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TESTIS BIOPSY FINDINGS IN THE SPINAL CORD INJURED PATIENT

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ABSTRACT

Purpose: Azoospermia after electroejaculation in spinal cord injured men may be due to testicular failure or obstruction. These men can initiate pregnancy with assisted reproductive techniques, such as intracytoplasmic sperm injection, but only if sperm are present in the testis biopsy. We analyzed the histopathology of testis biopsies from spinal cord injured men and assessed whether patient factors were predictive of testis biopsy pathology.

Materials and Methods: A total of 50 paraplegic men undergoing testis biopsy were divided into 2 groups based on normal or abnormal testis histopathology. Patient age, post-injury years, level of lesion, hormonal status and semen analysis results were compared.

Results: Spermatogenesis was normal in 28 of the 50 patients. Hypospermatogenesis was exhibited in 15, maturation arrest at the spermatid stage in 6 and maturation arrest at the spermatocyte stage in 1 of the 22 abnormal cases. Nevertheless, mature sperm were identified in 43 of 50 biopsies (normal spermatogenesis and hypospermatogenesis). Men with normal spermatogenesis had better forward progression of sperm and a higher testosterone-to-luteinizing hormone ratio. Otherwise, there was no statistically significant correlation between study variables and testis biopsy results. No factors were predictive of testis biopsy histopathology.

Conclusions: The documentation of mature sperm in 43 of 50 biopsies from spinal cord injured patients suggests that a high rate of sperm retrieval is possible using testicular sperm extraction if sperm cannot be retrieved from the ejaculate. With intracytoplasmic sperm injection techniques the majority of spinal cord injured men retain fertility potential, even if azoospermic following electroejaculation.

KEY WORDS: testis, biopsy, electric stimulation, spinal cord injuries, spermatogenesis

Spinal cord injured patients, typically young men, represent the largest physical medicine and rehabilitation population with fertility related disorders. Although 3% to 20% of these men can ejaculate with self-stimulation, this ability tends to be unpredictable and is rarely effective to initiate pregnancy.[1] In 85% to 97% of these men loss of ejaculatory capability is permanent.[2] Rectal probe electroejaculation and vibratory penile stimulation provide an approach to obtain semen. However, even when semen is produced azoospermia is not uncommon and asthenospermia is common. The cause of poor semen quality following electroejaculation is unclear.

We previously demonstrated that the electroejaculation procedure has no effect on sperm motility or forward progression in vitro.[3] Brackett et al demonstrated that the seminal plasma of spinal cord injured men may be detrimental to sperm.[4] Poor sperm quality after electroejaculation of spinal cord injured patients has resulted in low fertilization and pregnancy rates, despite use of in vitro fertilization (IVF) or intrauterine insemination.[5] However, with assisted reproductive techniques, such as intracytoplasmic sperm injection (ICSI), it is now possible to achieve fertilization more effectively using sperm even from a specimen obtained by electroejaculation.[6] Additionally, even if the azoospermic spinal cord injured male has only a few mature sperm on testis biopsy, fertilization is still possible with ICSI using sperm obtained by testicular sperm extraction (TESE).[7,8] Thus, we sought to identify factors predictive of testicular biopsy findings to help identify spinal cord injured patients who would be good candidates for TESE and subsequent IVF-ICSI.

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MATERIALS AND METHODS

Study design and subjects. A prospective analysis was performed based on a cohort of 50 spinal cord injured patients who presented to our tertiary care centers for evaluation of infertility and underwent electroejaculation with semen analysis. A subset of patients cared for by one of us (I. H. H.) underwent testis biopsy regardless of semen analysis results and the remainder underwent biopsy only if the sample indicated oligospermia or azoospermia. Patients were categorized into 2 groups based on normal or abnormal testis histology, and demographic characteristics and laboratory values were compared. Study variables consisted of patient age, post-injury years, level of injury, serum follicle-stimulating hormone (FSH) (normal 4 to 10 ng./ml.), serum luteinizing hormone (LH) (normal 6 to 19 mIU/ml.), total serum testosterone (normal 200 to 1,000 ng./dl.), testosterone-to-LH ratio and semen analysis, including volume, sperm density, sperm motility and forward progression (0 to 4). Serum hormones were measured within 6 months of biopsy. Some patients received several trials of electroejaculation and, therefore, the specimen with the best overall semen analysis (highest sperm density, motility and forward progression) was considered representative.

Electroejaculation technique. The electroejaculation preparation and procedure have been described previously.[9] Briefly, rectal probe stimulations were delivered with a

G & S Model 12† electroejaculator. An increasing series of voltages was delivered, using 2 stimulations for each voltage, with each stimulation lasting approximately 2 to 4 seconds. If the patient had not ejaculated at a maximum of 10 V., then 3 stimulations were used at each voltage before proceeding to the next higher voltage. Most patients ejaculated at less than 20 V. and less than 50 stimulations but occasionally up to 30 V. were required. Antegrade and retrograde samples were collected. Only the antegrade semen analysis is reported as consistent assessment of retrograde volume and sperm density was difficult.

Testis biopsy. The testis biopsy procedure has also been described previously.[10] During the procedure patients received no or general anesthesia depending on the completeness of the spinal cord lesion. After a 1 to 2 cm. incision was made through the parietal tunica vaginalis, the tunica albuginea was incised for approximately 5 mm. with a No. 11 scalpel blade. Gentle pressure on the testicle extruded a small amount of testicular parenchyma, which was carefully excised with fine, sharp scissors and placed promptly in an appropriate fixative or processed first for cytological analysis. After hemostasis was obtained the tunica albuginea and layers of the scrotal wall were closed with fine absorbable running suture. Based on standard pathological interpretations of the hematoxolin and eosin sections, biopsies were classified as Sertoli-cell-only, maturation arrest-spermatocyte stage (spermatocytes but no spermatids), maturation arrest-spermatid stage (spermatids but no mature spermatozoa), hypospermatogenesis or normal spermatogenesis. Hypospermatogenesis indicates that all stages of spermatogenesis, including mature sperm, are present but the relative numbers of cells are decreased.

Statistical analysis. Statistical analysis was performed in collaboration with a professional biostatistician using computer software. Data are presented as mean plus or minus standard error unless otherwise stated. Results for continuous variables were compared between groups using the unpaired Student t test or Mann-Whitney rank sum test when normalcy failed. Using a contingency table of independence a chi-square test was performed for the categorical variable (level of lesion). Predictability of testis biopsy results using demographic data, serum hormones and semen analyses was assessed by multiple logistic regression analysis.

RESULTS

Patient age at biopsy was similar between the 2 groups. Similarly, the delay between spinal cord injury and testis biopsy, and the level of spinal cord injury between groups was not significantly different (table 1). Spermatogenesis was normal in 28 men (group 1). Hypospermatogenesis was noted in 15 (30%), maturation arrest-spermatid stage in 6 (12%) and maturation arrest-spermatocyte stage in 1 (2%) of the 22 abnormal cases (group 2). There was no significant difference in serum FSH, LH or total testosterone between the 2 groups. The testosterone-to-LH ratio was significantly higher in group 1 patients (table 1). Sperm density and motility in the antegrade fraction were not statistically different between groups. Forward progression was significantly higher in group 1 (table 1). All data points were available in 22 cases. Multiple logistic regression analysis revealed no variable to be predictive of testis biopsy outcome (table 2).

† G & S Instrument Co., Duncanville, Texas.

DISCUSSION

Our significant practical finding is that the majority of spinal cord injured men presenting for electroejaculation have mature sperm on testis biopsy. Of 50 patients 43 had mature sperm on histological examination of the testicular tissue and this ratio was no different for those with severe oligospermia or azoospermia (less than 1 million sperm per ml., 9 of 11 patients). The clinical application of this observation is that even if electroejaculation results in an azoospermic semen specimen patients should be counseled that they may be good candidates for TESE with ICSI, or sperm retrieval from the epididymis or vas deferens if there is obstruction.[11] Consistent with previous studies of spinal cord injured men we found no factor to be predictive of testis biopsy findings. Forward progression and testosterone-to-LH ratio, although significantly higher in men with normal findings, were not predictive of testis biopsy results. Therefore, if other methods of sperm retrieval have failed, TESE with ICSI may be pursued regardless of the level of lesion, patient age, time since injury, hormone concentrations and semen analysis.

Our results demonstrate that a higher proportion of spinal cord injured men possess at least some mature spermatozoa (hypospermatogenesis or normal) on testis biopsy than previously reported. Stemmermann et al reported normal findings in 6 of 16 spinal cord injured patients, hypospermatogenesis in 3, various phases of maturation arrest with no apparent spermatozoa in 6 and the Sertoli-cell-only syndrome in 1.[12] Bors et al found normal spermatogenesis in 3 of 34 paraplegic men, hypospermatogenesis in 17 and varying degrees of maturation arrest in the remaining 14, including 1 with the Sertoli-cell-only syndrome.[13] Leriche et al reported that 27 of 54 biopsies in spinal cord injured men showed tubular atrophy and maturation arrest, with the other 50% demonstrating atypical findings of normal spermatogenesis but obstruction of the tubules and hyperplasia of the Sertoli cells.[14] A smaller study by Perkash et al yielded results similar to ours as spermatogenesis was normal in 6 of 13 spinal cord injured patients (46%) and the remainder had hypospermatogenesis.[15]

A possible explanation for our higher frequency of mature sperm is that we specifically sought to identify even small numbers of mature sperm. This distinction is important be-

TABLE 1. *Testis biopsy results versus study parameters*

	Normal	No. Group 1	Abnormal	No. Group 2	p Value
Pt. demographics:					
Mean age ± SE	31.6 ± 1.4	24	32.1 ± 1.3	20	0.730
Mean post-injury yrs. ± SE	8.8 ± 1.4	23	8.7 ± 1.8	17	0.853
No. lesion level	10 Cervical, 16 thoracic, 2 lumbar	28	6 Cervical, 12 thoracic, 3 lumbar	21	0.556
Mean serum hormones ± SE:					
FSH (ng./ml.)	6.5 ± 1.1	25	10.0 ± 2.5	19	0.530*
LH (mIU/ml.)	7.4 ± 1.9	25	14.4 ± 3.6	17	0.096
Testosterone (ng./dl.)	464.7 ± 35.5	25	450.8 ± 44.7	17	0.807
Testosterone-to-LH ratio	168.6 ± 30.2	24	84.4 ± 21.7	16	0.029
Mean semen analysis ± SE:					
Density (million sperm/ml.)	179.2 ± 75.2	19	75.0 ± 44.1	16	0.552*
% Motility	13.3 ± 3.1	19	12.5 ± 4.1	17	0.877
Forward progression	2.0 ± 0.2	19	1.1 ± 0.2	14	0.004

* Mann-Whitney rank sum test.

TABLE 2. *Multiple logistic regression analysis*

Parameter	p Value
Pt. demographics:	
Age	0.3928
Post-injury yrs.	0.6161
Lesion level	0.9190
Serum hormone concentrations:	
FSH	0.2671
LH	0.0714
Total testosterone	0.5575
Testosterone-to-LH ratio	0.1143
Semen analysis:	
Density	0.2014
Motility	0.2600
Forward progression	0.1540

cause today only a few sperm are required to initiate a pregnancy with ICSI. Interestingly, in contrast to some earlier studies but in agreement with Perkash et al we found that testis biopsies in spinal cord injured men rarely demonstrated maturation arrest at the spermatocyte stage, and that the Sertoli-cell-only syndrome was nonexistent.[15] Instead, biopsies from some spinal cord injured men were characterized by a marked decrease in sperm production, suggesting a difference in pathogenesis between testis failure due to spinal cord injury and causes seen in otherwise healthy but infertile men. A possible etiology of testis histopathology particular to spinal cord injured men is testicular hyperthermia.[16]

Previous evaluations of various sex hormones in spinal cord injured patients have yielded a variety of findings, from normal serum concentrations to chronic elevations of LH, FSH and testosterone.[13,15,17,18] No correlation in other series or ours was found between these hormone concentrations and testis biopsy results.[14] Regardless, none of the patients presented with serum FSH greater than 3 times normal, which is the criterion commonly used to describe primary testicular failure. The ratio of serum testosterone-to-LH was higher in men with normal biopsies. Although to our knowledge testosterone-to-LH ratio has not been previously evaluated in the spinal cord injured population, it has been widely used in others as a more sensitive indicator of Leydig cell function than testosterone or LH alone.[19,20] Despite a slightly depressed testosterone-to-LH ratio in men with abnormal biopsies, serum testosterone and LH were normal, which could represent an adaptation of the pituitary gland to relative failure of the Leydig cells to maintain normal serum testosterone.

It is believed that cauda equina lesions (below T10) may result in a different pathological condition, with relative sparing of the testis. Although Bors et al demonstrated that men with cauda equina injury had more normal biopsy results compared to those with upper cord lesions, subsequent studies have failed to corroborate this correlation.[12–15] We found no significant association between spinal lesion level (cervical, thoracic, lumbar) and testis biopsy results. Additionally, when cases were grouped as cauda equina and upper cord lesions, there was no sparing of testis pathology in the cauda equina group (48% normal versus 62% normal in the upper cord lesion group). Previous studies demonstrated no correlation between the number of years since spinal cord injury and biopsy results.[12,13,15,21] Our study also found no significant difference in post-injury years between patients with normal and those with abnormal biopsies. Additionally, multiple logistic regression analysis using patient age, post-injury years, serum hormones, sperm density, sperm motility and forward progression revealed no significantly predictive factors of the degree of testis histopathology. However, complete data were available for only 22 men.

Treatment of the infertile spinal cord injured male begins with vibratory stimulation or electroejaculation for sperm retrieval, which is productive in the majority. However, at a tertiary care facility the spinal cord injured patient often presents with azoospermia after assisted ejaculation. We demonstrated that the majority retain fertility potential, even when azoospermia is found. Our biopsy results suggest that spinal cord injured men will likely have sperm on TESE, despite the indication of testicular failure based on semen analysis and hormone concentrations.[7,8] Although some azoospermic spinal cord injured patients had obstruction, the standard care for most is sperm retrieval rather than correcting the cause of the obstruction. Thus, spinal cord injured men should be counseled about reproductive possibilities using TESE with ICSI.

CONCLUSIONS

Testis histopathology in the spinal cord injured patient is commonly normal or hypospermatogenic. Maturation arrest was rare, and the Sertoli-cell-only syndrome was not found in our series. Sperm can be retrieved from the majority of spinal cord injured men using a combination of vibratory stimulation and electroejaculation. However, vasal aspiration or testis biopsy is indicated for patients who do not produce sperm with these procedures. If mature sperm can be found on testis biopsy in most spinal cord injured patients as in ours, TESE with ICSI offers those with electroejaculation failure an important alternative means of fathering a biological child.

Electroejaculation Study Group members Stanton Honig, Dana Ohl, Steven Shaban, Samuel Thompson and Michael Witt contributed to the database.

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