Impact of repeated testicular fine needle aspirations (TEFNA) and testicular sperm extraction (TESE) on the microscopic morphology of the testis: an animal model

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BACKGROUND: The aim of this work was to compare testicular histological changes occurring following testicular fine needle aspiration (TEFNA) and testicular sperm extraction (TESE). METHODS: TESE or TEFNA were performed on normal male rats, in a similar manner to azoospermic men. The animals were killed after 7, 14 and 31 days and their testes were examined. RESULTS: TESE caused chronic inflammation, occasional necrosis and degenerative changes in testicular germ cells in only ~10% of the remaining testicular tissue. TEFNA caused widespread architectural distortion of seminiferous tubules into irregular and deformed lumens lined by Sertoli cells only, as well as focal chronic inflammation, necrosis and degenerative changes accompanied by decreased spermatogenesis. Similar but less extensive changes were noted when fewer punctures were performed. When negative suction pressure was not applied during TEFNA, similar histological changes occurred, indicating that testicular damage was related to the puncture itself. Following either procedure, the contralateral non-operated testes were unaffected. CONCLUSION: In this animal model, TEFNA inflicts severe, progressive and irreversible damage on the architecture of the tubules in the needle’s path. The multi-focal nature of this technique eventually leads to widespread tubular atrophy that is proportional to its extent. In contrast, TESE causes localized scarring and fibrosis, rendering most of the remaining testicle intact. The clinical relevance of such findings, produced in normal animal testes, to testes of azoospermic men, is yet to be determined.

Key words: animal model/non-obstructive azoospermia/TEFNA/TESE/tissue damage

Introduction

Intracytoplasmatic sperm injection (ICSI) enables fertilization and pregnancy when only few testicular sperm are available, following surgical sperm recovery from azoospermic men. Non-obstructive testicular azoospermia can be a primary condition in cases of Klinefelter’s syndrome, spermatogenesis maturation arrest, Sertoli cell-only syndrome, focal hypoplasmatogenesis and microdeletions of Y chromosome fragments. Testicular azoospermia can also result from environmental events such as trauma, ionizing irradiation and exposure to cytotoxic drugs. ICSI of surgically recovered sperm is the only way in which men suffering from these disorders can share the genetic parenthood of their offspring (Devroey et al., 1995).

Several surgical methods for obtaining testicular sperm needed for fertilization have evolved in different centres. The main approaches are open biopsy by testicular sperm extraction (TESE) (Devroey et al., 1995; Silber et al., 1995) and closed percutaneous testicular fine needle aspiration (TEFNA) (Craft et al., 1997; Lewin et al., 1999). TESE is performed by opening a slit in the scrotal skin and tunica albuginea, followed by excision of a small peripheral testicular tissue fragment, which is immediately processed for sperm recovery. Because sperm might be produced focally, performing biopsies at multiple sites can increase the retrieval yield of TESE (Hauser et al., 1998). Open biopsies can lead to several disturbing potential side effects emanating from the apparently traumatic nature of the procedure. Bleeding, inflammation, devascularization and fibrosis, manifested both clinically and sonographically, might cause irreversible damage to the testis, producing clinical complications and limiting the possibility to repeat the procedure (Schlegel and Su, 1997; Ron El et al., 1998). Closed TEFNA, on the other hand, is apparently less traumatic than TESE (Lewin et al., 1999). A 21 gauge (or thinner) needle is used in order to aspirate tissue without opening and traumatizing the testes. Multiple sites are punctured, including the center of the testes and rete. Sperm recovery, fertilization and pregnancy rates resulting from both techniques are variable as reported by different authors, averaging up to 50% depending on the etiology of azoospermia in the specific patient population.
(Friedler et al., 1997; Tournaye et al., 1998, 1999; Mercan et al., 2000). The assumed less traumatic nature of TEFNA is supported by the low rate of postoperative clinical complications and ultrasonographic abnormalities (Lewin et al., 1999), and the ability to re-perform the procedure successfully (Fasouliotis et al., 2002). However, the microscopic damage to testicular tissue caused by repeated punctures and the negative pressure applied are unknown. There is no histological work comparing the effect of needle aspiration and open biopsies on testicular tissue.

The aim of this study was to determine accurately, in a rat model, the type and extent of testicular damage caused by these two sperm retrieval techniques.

Materials and methods

This study was approved by the Hebrew University’s experimental animals ethical committee, and was performed under its supervision.

Mature male Sabra rats with a proven breeding history, weighing at least 500 g, were held in a regular animal facility. Anaesthesia was carried out by i.p. injection of ketamine (5 mg) (Ketalar; Parke-Davis, UK) and droperidol (1 mg) (Dehydrobenzperidol; Janssen Pharmaceutica, Belgium). TEFNA was performed percutaneously using a 23 gauge butterfly needle attached to a 20 ml syringe. Seven multidirectional punctures were performed on the right testicle applying negative pressure with the attached syringe (as described by Lewin et al., 1999). A fragment of testicular tissue was aspirated in each puncture of the right testicle. TEFNA was also performed in two variations; one or three multidirectional aspirating punctures and seven non-aspirating punctures. TESE was performed through a 3 mm incision in the scrotal skin and tunica albuginea and a piece of peripheral testicular tissue was removed using scissors. Haemostasis was achieved by application of gentle pressure only. The scrotal skin was sutured with 4/0 Vicryl sutures, leaving the tunica albuginea open. In all experiments the left testicle was left intact to serve as a control. At the scheduled time intervals (7, 14 or 31 days after the procedure), the rats were killed by carbon dioxide inhalation. The testes were excised by laparotomy, fixed in formalin, processed and embedded in paraffin. The blocks were sectioned at 4 mm and stained with hematoxylin and eosin. Three to six sections were examined in each sample. The following morphological findings were evaluated: spermatogenesis, presence of inflammation, necrosis and degenerative changes.

Results

TESE was performed on eight testes. Three were examined 1 week after the procedure, three at 2 weeks and the other two at 1 month. Macroscopically, local scarring was the only change noticed (Figure 1). Microscopically, focal chronic inflammation accompanied by granulation tissue formation was observed in all testes, more prominently 2 weeks and 1 month after the procedure. Coagulative necrosis of the seminiferous tubule cells was generally mild and focal. Mild degenerative changes of the epithelial component of the tubules were also noted, with karyolysis, and karyopyknosis of germ cells and occasional multinucleated cells. The structure and lumen of the seminiferous tubules was preserved. All these changes were exclusively limited to the area adjacent to the biopsy site. Most of the testicular tissue was well preserved with normal spermatogenesis, and showed no change at the various time intervals after the procedure (Figures 2A2, 2B2 and 2C2). Figure 2D shows a cross-section of a control testis for comparison.

TEFNA was performed on 15 testes. Five were examined 1 week after the procedure, five at 2 weeks and the other five at 1 month. Macroscopically, initial engorgement and a later gradual shrinkage were observed (Figure 1). The microscopic examination revealed chronic inflammation with foci of acute inflammation along with granulation tissue in all samples. Signs of haemorrhage and calcifications were seen in the late stages following the procedure. Foci of coagulative necrosis were found in almost all the testes, ranging from minimal foci in most cases, to extensive necrosis involving most of the tissue.

Unique degenerative changes of the epithelial component of the seminiferous tubules were found in all testes. These were characterized by karyopyknosis, karyolysis of immature germ cells, multinuclear cell formation and depletion of epithelial component. These changes resulted in destruction and transformation of the seminiferous tubules into enlarged, irregular and deformed cavities with empty lumens, lined predominantly by flattened Sertoli cells. Spermatogenesis was universally absent from these deformed tubules. The extent of this unique degeneration process of the seminiferous tubules correlated directly with the time elapsing from the procedure, indicating a gradual progressive evolution following the initial injury. While a minority of tubules had undergone this change 1 week after the aspiration, at 1 month after TEFNA most of the testicle was occupied by these degenerated tubules (Figures 2A1, 2B1 and 2C1).

In order to assess the effect of the number of punctures on the extent of damage in a semi quantitave way, two experiments were carried out. In the first, one puncture was performed and in the second, three punctures were performed on nine testes. The testes were subjected to histological examination at 1 and 2 weeks and at 1 month interval (three testes for each time point) following the TEFNA procedure. In both experiments, testicular micro-architectural damage was similar to that described above and extended considerably beyond the path of the needle. However, testicles with one puncture were substantially less affected then those with three punctures at any time point examined.
Testicular histological changes following TEFNA and TESE

Figure 2. Photomicrographs of rat testes 7 (A1, A2), 14 (B1, B2) and 31 days (C1, C2) following TEFNA (A1, B1, C1) and TESE (A2, B2, C2). (D) Cross section of an unaffected control testicle. Optical magnification ×50 on the left and ×200 on the right.
In order to evaluate the effect of negative suction on testicular damage, three testes were punctured without applying negative pressure and assessed histologically at 1 month after the procedure. The histological changes found were similar to those observed when negative suction pressure was applied except that the empty flat lined tubules were symmetrically round and not as distorted as in the testes that were punctured and aspirated (Figure 3).

No changes were observed in the Leydig cell population of the operated testes at any time after the performance of either TEFNA or TESE. The contralateral non-operated testes were unaffected following either TEFNA or TESE. No inflammation, necrosis, anomaly of testicular micro-architecture or spermatogenesis was found in these testes.

Discussion

ICSI of testicular sperm is the only hope of genetic parenthood for men with testicular non-obstructive azoospermia. The critical step in this combined and complex treatment is obtaining sperm of sufficient quality and quantity for ovum fertilization. These cells are scarcely found in the testicular tissue and have to be removed surgically. Several sperm extraction techniques from the testes of azoospermic men have evolved since ICSI became routinely available for clinical use. Open biopsies and needle aspiration are the two basic techniques. TEFNA is apparently less traumatic than open biopsy and has a lower reported clinical complication rate (Lewin et al., 1999). Since low sperm production in cases of testicular azoospermia is focal, needle aspiration might be advantageous due to its unlimited access to multiple superficial and deep testicular sites, in contrast to the local, superficial and limited nature of biopsies. Successful testicular sperm retrieval in varying rates was reported for both methods (Tournaye, 1999). In most reports, TESE was performed. Similar success rates of TEFNA were reported by others (Craft et al., 1997; Rosenlund et al., 1998; Lewin et al., 1999), but in comparative studies TESE was found to be more efficient in extracting testicular sperm for ICSI (Friedler et al., 1997; Ezeh et al., 1998; Mercan et al., 2000).

The extent of damage inflicted to the testes by the application of these techniques has never been directly investigated. The existing data consist of reported clinical complications and functional hormonal performance following the procedures. The reported complications of open biopsies are similar to those following other types of open surgery (infection, hemorrhage, and fibrosis) (Schlegel and Su, 1997). The reported rate and severity of complications following TEFNA is lower. Following TEFNA, normal sexual function and hormonal profile had been reported (Lewin et al., 1999; Westlander et al., 2001). When repeated, TEFNA was found to have a similar sperm retrieval yield; and when sperm were found in the first aspiration attempt there was a 70% chance to find sperm cells in the following aspiration as well (Fasouliotis et al., 2002).

In this study, we evaluated in an animal model the microscopic damage caused by these procedures. In addition, the evolution of tissue damage and nature of the repair processes over time was also studied.

We demonstrated that the damage to the seminiferous tubules and Sertoli cells is greater following needle puncture and aspiration than after an open biopsy. Following TEFNA, permanent destruction of each aspirated tubule, and of the neighbouring tubules as well, eventually occurs. The tubular infrastructure of Sertoli cells was gradually, but totally, destroyed and 1 month after the procedure, the vast majority of the tubules were found to be severely distorted and devoid of any spermatogenetic activity. This damage did not occur immediately, but rather gradually, weeks after the aspiration, indicating a major role for late inflammatory and repair cellular processes. It may be hypothesized that these processes, which were investigated thoroughly in other tissues, are initiated by a relatively minor (but widespread) trauma and are responsible for most of the tubular damage evolving afterwards. The performance of fewer repetitive punctures was less traumatic, but would probably decrease the success rate in a clinical setup. Avoiding negative pressure aspiration did not decrease the damage significantly, indicating that the main factor affecting the testicle during TEFNA is the puncture itself and not the high negative pressure applied. Open biopsy causes similar damage, leading to permanent scarring, which is restricted to the biopsy site, leaving the remaining testicle almost intact. Paradoxically, in this animal model, the less traumatic procedure, from a clinical point of view, eventually causes a greater degree of late occurring tissue damage.

With regard to TEFNA, the relevance of this animal model to human clinical practice is not clear. It is impossible to draw direct parallels between the changes occurring after aspiration in normal rat testicles, on which this study was performed, and those occurring in aspirated testicles of azoospermic men. It is not mandatory that these changes actually occur in human testes with an already primarily severely impaired spermatogenesis. The damage inflicted by needle aspiration in this animal model mainly affects Sertoli cells, which are the backbone of spermatogenesis, but not the testosterone-producing Leydig cell population. Even if these changes do
occur after aspiration of human testes, they might not be clinically relevant to men who have an already existing absolute impairment of spermatogenesis. Moreover, if human TEFNA causes similar damage to the testes as found in our animal model, it would conflict with the clinical finding that TEFNA can be repeated successfully (Fasouliotis et al., 2002). Therefore, one should interpret the results of this study cautiously before applying them to patients undergoing TEFNA. Although the damage observed in this animal model may not occur to the same extent in aspirated human testes, it should be borne in mind that TEFNA may not be free from testicular damage. On the other hand, this study demonstrates the safety of open unaided testicular biopsies. The damage inflicted by these procedures is local and limited to the biopsy site, without affecting the remaining testicle.

References


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