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Fertility of testicular cancer patients after anticancer treatment – experience of 11 years

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Testicular cancer affects men mostly in their reproductive age with a cure rate over 90%. Preserved fertility is one of the main concerns of the survivors. To further elucidate the question of fertility after anticancer treatment for testicular cancer, we performed a survey among patients who underwent sperm cryopreservation procedure in our department. A structured questionnaire was designed to collect data on demography, anticancer treatment, histological type of cancer, family planning intentions and fertility prior to and after treatment. During a period of 11 years 86 men underwent semen cryopreservation before starting chemo- or radiotherapy. Fifty-nine of them consented to participate in the study. The average length of follow up was 4.6 ± 3.8 years. In case of 11.9% of the patients their banked sperm was used, which led to live birth in 57% of the couples. The partners of 6 patients became pregnant after *in vitro* fertilization (IVF) resulting in 4 live births and 2 miscarriages. The spontaneous pregnancy rate was 22%. Spontaneous pregnancy occurred in 13 partners resulting in 18 pregnancies followed by 12 live births, 2 artificial abortions and 4 miscarriages. We could not prove any association between preserved fertility and anticancer treatment or the histological type of the cancer. In conclusion, although spontaneous pregnancy rate is remarkably high after anticancer treatment for testicular cancer, the risk of infertility after receiving gonadotoxic treatment cannot be predicted. Cryopreservation is a safe and effective method to preserve fertility in these cases. As a result we strongly recommend discussing the advantages of semen cryopreservation with all patients awaiting treatment for testicular cancer.

1. Introduction

In Western Europe the number of testicular cancer patients has almost doubled in the past 15 years. The reasons of this increased incidence are still unclear, although prenatal environmental factors are strongly suspected (Huyge et al. 2007). The introduction of cisplatin-based chemotherapy has dramatically improved the survival rate for testicular cancer patients (Bray et al. 2006).

According to the literature the majority of men suffering from testicular cancer are in their reproductive age (19–39 years). Long-term side effects like gonadotoxicity became increasingly important and the question of future fertility is gaining a greater part of their managements (Wallace et al. 2005; Hallak et al. 1999). Cisplatin based chemotherapy results in the majority of male patients in temporary azoospermia, which becomes more often permanent in patients receiving a cumulative dose over 400 mg/m^2 (Howell and Shalet 2005).

Previous studies have shown that the majority of patients with testicular germ cell tumours have reduced sperm counts at the time of diagnosis (before any anticancer therapy), and this will further deteriorate during treatment (Fossa et al. 1989; Meirow and Schenker 1995). The duration and severity of spermatogenic damage cannot be predicted, because it depends on various factors such as the dose and the duration of chemotherapy, testicular function prior to therapy, type of cancer etc. (Fossa et al. 1993; Morrish et al. 1990). Despite an early depression in spermatogenesis, a reasonable number of patients show recovery within 1–2 years after treatment with variable sperm counts in their ejaculates (Howell and Shalet 2005).

The present study was designed to assess information on fertility outcomes among testicular cancer patients (who requested semen cryopreservation at our unit). To follow up the recovered cancer patients is difficult, as different clinical centers are simultaneously taking part in their treatments, oncological and reproductive follow-ups. Consequently the evaluation of the reproductive outcome is a complex task, although collecting data on this field is principal in order to maintain the patients properly informed about the opportunity and advantages of semen cryopreservation. This information could help us to make appropriate decision to proceed or not semen cryopreservation and help to choose the proper reproductive technique for the couple.

As an exploration of future fertility options of young testicular cancer patients we constructed a questionnaire with special reference to fertility status before and after the diagnosis of cancer, and collected data about utilization rate and reproductive outcome. The reports on future fertility of testicular cancer patients are rare and the examined groups are too small. That is why it is so difficult for the clinician to answer the patient's questions

Table 1: Semen parameters of patients with different hystological types of testicular tumor at the time of cryopreservation (after semicastration and before anticancer therapy)

	sample (%)	Mean age	Azoospermic (%)	Normozoospermic (> 15 M/ml sperm cc.)	Oligozoospermic (< 15M/ml sperm cc.)	Total sperm count > 40M
Seminoma	27 (45.8%)	29.2 (22–42)	7 (25.9%)	17 (63%)	3 (11.1%)	15 (55.5%)
Non-seminoma	32 (54.2%)	25.9 (16–39)	5 (15.6%)	18 (56.3%)	9 (28.1%)	16 (50%)

about the recovery time of damaged spermatogenesis, the risk of further need of assisted reproductive techniques (ART). The chance of natural conception is also an under-examined field.
It is also doubtful whether classical semen analysis gives the right information about the status of semen from men with testicular carcinoma. Besides, it is not clear whether the cancer itself is also responsible for inducing changes in the genomic integrity of the spermatozoon.

2. Investigations and results

We retrospectively assessed the database in the University of Debrecen, Hungary, Medical and Health Science Centre, Department of Obstetrics and Gynecology, Andrology and Cryopreservation Laboratory searching for all male testicular cancer (TC) patients who were referred to the institute to cryopreserve semen before starting chemo- or radiotherapy during a period of 11 years, a total number of 86 male patients.
Written informed consent was obtained from all patients after that they were asked to complete our questionnaire. We collected data on age, marital status, family history, type of the surgical procedure, hystological type of the cancer, the type of anticancer treatment and the couples' obstetric history, diseases of the children conceived before and after the diagnosis of TC and whether cryopreserved semen was used. Data were analyzed and matched to the patients' sperm parameters and to the TC histological types. The questionnaires used in our study were previously validated and widely used in the literature (Bosetti et al. 2001).
During the 11-year follow-up 86 patients were referred to our institute for semen cryopreservation after surgery for testicular cancer and before starting chemo- or radiotherapy. The questionnaire was completed and returned by 59 (68.6%) of the 86 patients.
The mean age of responders to our survey was 27 years (range: 16–41 years) at the time of cryopreservation and 32 years (range: 21–47 years) at the time of the study. 71% of the patients were under 30 years at the time of the study. Testicular cancer affected the left testis in 31 patients, the right testis in 25 patients and 3 patients (5%) had bilateral testicular cancer. Family history was positive for testicular cancer in 4 cases (6.7%). The histological findings were divided into two groups according to the type of cancer: seminoma and non-seminoma cancer (for other hystological types). The data of the two groups according to the cryopreserved semen parameters are presented in Table 1.
The average age in the non-seminoma group was significantly lower ($p = 0.05$), while the rate of azoospermic patients was significantly higher in the seminoma group ($p < 0.05$). Values under the reference range (oligozoospermia) were more characteristic to non-seminoma patients. Total sperm count was higher than the reference range in half of the patients in both groups. After orchydectomy 33 of the 59 patients received chemotherapy, 23 underwent radiotherapy and 3 patients received both treatment modalities (Table 2).
Retroperitoneal lymph node dissection can impair fertility by causing failure of emission, retrograde ejaculation or both. In our study group retroperitoneal lymph node dissection was necessary in 19 (32.2%) cases, among whom only one patient (1/19, 5.3%) developed retrograde ejaculation.
At the time of cryopreservation 44%, at the time of study 59% of our patients were married or in a stable relationship. Before the diagnosis of testicular cancer 22 patients had reproductive plans which resulted in 13 cases of pregnancy, the infertility rate was 41%. At the time of the study 31 couples had reproductive plans, 13 natural and 8 assisted conceptions occurred. At the time of the study 58% of the couples were infertile. According to literature the prevalence of infertility in the normal population is about 10% (Gnoth et al. 2005). We detected 18% elevation in the infertility rate after anticancer treatment (Table 3).
Thirteen testicular cancer patients (22%) reported natural conception with a mean of 4.4 years after treatment. The total number of pregnancies was 18, out of which 4 ended in miscarriage prior to the 12th week of gestation (22.2%). One of these was a twin pregnancy. There were two unwanted pregnancies which were terminated for social reasons. The successful pregnancies resulted in the birth of 8 girls and 4 boys. Those patients who reported spontaneous pregnancies after treatment for testicular cancer had a sperm-counts below 20 M/mL in 7 cases, 15 M/mL in 3 cases and less than 1 M/mL in one case at the time of semen cryopreservation. Two birth cases occurred within 1 year of the completion of anticancer treatment. No congenital malformation was reported (Table 4).
Altogether 7 patients utilized cryopreserved semen sample for *in vitro* fertilisation (IVF) (11.9%). The mean time was 4.1 years. The use of the specimens resulted in six pregnancies, from which 4 resulted in live births (2 girls and 2 boys) and 2 miscarriages during the first trimester. No malformation was reported in these pregnancies after IVF (Table 5).

3. Discussion

The deleterious effects of anticancer treatment modalities are well known. Disruption of fertility can have a devastating effect on patients who have not completed their family planning. Testicular damage can affect the somatic cells of the testis (Sertoli and Leydig cells) as well as the germ cells. Cytotoxic treatment targets rapidly dividing cells and as a result spermatogenesis can be disrupted. Most of the patients undergoing cytostatic chemotherapy become azoospermic approximately 7 to 8 weeks after the beginning of the treatment (Pont and Albrecht 1997).
The commonly used anticancer agents act mostly on germ cells during cell division and thus destroy mainly the rapidly proliferating type B spermatogonia. If all spermatogonial stem cells (type A spermatogonia) survive, spermatogene-

Table 2: Number of patients according to the received anticancer treatment

	Chemotherapy	Irradiation	Both
Seminoma	3	23	1
Non-seminoma	30	0	2
Total	33	23	3

Table 3: Changes in the reproductive plans of our patients with testicular cancer during the follow-up

	Before the diagnosis of testicular cancer	At the time of our study
With reproductive plans	22 (37%)	31 (53%)
achieved pregnancy	13	13
no pregnancy (infertile)	9	18
Without reproductive plans	37 (63%)	28 (47%)

Table 4: Spontaneous pregnancies after diagnosis of testicular cancer in our samples

Nr	Time to pregnancy (years)	Number of pregnancies	Number of abortions	Number of deliveries (boy/girl)	Cryopreserved sperm cc. (M/ml)	Hystological type	Anti-cancer treatment
1	3	1	–	1 girl	30.6	non seminoma	chemoth
2	10	1	–	1 girl	30.6	non seminoma	chemoth
3	4.6	3	1	2 girls	16.6	seminoma	irrad
4	3	1	–	1 girl	39.2	seminoma	irrad
5	8,9	3	2	1 boy	47.5	seminoma	irrad
6	6	1	1	-	18	seminoma	irrad
7	4	1	–	1 girl	7	non seminoma	chemoth
8	1.3	2	–	1 girl, 1 boy	40	seminoma	irrad
9	5	1	–	1 girl	< 1	seminoma	irrad
10	5	1	1	–	15.6	non seminoma	chemoth
11	1	1	–	1 boy	16.5	seminoma	chemoth
12	3	1	1 (twins)	–	14	non seminoma	chemoth
13	3	1	–	1 boy	25	seminoma	irrad

(chemoth: chemotherapy, irrad: irradiation)

sis will presumably recover 12 weeks after treatment. Partial or complete destruction of type A spermatogonia leads to sustained or irreversible loss of sperm cell production. The severity and duration of long-term impairment of spermatogenesis after cytotoxic therapy correlates well with the number of destroyed type A spermatogonial (Howell and Shalet 2005).

The nature and extent of this damage depends on the cancer type, the drug given, the dose received and the age of the patient. Although many features of the treatment could affect fertility, disease itself might cause impaired gonadal function. Azoospermia was found in 9.7–17.3% of males referred for sperm cryopreservation (Lass et al. 2001; Jakab et al. 2010). Severe abnormalities in sperm concentration and lower motility were frequently detected (Blackhall et al. 2002, Brydoy et al. 2012, Fossa et al. 1993, Huyge et al. 2004, Meseguer et al. 2006). The etiology of impaired spermatogenesis in cancer patients is not fully understood, it is thought to be associated with the involvement of the immune system due to damage to sperm DNA. The correlation between sperm pathology and testicular tumours is also known. Impaired quality of sperm production is most likely associated with disturbed differentiation of testicle during the embryonic development of the gonad. Testicular dysgenesis syndrome is manifested by the increased incidence of developmental defects of the genitalia (cryptorchism, hypospadias), spermatogenesis disorders, testicular microcalcification and testicular carcinomas (Skakkebeak et al. 2001; Jacobsen et al. 2000a).

Up to date no strong association between fertility after treatment and factors such as pretreatment sperm concentration or used anticancer agents was found, so infertility or permanent azoospermia after anticancer therapy can not be predicted (Brydoy et al. 2012).

Quality-of-life studies revealed that male factor infertility is one of the most devastating long term side effects of anticancer therapy. Although a majority of survivors father children, many report difficulties in the field of completion after treatment as compared to the general population.

According to the literature the mean time from diagnosis to the birth of the first child after treatment is about 7 years but that varies according to treatment. Assisted reproductive technologies are used in 5–22% of cases (van Casteren et al. 2008; Chra et al. 2009).

As cancer survival has increased dramatically during the last decades, oncologists' focus is on the long-term quality of life. Adequate patient information and the increasing incidence of testicular cancer, which is the most common cancer in men younger than 45 years (Bray et al. 2006; Aitken et al. 2004), results in an increasing demand for semen cryopreservation (Johnson et al. 2013).

Table 5: Utilization of cryopreserved semen samples and pregnancies by ART after anticancer treatment

Nr	Time to pregnancy (years)	Number of pregnancies	Number of abortions	Number of deliveries (boy/girl)	Cryopreserved sperm cc. (M/ml)
1	9	1	–	1 girl	< 1
2	1	1	–	1 boy	39
3	9	1	–	1 girl	6.8
4	4	1	1	–	< 1
5	2	1	1	–	21
6	3	1	–	1 boy	60
7	1	–	–	–	1.4

This study summarizes more than 11 years of experience in sperm cryopreservation in cancer patients. It specifically addressed the issue of sperm usage, the outcome of various treatments and the spontaneous pregnancy rate. Only few publications are available in the literature on fertilization results and spontaneous pregnancies which may be caused by the low cryopreserved sperm utilization rate and the low number of follow-up studies.

In our clinic the number of male cancer patients referred for elective semen cryopreservation extends year by year. Our data showed that the majority of our patients have not used their cryopreserved semen (11.9 % utilization, 8% disposal of frozen semen sample), the reasons being not fully understood. Factors such as disease status after treatment, age, marital status, number of children at the time of diagnosis might influence the patients making reproductive decisions.

In our retrospective study, the mean age of patients was 32 years and 71% of them was younger than 30 years. From the time of cryopreservation to the time of completing the questionnaire the proportion of men living in legal relationship increased by 15%, therefore, utilization rate is expected to increase in the following years. The utilization rates found in the literature ranged from 4.7% up to 12.5% (Blackhall et al. 2002; Meseguer et al. 2006; Botchan et al. 2013). Accordingly the 11.9% rate of frozen-thawed sperm usage in our center is among the highest reported. A large Danish cohort study proved the reduction in offspring sex ratio from 51.3% to 49.2% males (Jacobsen et al. 2000b). Our results are consistent with this hypothesis. The frequency of spontaneous pregnancy after anticancer treatment for testicular cancer was remarkably good in our series (22%).

In one study, 20 of 218 cancer patients were reported to father spontaneous pregnancies (9.17%) (van Casteren et al. 2008). Although the time to conception and the rate of conception are the best measures of fertility in couples seeking parenthood, this approach is hardly helpful in the practical setting because no more than one third of testicular cancer patients express a wish to father a child after completing the treatment (Carpentier and Fortenberry 2010).

In a large study only 7.5% of the patients used their banked sperm, which led to live birth in 49% of the couples. The success rate differs widely between studies, ranging from 33% to 73% (van Casteren et al. 2008). Based on our results the frequency of miscarriage after anticancer treatment did not increase, in addition, no fetal malformation was reported in our series. A multicenter study, so far the largest, proved that fertility decreased from 91% to 67% after anticancer treatment (Huyghe 2004).

In conclusion, although the spontaneous pregnancy rate is remarkably high after anticancer treatment for testicular cancer, cryopreservation is a safe and effective method to preserve fertility. We strongly recommend offering the possibility and discussing the future advantages of semen cryopreservation with every patient awaiting treatment for testicular cancer. Semen can be stored for long time without affecting pregnancy rates. The use of cryopreserved semen provides hope for men undergoing chemotherapy, radiation or radical surgery who once had no chance for future fertility (Anger et al. 2003). According to our findings, more than half of the couples can welcome successful pregnancies.

4. Experimental

The chemotherapy treatment was performed according to the staging of the patients. Good-risk patients mean those patients with cancer at any primary site and with no nonpulmonary visceral metastases and normal tumour markers. Intermediate-risk patients mean those patients with cancer at any primary site and nonpulmonary visceral metastases and normal tumour markers value. Advanced testis cancer is disease of clinical stage 2 or greater.

In good-risk disease the standard treatment consists of three cycles of bleomycin, cisplatin and etoposide (BEP) given over 5 days at intervals of 22 days. (BEP: cysplatin 20 mg/m^2 i.v., etoposide 100 mg/m^2 i.v., bleomycin 30 units i.v.) The overall cure rate after this regimen is > 90%. Bleomycin which is cumulatively toxic can cause fatal pulmonary toxicity. In intermediate-risk disease the treatment accomplishes four cycles of BEP given over 5 days. Cure rates are about 80%. Finally, in poor-risk disease the treatment varies in different departments but a common regimen uses four cycles of BEP given over 5 days, or four cycles of etoposide 75 mg/m^2 i.v, ifosfamide 1.2 mg/m^2 i.v and cisplatin 20 mg/m^2 i.v given over 5 days.

Semen samples of patients with testicular cancer undergoing orchydectomy (referred for semen cryopreservation to the Andrology Laboratory of the Department of Obstetrics and Gynecology Medical and Health Science Center University of Debrecen) were studied before the initiation of the adjuvant radio- or chemotherapy. Semen specimen was collected after a requested abstinence of two to five days. Semen analysis was performed manually according to WHO guidelines and morphology was examined using strict criteria (WHO, 2010).

The statistical analysis was performed with Microsoft Excell and Statistica for Window programs. For correlation analysis we used the Anova program and the "t" probe was performed. We considered the results significant when p was < 0.05.

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