Testicular sperm extraction in cancerous testicle in patients with azoospermia: A Case Report

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The aim of this study is to evaluate the feasibility of testicular sperm extraction (TESE) in a cancerous testicle during orchietomy for testicular neoplasm. This is a retrospective study and includes case histories of four azoospermic patients with incidental testicular cancer. None of the patients had fathered children prior to surgery and all patients were strongly motivated by the desire to have offspring. Patients underwent surgical exploration via inguinotomy and spermatic cord clamping. After nodule excision, micro-TESE was performed from the same albinoital incision, under microscopic guidance. Frozen section examination was not performed in the case of large nodules (>3 cm in diameter). Two patients showed classic seminoma and underwent orchietomy. In two patients, a Leydig cell tumour was found (one patient underwent orchietomy for large nodule size). Micro-TESE was performed in four patients. Spermatozoa were found in three patients and the retrieved sperm was cryopreserved. One ICSI cycle was performed, but pregnancy failed. In azoospermic patients with testicular nodules, TESE in the cancerous testis is feasible and may avoid further surgery, without any oncological risk.

Key words: azoospermia/infertility/testicular cancer/testicular sperm extraction

Introduction

Several articles have recently pointed out a correlation between infertility and testicular cancer (Møller and Skakkebaek, 1999; Jacobsen et al., 2000; Raman, 2005). According to some authors, a dysgenesis syndrome links infertility and testicular cancer (Skakkebaek et al., 2001). More and more often nowadays, diagnosis of testicular cancer is made in the course of a visit or ultrasound for infertility and the contralateral testis is frequently small in size in infertile patients suffering from testicular cancer. In patients with non-obstructive azoospermia or obstructive azoospermia that cannot be corrected, the only possibility of fertilization is represented by sperm retrieval by means of testicular sperm extraction (TESE) (Schlegel, 1999).

TESE, even when performed microsurgically Schlegel and Li (1998), can cause small haematomas or infections, further compromising the quality of an already poor testicle (Wood et al., 2003). Sporadic cases of TESE in cancerous testicles are reported in the literature (Novero et al., 1996; Kohn et al., 2001; Choi et al., 2005). The aim of this study is to evaluate the feasibility of TESE in the cancerous testicle during orchietomy or of conservative therapy for testicular nodules with benign histology through retrospective analysis of a small sample of consecutive patients.

Materials and methods

From April 2000 to February 2006, an intratesticular mass was found in four patients during investigations for azoospermia. The patients were referred to us for performance of explorative testicular surgery. Azoospermia was confirmed by at least two semen analyses.

The patients underwent blood tests and viral tests, tumour markers (alpha fetoprotein, beta HCG and lactic dehydrogenase), hormone assays (testosterone, LH and FSH), scrotal ultrasound, chest X-ray, screening for cystic fibrosis, Y chromosome microdeletion analysis and karyotype.

All patients were strongly motivated by the desire to have offspring. They all signed an informed consent form that included both the time needed for oncological surgery and the time for sperm retrieval.

One patient (Case 1) had already been cited in the work of Colpi et al. (2005). We decided to include this patient because the two studies have completely different aims.

Surgical technique

The microsurgical technique is very similar to the one already described by Hoppes and (2002) Goldstein, with a few variants (Colpi et al., 2005). The testicular lesion is identified by ultrasound prior to surgery with 3D coordinates to aid intraoperative recognition. A traditional inguinal incision is performed, and the spermatic cord is clamped subinguinally without opening the canal. If the lesion is impalpable, the lesion site is checked intraoperatively with a
15 MHz ultrasound probe. An equatorial incision of the tunica albuginea is then performed on a separate operative field, using a × 6–20 magnification operating microscope, on a plane orthogonal to the major axis of the testicle, from one-quarter to about three-quarters of its circumference depending on the depth of the lesion, sparing the subtunical vasa. The parenchymal lobuli are dislodged and the seminiferous tubules dissociated. The nodule is then identified and removed completely, along with ~1 mm of surrounding healthy tissue. The tissue is then sent for frozen section examination together with biopsy of the resection margins. From the same incision, micro-TESE is then performed by taking about 30 microsamples of the largest seminiferous tubules from the two sides of the open parenchyma, as described by Schlegel (1999).

If there is histological evidence of malignancy, the inguinal canal is opened while the spermatic cord is still clamped, and the inguinal portion of the spermatic cord is recovered. If histological results show benign nodules and negative resection margins, the tunica albuginea is sutured with a 5/0–6/0 slow absorption monofilament, using anatraumatic needle.

Protocol for the sperm cryopreservation procedure

The biopsied testicular tissue specimens are transferred to the laboratory in human tubal fluid (HTF)–HEPES medium (Irvine, Santa Ana, CA, USA) and then rinsed in the same medium. The tissue is immediately dispersed and finely minced with scalpel blades and an 18 G needle into a Petri dish; the effluent is placed into a 10 ml Falcon tube and centrifuged at 1300g for 10 min. The supernatant is removed and the pellet is resuspended by adding 250 μl of fresh HTF–HEPES medium. A 5 μl droplet is examined for the presence of spermatozoa and an eosin vitality test is performed. If sufficient numbers of vital spermatozoa are present in the testicular extraction fluid, the pellet is diluted to at least 1 ml with HTF–HEPES medium; the same volume of cryopreservation medium (Test Yolk Buffer; Irvine) is added dropwise and carefully mixed. Subsequently, 0.3 ml high security straws (Cryo Bio System, Paris, France) are loaded with the mixture, sealed, kept for 30 min at 4°C, and then frozen in liquid nitrogen vapor at −180°C. After a 10 min centrifugation (1300g), 2 μl aliquots of the pellet are pipetted in the centre of 5 μl HTF–HEPES droplets in a Petri dish and examined for the presence of spermatozoa and an eosin vitality test is performed. If sufficient numbers of viable spermatozoa are present in the testicular extraction fluid, the pellet is diluted to at least 1 ml with HTF–HEPES medium; the same volume of cryopreservation medium (Test Yolk Buffer; Irvine) is added dropwise and carefully mixed. Subsequently, 0.3 ml high security straws (Cryo Bio System, Paris, France) are loaded with the mixture, sealed, kept for 30 min at 4°C, then transferred into liquid nitrogen vapours (~80°C) for 20 min and finally plunged into liquid nitrogen and stored until ICSI. For thawing, one straw is allowed to equilibrate at room temperature for 10 min and then the content is expelled into a 10 ml Falcon tube and diluted dropwise with 10 ml of HTF–HEPES medium. After a 10 min centrifugation (1300g), 2 μl aliquots of the pellet are pipetted in the centre of 5 μl HTF–HEPES droplets in a Petri dish and examined for the presence of spermatozoa. If no spermatozoa are seen, another straw is thawed.

The samples are then incubated at 37°C until the moment for individual spermatozoa pickup for ICSI procedure.

Morphological evaluation was carried out at a magnification of ×400 under an inverted microscope (Nikon TE200) equipped with a Hoffman modulation contrast system.

Only spermatozoa exhibiting a head as near as possible to normal and a single tail of normal length are used for microinjection of oocytes. Mature spermatids are also considered.

Results

Case history

Case 1
Medical history. Monorchid since the age of 6 after hernioplasty with consensual left radical orchiectomy. Reporting no genital traumas. Former smoker. Karyotype 46XY. Had been attempting to conceive for 3 years. Azoospermia found. No varicocele.

Clinical examination immediately revealed a 5 mm palpable nodule at the superior pole. Explorative surgery was performed. The nodule was removed, and a biopsy of the resection margins and micro-TESE were performed. Cytological analysis revealed the absence of spermatozoa, mature spermatids and the presence of Sertoli cells, making it impossible to cryopreserve any sperm. The testicle was, therefore, preserved. The definitive histological result showed the presence of a benign Leydig cell tumour and Sertoli cell only syndrome.

Case 2
Medical history. Scrotal trauma 3 years prior to surgery. Cyclist. Former smoker. Reporting no genital infections. Karyotype 46XY. Had been attempting to conceive for 1 year. Azoospermia found. No varicocele. Clinical examination revealed an increased consistency at the middle third of the right testicle. Explorative surgery was performed with exposure of a lardaceous mass of ~3 cm. Considering the size of the nodule, no frozen section examination was performed.

A sample of macroscopically intact testicular pulp was obtained for TESE, and radical orchietomy was performed. Cytological analysis of the micro-TESE sample revealed the presence of rare spermatozoa and rare mature spermatids. The sample was cryopreserved. The definitive histological result showed the presence of a benign Leydig cell tumour and germ line aplasia. The patient has chosen not to use the cryopreserved sperm yet.

Case 3
Medical history. Reporting no genital traumas or infections. Had been attempting to conceive for 1 year. Azoospermia found. Karyotype 46XY. Contralateral testicular hypotrophy. No varicocele. Clinical examination revealed an increase in the consistency and volume of the right testicle, which did not appear recognizable in its components. Explorative surgery was performed, with exposure of a neoformation of ~3.5 cm that occupied almost the entire volume of the testis. Micro-TESE was performed, followed by radical orchietomy. The definitive histological result showed the presence of seminoma (pT1) with remaining spermatogenesis in 30% of the tubules in the parenchyma untouched by the neoplasia.

Figure 1 shows the presence of germinal elements in all stages of maturation (mature spermatids: red arrow), but with a disorganized maturation and an intratubular sloughing of spermatogonia (black arrow) and spermatocytes (green arrow) in the intraluminal compartment. Haematoxylin–eosin (×20). Cytological analysis of the micro-TESE sample showed the presence of rare spermatozoa and rare mature spermatids. The sample was cryopreserved. Owing to the recent surgery, the couple has not yet made use of the cryopreserved sperm.

Case 4
Medical history. Left orchiopexy for a mobile testicle at age 8. Reporting no genital traumas or infections. Had been attempting to conceive for 1 year. Azoospermia found. Karyotype 46XY. Y chromosome microdeletion analysis negative. No varicocele. Clinical examination revealed a painless nodule with increased consistency at the inferior pole of the right
testicle. Explorative surgery was performed, and a lardaceous mass of \( \frac{1}{2} \) cm was found at the inferior pole of the testis; the neoformation was radically removed. Micro-TESE was performed, taking a macroscopically intact sample of the testicular pulp. Radical orchiectomy was then performed. Definitive histological results showed the presence of seminoma (pT1).

Cytological analysis of the micro-TESE sample showed the presence of rare spermatozoa and rare mature spermatids. The sample was, therefore, cryopreserved. The couple underwent one ICSI cycle with transfer of one embryo, but pregnancy failed. Subsequently, the couple underwent artificial insemination by donor and gave birth to a child.

Table I sums up the most significant characteristics of the four patients.

**Discussion**

All patients in our case series were strongly motivated by the desire to have offspring.

In our experience, it was possible to retrieve sperm for cryopreservation with micro-TESE in three out of four cancerous testicles in azoospermic patients. Although very low in number, the spermatozoa and mature spermatids retrieved were adequate for cryopreservation. We measured the vitality of the sperm retrieved with micro-TESE by eosin test, which was positive in all patients in whom spermatozoa were found. In our case series, a single ICSI cycle has been attempted to date, but unfortunately the pregnancy achieved through ICSI failed.

With respect to hormones, all patients had high FSH levels. This is explained by the presence of hypogonadism due to testicular insufficiency. Two Leydig cell tumour patients showed low testosterone levels. This is not in contrast to the high FSH level, since only few Leydig cell tumour patients show high levels of testosterone with consequent negative feedback on sexual hormones, and usually these patients are children with signs of sexual pseudoprecocity such as pubic hair, voice change or enlarged genitalia (Thrasher and Frazier, 1994).

Pre-orchiectomy semen cryopreservation is clearly the method of choice in the case of testicular cancer in fertile patients. Pont and Albrecht (1997) have reported that a consistent proportion (10%) of patients with unilateral testicular neoplasia is azoospermic prior to the start of chemotherapy. Cryopreservation is feasible in these patients only through TESE.

Although in low percentages, possible complications for TESE are reported in the literature, even when TESE is performed microsurgically. Possible complications include infections, pain from the surgical wound and subsequent orchialgia (Wood et al., 2003). Complications for TESE performed on its own are \( \sim 33\% \).

Finally, it has been reported that monorchid patients more frequently experience late-onset hypogonadism (Huddart et al., 2005). In a recent paper, Huddart et al. report that 38% of patients who undergo chemoradiotherapy show hypogonadism after a few years and that 11% of monorchid patients present testosterone values at the lower end of the normal range. Performing TESE simultaneously with orchiectomy avoids patients further surgery on the contralateral healthy testis, which is the only remaining Leydig cell and hormonal asset.
<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Nodule size (mm)</th>
<th>Clinical examination</th>
<th>Chest X-ray</th>
<th>Markers</th>
<th>Echostucture</th>
<th>FSH (1.7–12.0 U l⁻¹)</th>
<th>LH (1.1–7.0 U l⁻¹)</th>
<th>Testosterone (3–10.6 ng ml⁻¹)</th>
<th>Frozen section histology</th>
<th>Definitive histology</th>
<th>Surgery</th>
<th>Micro-TESE</th>
<th>Eosin test</th>
<th>Cryo test</th>
<th>Paternity post-TESE</th>
<th>Onco FU (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34</td>
<td>5</td>
<td>Positive</td>
<td>Neg</td>
<td>Neg</td>
<td>Hypo-echoic</td>
<td>20.37</td>
<td>9.82</td>
<td>6.25</td>
<td>Benign</td>
<td>pT1 benign Leydig cell tumour</td>
<td>Sparing absence of spz</td>
<td>–</td>
<td>No</td>
<td>No</td>
<td>Neg</td>
<td>(53)</td>
</tr>
<tr>
<td>2</td>
<td>39</td>
<td>25</td>
<td>Positive</td>
<td>Neg</td>
<td>Neg</td>
<td>Mixed</td>
<td>21.7</td>
<td>8</td>
<td>2.57</td>
<td>Not performed</td>
<td>pT1 benign Leydig cell tumour</td>
<td>Radical Rare spz²</td>
<td>Pos</td>
<td>Yes</td>
<td>No³</td>
<td>Neg</td>
<td>(59)</td>
</tr>
<tr>
<td>3</td>
<td>38</td>
<td>45</td>
<td>Positive</td>
<td>Neg</td>
<td>Neg</td>
<td>Hypo-echoic</td>
<td>26.59</td>
<td>5.13</td>
<td>2.99</td>
<td>Malignant</td>
<td>pT1 seminoma</td>
<td>Radical Rare spz²</td>
<td>Pos</td>
<td>Yes</td>
<td>No⁴</td>
<td>Neg</td>
<td>(5)</td>
</tr>
<tr>
<td>4</td>
<td>34</td>
<td>14.3</td>
<td>Positive</td>
<td>Neg</td>
<td>Neg</td>
<td>Mixed</td>
<td>19.43</td>
<td>10.2</td>
<td>5.9</td>
<td>Malignant</td>
<td>pT1 seminoma</td>
<td>Radical Rare spz²</td>
<td>Pos</td>
<td>Yes</td>
<td>One ICSI cycle, embryo transfer, failed pregnancy</td>
<td>Neg</td>
<td>(53)</td>
</tr>
</tbody>
</table>

Spz, spermatozoa; p, patient; cryo, sample cryopreservation; onco FU, oncological follow-up; positive clinical examination, presence of palpable testicular nodule; Neg, chest X-ray, absence of pulmonary nodules; Neg, markers, normal levels of alpha-FP, lactate dehydrogenase and human gonadotropin; onco FU neg, absence of progression.

²Rare means one spermatozoon every two to three magnification fields × 400.

³Patient choice.

⁴Recent surgery.
Some criticism has already been expressed with respect to this method, owing to the possible transmission of altered genetic inheritance (Kohn et al., 2001). To this date, however, no definite data exist with regard to the transmissibility of testicular tumour from father to son. In any case, even during routine pre-operative semen cryopreservation, it is not possible to avoid that a part of the sperm come from the cancerous testicle.

Conclusions

TESE during orchiectomy for testicular cancer in patients with azoospermia is a feasible procedure that saves patients from having to undergo further surgery. All azoospermic patients who need to have scrotal explorative surgery should be offered the possibility of intraoperative sperm retrieval within the same operation.

References


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