CASE REPORT

Testicular sperm extraction in a patient with metachronous bilateral testicular cancer

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A new indication for testicular tissue cryopreservation is demonstrated in a patient with metachronous bilateral testicular tumours and azoospermia. At the age of 18 (1982) the patient underwent left orchidectomy and radical retroperitoneal lymphadenectomy for a testicular teratoma (pT1N0M0). Semen samples were not cryopreserved because of absence of motile spermatozoa after thawing. Seventeen years after the primary testicular cancer, a seminoma of the contralateral right testis was diagnosed (pT1N0M0). Since the patient was azoospermic, no semen samples could be cryopreserved. However, spermatozoa were detected in testicular biopsy material of the right testis and were cryopreserved for ICSI. Since all spermatozoa were dead after thawing, testicular sperm extraction (TESE) was performed in the remaining tissue samples at the time of ICSI treatment. Only spermatids could be extracted from frozen–thawed samples due to the inhomogeneous distribution of spermatogenic activity in the testicular tissue. Although one oocyte was fertilized with these spermatids, a clinical pregnancy was not achieved. Despite the disappointing results of ICSI in the couple presented here, this case report demonstrates that cryopreservation of testicular tissue and TESE should be considered in patients with bilateral testicular tumours and azoospermia, if frozen semen samples are not available.

Key words: azoospermia/seminoma/teratoma/TESE/testicular cancer

Introduction

The incidence of germ cell testicular tumours is increasing worldwide with the highest rate in men between 20 and 40 years (Giwercman and Skakkebaek, 1992). Non-seminomas were most frequently found in the age group 10–34 years, whereas seminomas were the predominant testicular tumours in older men (35–59 years) (Moller, 1993). In Germany, only 11.8% of urologic tumours are testicular, with 53.6% seminomas and 46.6% non-seminomas (Fischer et al., 1998). However, testicular cancer is the most common malignancy in young men. Since medical treatment has been improved, maintenance of reproductive health is extremely important to these patients. Therefore, semen cryopreservation prior to therapy is generally recommended.

Carcinoma in situ of the contralateral testis has been reported in 5–6% of patients with primary germ cell tumours (Herr and Sheinfeld, 1997). Van Basten et al. (1997) demonstrated contralateral testicular tumours in 3% of 365 patients with non-seminoma testicular tumours (Van Basten et al., 1997). This risk was 60-fold the expected incidence rate of testicular tumours. Other authors discussed 500–1000-fold increased risks for patients with one testicular cancer to develop a contralateral testicular neoplasm (Coogan et al., 1998; Sonneveld et al., 1998). Schreiber et al. (1987) and Sonneveld et al. (1998) reported 412 and 445 men with unilateral testicular cancer, of whom 4.3 and 3.6% suffered from a second primary germ cell tumour (Schreiber et al., 1987; Sonneveld et al., 1998). Consecutive development of bilateral testicular tumours is more frequently observed than are synchronous bilateral testicular cancers (Ondrus et al., 1993). Approximately 80% of bilateral testicular cancers develop metachronously (Coogan et al., 1998). Consecutive tumours were found 0.5–18 years after diagnosis of the primary germ cell cancer with highly varying histological classifications (Patel et al., 1990; Ondrus et al., 1993; Sonneveld et al., 1998). Testicular cancer is known to impair semen quality significantly (Botchan et al., 1997), but even bilateral testicular tumours do not necessarily result in total loss of fertility.
(Heidenreich et al., 1997). However, Kliesch et al. (1997) reported azoospermia in four of 14 patients with testicular cancer and contralateral carcinoma in situ or bilateral testicular malignancies (Kliesch et al., 1997). In two azoospermic men they performed testicular sperm extraction (TESE) without finding spermatids. On the other hand, Novero et al. (1996) achieved successful fertilization of oocytes and a pregnancy after intracytoplasmic injection of spermatozoa extracted from testicular tissue samples with seminoma (Novero et al., 1996). Scher et al. (1999) retrieved spermatozoa from a solitary testis at the time of radical orchidectomy for cancer (Scher et al., 1999). Recently, successful sperm extraction from the epididymis and vas deferens at orchiectomy for testis cancer was described in three patients with azoospermia (Baniel and Sella, 2001).

The present case report demonstrates that both testicular spermatozoa and spermatids could be obtained from a man with azoospermia and consecutive testicular cancers. Therefore this procedure is an option for selected patients with testicular tumours.

Case report

Medical history

At the age of 18 the patient underwent orchidectomy and radical retroperitoneal lymphadenectomy after diagnosis of a left-sided testicular teratoma (pT1N0M0). In 1982 his ejaculate was examined for cryopreservation (volume: 1.4 ml; sperm left-sided testicular teratoma (pT1N0M0). In 1982 his ejaculate was examined for cryopreservation (volume: 1.4 ml; sperm concentration: 14.2×10^6/ml; total motility: 35%). Two years later no motile spermatozoa were detectable in the patient’s cryosamples after thawing. Since ICSI had not yet been established, the ejaculate was no longer cryopreserved. Between 1982 and 1996 no further semen analyses were performed. Seventeen years after the primary testicular cancer, a tumour of the contralateral testis was diagnosed by ultrasonography. Intra-operative pathological examination of the tumour revealed a seminoma (pT1N0M0).

Therefore orchidectomy of the right testis was performed. Prior to surgery the patient was asked to produce an ejaculate for cryopreservation.

Physical examination, semen analysis and hormones

The volume of the right testis was 25 ml with a central induration. Ultrasonography showed a 32×20 mm hypodense region. Body configuration, body hair distribution and examination of penis and breasts were without pathological findings. Standard semen analysis according to the World Health Organization (World Health Organization, 1999) was performed twice with the following results: volume: 0.7–0.8 ml; pH: 7.5–7.7; fructose: 9.4 μmol/ejaculate (reference values: ≥2.0 ml, ≥pH 7.2, ≥13 μmol/ejaculate respectively). No spermatozoa were found in the post-ejaculatory urine and both ejaculates, either in the native specimen or after centrifugation (800 g, 10 min).

Microbiological examination of the ejaculate did not reveal growth of pathological bacteria. The hormone profile was as follows (normal): FSH: 44.0 mU/ml (1.2–10.0 mU/ml); LH: 14.3 mU/ml (0.8–9.0 mU/ml); testosterone: 2.11 ng/ml (2.2–9.2 ng/ml); sex hormone-binding globulin (SHBG): 38.0 nmol/l (11–71 nmol/l); prolactin: 11.2 ng/ml (1–15 ng/ml).

Removal of testicular tissue

Immediately after orchidectomy (malignancy was proven by frozen section in the tumour biopsy), the solid tumour (2×3×2 cm) was totally resected including a rim of 0.5–1 cm tissue surrounding the tumour. In the remaining tissue adjacent to the tunica albuginea, six biopsies (about 1 ml each) were cut off from the tunica and divided into three parts. In order to exclude seminoma or carcinoma in situ, one part of each biopsy was examined immunohistochemically (placental alkaline phosphatase) without pathological findings. The remaining 12 biopsies were transferred to the andrology laboratory for cryopreservation. One biopsy was fixed in Bouin solution; multiple paraffin sections were exclusively examined for spermatogenic cells.

Testicular sperm extraction

Eleven samples of testicular tissue unrelated to the tumour were suspended in Ham’s F10 medium and mixed with equal volumes of cryoprotectant (SpermFreeze™, FertiPro N.V., Sint-Martens-Latem, Belgium). This suspension was frozen (5 min to −60°C, 55 min to −120°C) by means of Nicool LM10® (Air Liquide, Wiesbaden, Germany) and stored in liquid nitrogen. After thawing one sample, TESE was performed according to Salzbrunn et al. (Salzbrunn et al., 1996). Briefly, testicular tissue was incubated in Ham’s F-10 medium at 37°C; after 2 h 0.8 mg collagenase (Sigma, Deisenhofen, Germany) were added. After further incubation for 2 h at 37°C, the cell suspension was centrifuged (800 g, 10 min). The supernatant was discarded and the pellet resuspended in Ham’s F-10 medium. The cell suspension was examined for testicular spermatozoa in the Neubauer chamber. Four tissue samples were thawed consecutively, but no spermatozoa were found. This was due to the inhomogeneous distribution of tubules with intact spermatogenesis, as also shown by testicular histology (Figure 1).

However, spermatozoa were extracted from the fifth sample. All spermatozoa were immotile. At the time of two ICSI cycles, the remaining testicular tissue samples were thawed and prepared for TESE. Only late spermatids were found in these samples.

Intracytoplasmic sperm injection

Follicular stimulation was induced by nafarelin acetate combined with recombinant hFSH. Ovarian response was monitored by transvaginal ultrasound and determination of oestradiol serum concentrations. The technique for oocyte retrieval, ICSI and embryo transfer was described in detail by Montag et al. (1997). Two ICSI cycles were performed. The first time, 18 oocytes were retrieved; ICSI with spermatids was performed in 10 mature oocytes without fertilization. The second time, 11 mature oocytes were found; ICSI was performed in all oocytes. However, fertilization was observed only in one oocyte followed by transfer of an 8-cell stage embryo grade B at day 2 after ICSI. Implantation and clinical pregnancy did not occur.
Figure 1. Histology of a testicular tissue sample from the right testis. Peripheral parts of the right testis, apparently unrelated to the testicular tumour, were cut into 11 pieces for histological examination and cryopreservation. Low magnification (×400) shows inhomogeneous distribution of tubuli with spermatogenesis (a, arrow) which, however, is reduced qualitatively and quantitatively (b, arrows, original magnification: ×400).

Discussion

The present case report demonstrates critical aspects of the TESE procedure in a patient with metachronous bilateral testicular cancer. It also reflects how ICSI has changed the criteria for semen cryopreservation.

Normal pregnancies have been achieved with frozen–thawed ejaculated spermatozoa from patients with testicular tumours and reduced semen quality (Chan et al., 1996). Before ICSI had been established, ejaculates with low quality were not cryopreserved. Since no motile spermatozoa were detectable in our patient’s cryosamples after thawing in 1984, the ejaculate was no longer cryopreserved. Therefore, no cryopreserved ejaculated spermatozoa were available for ICSI after diagnosis of contralateral testicular cancer.

Successful ICSI has already been reported after TESE in a patient with seminoma and azoospermia. Testicular tissue was obtained after biopsy of the healthy testis and was cryopreserved until ICSI (Res et al., 2000).

Novero et al. (1996) reported fertilization and pregnancy after TESE and ICSI in two patients with incidental findings of testicular seminoma at the time of TESE (Novero et al., 1996).

In contrast, the present case report demonstrates that TESE is also possible in patients with azoospermia and bilateral testicular cancers. Of course, pre-treatment cryopreservation of ejaculated spermatozoa must always be the first option for patients with testicular cancer. However, reduced semen quality is a common phenomenon in these men. More than 10% of patients with unilateral testicular cancer are azoospermic before cytotoxic chemotherapy (Pont and Albrecht, 1997). The rate of azoospermia is even higher in patients with bilateral tumours (Kliesch et al., 1997), although spontaneous pregnancy occurred in a patient with metachronous bilateral testicular germ cell tumour (Heidenreich et al., 1997). In these cases, and for patients with bilateral testicular cancers in whom cryopreserved semen samples are not available, TESE from tissue unrelated to the tumour is a reasonable option.

Several aspects need to be critically addressed. Does the offspring of patients suffering from testicular tumours have a higher genetic risk of the same disease, especially when spermatozoa from the affected testis are used? Although inheritance of testicular cancers in general has not been reported in the literature, it is known that brothers of testicular cancer patients have a 3- to 13-fold increased relative risk of developing testicular neoplasms. The relative risk to fathers of testicular cancer patients ranges between 2 and 4. The prevalence of familial testicular cancers varies between 1.0 and 2.8% (Sonneveld et al., 1999). Inheritance of testicular cancers may also be possible because the chance of occurrence of a second primary testicular cancer is higher than expected by chance alone. However, no data are available that would allow an estimate of the testicular cancer risk in sons from fathers who had spermatozoa extracted from the neoplastic testis for ICSI.

Another problem is the inhomogeneous intratesticular distribution of tubules with intact spermatogenesis in the present case. No spermatozoa were found in the first four testicular tissue samples that had been thawed. Immotile testicular spermatozoa were obtained from only one tissue sample. The remaining biopsies contained only spermatids but no mature testicular spermatozoa.

Discrepancies between the presence of testicular spermatozoa in histological preparations and after TESE have been described previously (Schulze et al., 1999). In the present case only spermatids were found in the testicular tissue samples.
thawed for the ICSI procedure. Although pregnancies have been reported by using spermatids (Antinori et al., 1997; Vanderzwalmen et al., 1997; Sofikitis et al., 1998), fertilization of only one oocyte without implantation was observed in our patient. This may also be due to non-optimal conditions of the cryopreservation procedure. Yamamoto et al. (1999) recommended a mixture of seminal plasma and test yolk buffer as an extender prior to cryopreservation of spermatids and achieved better fertilization rates, cleavage rates and embryonic development.

The present case also raises the question of whether TESE should be performed before or after cryopreservation. Our procedure was according to the ‘cryo-TESE concept’ described by Schulze et al. (1999) with the combination of histology, trial TESE and cryopreservation of testicular tissue for later ICSI treatment (Schulze et al., 1999). Cryopreservation of testicular tissue was suggested to prevent spermatozoa or spermatids from damage during freezing and thawing (Salzbrunn et al., 1996). Since no spermatozoa could be found in the first ‘trial TESE’ of our patient (Schulze et al., 1999), other testicular tissue samples were thawed consecutively. After identification of testicular spermatozoa in the fifth sample, ICSI was recommended. The critical aspect of this ‘trial TESE’ procedure was the lack of information about the quality of the remaining biopsies which were used for ICSI later.

In contrast, Verheyen et al. (1997) have recommended extraction of testicular spermatozoa before cryopreservation (Verheyen et al., 1997). The advantage of this procedure is to obtain data about number and motility of spermatozoa that can be used for ICSI. The present case demonstrates that the procedure by Verheyen et al. (1997) should especially be preferred in patients with obviously inhomogeneous distribution of spermatogenic activity.

Whether cryopreservation of at least one remaining testicular tissue sample should have been suggested to our patient to permit germ cell culture and transplantation in the future has to be discussed critically.

Despite the disappointing results of ICSI in the couple presented here, this case report demonstrates that cryopreservation of testicular tissue and TESE should be considered in patients with bilateral testicular tumours and non-obstructive azoospermia, if frozen semen samples are not available. However, offspring of these patients should be followed up carefully.


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References

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