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The study of detecting sperm in testis biopsy in men with severe oligospermia and azoospermia by two methods of wet prep cytologic and classic histopathologic

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Abstract

Background: Many azoospermic patients with non obstructive azoospermia (NOA) are candidate for testicular sperm extraction (TESE) and in vitro fertilization. Because sperm might be present in some but not all parts of the testes of such men, multiple sampling of testicular tissue are usually necessary to increase the probability of sperm finding. Sperm finding can be done by two methods: 1) classic histopathology and 2) wet smear.

Objective: Comparative study of pathology and wet smear methods for discovering sperm in testis biopsy of azoospermic men.

Materials and Methods: We prospectively studied 67 consecutive infertile men who referred to Fatemieh Hospital, Hamedan, Iran between April 2002 and September 2004. All patients were either azoospermic or severely oligozoospermic. They underwent intraoperative wet prep cytological examinations of testis biopsy material and then specimens were permanently fixed for pathologic examination too.

Results: Among the 67 testes that underwent wet prep cytological examination, 44 (65.7%) were positive and 23 (34.3%) had no sperm in their wet smear. On the permanent pathologic sections, 19 (28.4%) were positive and 48 (71.6%) cases were with no sperm in their sections. Among all the individuals 18 (26.8%) were negative in both studies, while 14 (20.8%) had minimum 1 sperm in their smears in both examinations. The positive cases in wet prep cytological examination were significantly more than the cases in the permanent histopathologic sections (p-value=0.000).

Conclusion: It seems that wet prep cytological examination is more reliable than permanent histopathologic sections in detecting sperm in testis biopsy of azoospermic men.

***Key words**: TESE, Histopathology, Wet smear.*

Introduction

Azoospermia affects approximately 1% of the male population and 10% of men who seek fertility evaluation (1, 2). Until recently, it was assumed that men with non-obstructive azoospermia were untreatable.Indeed, these patients were often referred to as being sterile or having testicular failure. The only way these couples could have children was to use donor spermatozoa or embryo reception or to adopt. Several observations have

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changed the approach to this condition. It was observed that direct evaluation of testis biopsy specimens often demonstrates sperm in men with non-obstructive azoospermia, despite severe defects in spermatogenesis (3). Mature sperm cells can be found in approximately 50% of the testes of men with nonobstructive azoospermia (NOA). Even when sperm cells exist, they may not be present in all the testicular samples because the testicular tissue structure may not be homogenous, and spermatogenesis may be present only in minute foci (focal spermatogenesis) (4).

According to current standards, a man is considered sterile and can not father his own genetic offspring if no sperm cells are detected in different locations in the testis. It stands to reason, then, that the most reliable method should be used to reduce the chance of misdiagnosis. There is, however, no reliable noninvasive method of predicting sperm production in the testis.

Testis biopsy for evaluation of male infertility was first reported over 50 years ago by Hotchkiss (5) and Charny (6). It is most useful in distinguishing between reproductive tract obstruction vs. primary testicular failure in azoospermic or severely oligozoospermic men (7). Nowadays, the wet prep studies are easy to perform and allow assessment of both the presence and motility of sperms.

Materials and methods

Between April 2002 and September 2004, we prospectively studied 67 consecutive infertile men who referred to Fatemieh Hospital, Hamedan, Iran. A detailed history and physical examination and required laboratory test was performed on all patients. All patients were either azoospermic or severely oligozoospermic according to multiple semen analysis tests and had serum follicle-stimulating hormone levels less than three times normal. All procedures were performed by the same surgeon and with the men under local anesthesia. They all presented with primary infertility and all had documentation of azoospermia in repeated semen analyses. Severe oligozoospermia was detected in 15% of cases followed by several semen analysis and in some of them azoospermia were reported and Percutaneous Epididimal Sperm Aspiration (PESA) of these men showed no sperm.Typically, the man with non-obstructive azoospermia will have small testes (< 15 cc) with a flat epididymis. Some men may have a history of cryptorchidism. Hormonal evaluation of a man with non-obstructive azoospermia (NOA) will typically demonstrate an elevated serum FSH, with normal or nearly normal testosterone levels for all patients with azoospermia, a complete history and physical examination is necessary to identify potentially correctable causes of male factor infertility. Men who were diagnosed as having obstructive azoospermia before the operation, based on physical examination and laboratory findings, were excluded from the study, as were those with an indefinite preoperative diagnosis and a postoperative testicular histology of normal spermatogenesis or PESA positive for sperm.The technique for diagnostic testicle biopsy (3) is very simple. For diagnostic testicle biopsy, the spermatic cord is injected with 5 ml of lidocain 2% solution via a 27 gauge needle just distal to the external inguinal ring. Then an additional 2 ml of local anesthetic is injected over the anterior scrotal skin. The testicular biopsies (TESE) were performed superficially. The tunica albuginea was incised transversely for about 5 mm in 3-4 locations in each testis. The testis was then gently squeezed and the protruding tissues were excised, each weighing approximately 50 mg. Tissue samples were removed until spermatozoa were identified or 3–4 biopsy pieces were extracted from each testis. The tunica albuginea was closed using 4/0 cat gut, and the layers of scrotum were sutured separately. The obtained testicular tissue samples were placed into Hamz F 10 and Bowan's solution (3,10). The specimens were dissected then compressed under a glass cover slip. The wet prep slide was immediately examined microscopically by embryologist under a high-dry (40 ×) objective. The use of phase-contrast microscopy is preferred but not imperative. The presence of minimum 1 sperm is called positive case and determination of motility was best evaluated just outside the margins of the compressed tissue (11). Specimens in Bowan's solution 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, and classified as demonstrating normal spermatogenesis, hypospermatogenesis (graded slight, moderate, or severe), maturation arrest (complete or partial), Sertoli cell-only pattern, or tubular and peritubular sclerosis.

Statistical analysis

Both examinations were performed on all the cases and the results of the two examinations were compared using Chi-Square test. p-value < 0.05 was considered as significance difference.

Results

Our patients ranged in age from 20 to 65, with a median age of 34 years. Azoospermia was present in 85% of the patients while the rest had severe oligozoospermia. Among the 67 cases that underwent wet prep cytological examination, 44 (65.7%) were positive and 23 (34.3%) had no sperm in their wet smear. On the permanent pathologic sections, 19 (28.4%) were positive and 48 (71.6%) cases were with no sperm in their sections. Among all the individuals, 18 (26.8%) were negative in both studies, while 14 (20.8%) had minimum 1 sperm in their smears in both examinations. Thirty of the patients (44.7%) were positive considering wet prep cytological examination and were negative on permanent histopathologic sections. In 5 cases (7.4%) no sperm was found on wet prep cytological examination but were positive considering permanent histopathologic sections. In 47.6% of cases the results were the same in both examinations.The positive cases in wet prep cytological examination were significantly more than the cases in the permanent histopathologic sections (p-value=0.05).

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 vasectomy and vasograms performed at the time of biopsy. In an attempt to offer a rapid intraoperative diagnosis, Coburn *et al* (8) 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* (9) 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 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%) (12). 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 (13), to recent observations on the fertilizing capacity of sperm that have not traversed the complete epididymis by Silber *et al* (3,14), 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 (13).

In defense of a role for epididymal functions, Cooper (15) 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* (16) 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: prolonged confinement within the reproductive tract, direct contact with refluxed epididymal factors, or 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, it suggests 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 sub zonal 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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