Furthermore, chronic obstruction may also cause adaptation of the testicular epithelium that allows acquisition of intratesticular sperm motility and maturation of fertilizing capacity to occur. It is plausible that a combination of these factors would indeed be necessary to account for our observations. Our findings regarding the presence of intratesticular sperm motility do not negate the role of the epididymis in sperm maturation. Nevertheless, they suggest that testicular sperm retrieved from men with unreconstructable obstruction might be utilized for *in vitro* fertilization of human oocytes, perhaps with the aid of an oocyte micromanipulation technique such as subzonal insertion. Clinically, we recommend that wet prep cytological examination be performed at the time of testis biopsy for assessing anatomical obstruction. Under such circumstances, the presence of motile sperm could justify immediate exploration and reconstructive surgery if indicated.

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Erratum

The editors of the *Journal of Andrology* regret that an error was made in the legend for the featured cover of the January/February issue. The caption for the micrographs should read:

Color confocal micrographs of a reconstructed area of the corpus epididymal epithelium immunostained for Y_f glutathion S-transferase P protein. This reconstruction was made up of 56 slices, each 0.3 μm thick. The reconstructed section is viewed at two different angles. Immunostaining intensity increases by approximately a factor of 10 between each color starting from blue, then green, red, and gray. In a cross-sectional view at 0° rotation (bottom), strong immunoreactivity (red, gray) is localized to the basal edge of the epithelium where basal cells reside, whereas very weak immunostaining (green, blue) is seen over principal cells or in the lumen. The extensive coverage of the tubule by the basal cells becomes more apparent as the tissue section is rotated through a 60° angle (top). ×1646. (See article by Veri et al.)