SPERM BANKING AND INFERTILITY TREATMENT IN MEN WITH TESTICULAR CANCER

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ABSTRACT

This study was designed to evaluate the use of sperm frozen before testicular cancer treatment and to analyse results of infertility treatment. A total of 166 male patients were referred for sperm freezing before testicular cancer treatment from 1995 to 2007. Cancer treatment, semen analysis, assisted reproduction techniques, and statistical evaluation were basic interventions. Sperm pathology analysis before and after cancer treatment, survival of patients, and pregnancy after infertility treatment were analysed. Testicular cancer was diagnosed and treated in 226 men from 1995 to 2007. A total of 166 men (73.5 %) decided to freeze their semen before cancer treatment. Seminoma was diagnosed in 87 men (52.4 %), nonseminomatous germ cell tumours (NSGCT) in 79 men (47.6 %). A total of 121 men (72.9 %) were of stage I. Azoospermia was diagnosed in 9 men (5.4 %), semen was cryopreserved in 157 patients. Median sperm concentration was 8.5 mil/ml in the seminoma group and 9.4 mil/ml in the NSGCT group – a non-significant difference. Until now, 5 patients (2.9 %) have died and 27 patients (17.2 %) have attended for infertility treatment. Median fresh sperm concentration was 4.4 mil/ml, median progressive sperm motility 3.7 %, azoospermia was found in 4 men (14.8 %). The confounding female infertility factor was found in 19 (70.4 %) female partners. Cryopreserved semen was used in 25 couples (92.6 %), intracytoplasmic sperm injection (ICSI) was the most effective procedure – 36 cycles resulted in 12 pregnancies and 9 deliveries. Testicular cancer survivors have a good chance of fathering a child by using cryopreserved sperm and ICSI procedure.

INTRODUCTION

Damage to reproductive function is a very frequent and well-documented side effect associated with the treatment

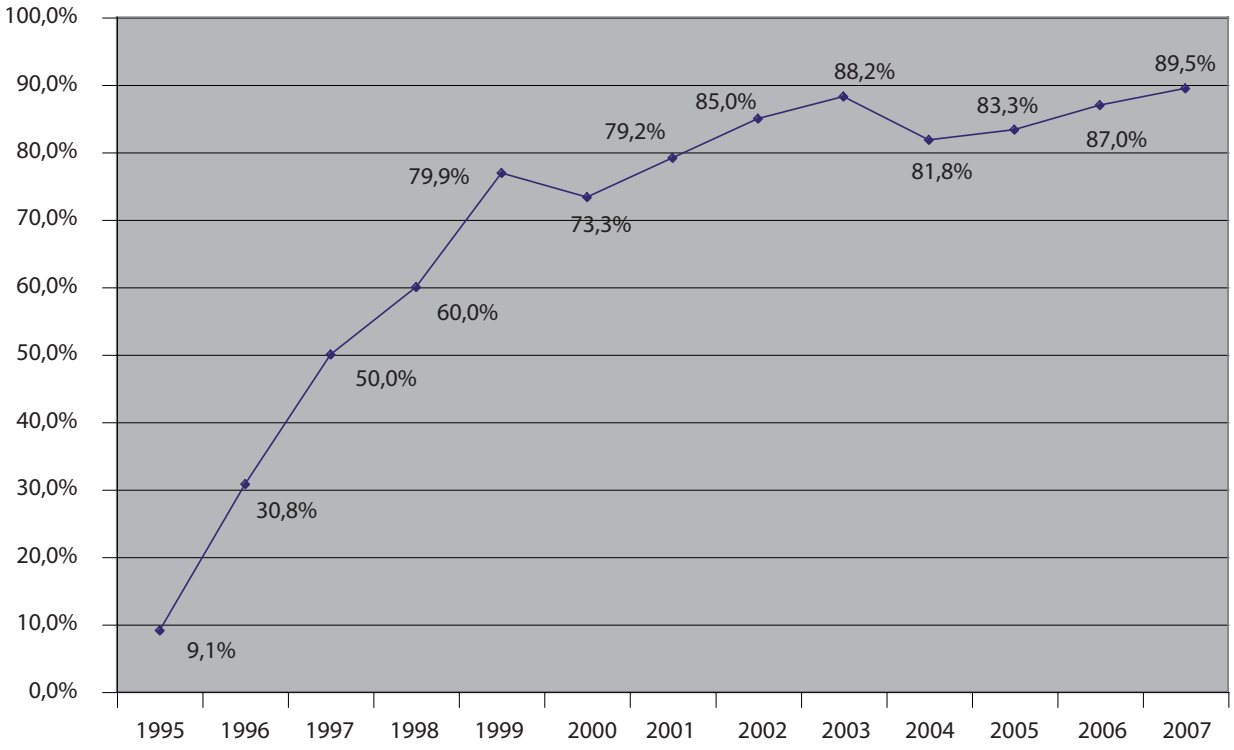

Figure 1
Sperm cryopreservation (%) in men with testicular cancer

of malignant testicular tumours. The first work describing chemotherapy-induced azoospermia was published in 1948 [1]. Variation in sperm quality in relation to the type of malignant tumour was also investigated [2]. The increasing success of testicular cancer treatment and determined efforts to improve the quality of life after successful treatment have turned attention to the preservation of reproductive function in young males [3]. The development of assisted reproduction techniques has brought about effective qualitative changes in this field [4, 5]. The collection, freezing, and long-term storage of sperm are currently considered to be the most effective method. The Assisted Reproduction Centre of the Department of Gynaecology and Obstetrics in collaboration with the Department of Urology launched their programme of sperm freezing and long-term storage before testicular cancer treatment in 1995.

PURPOSE

The objective of this study is to analyse the sperm counts of cancer patients and a possible correlation between sperm pathology and cancer diagnosis, and to make an overview of using frozen sperm during the 12 years of sperm banking.

MATERIAL AND METHODS

Between October 1995 and the end of December 2007 young men with testicular cancer were referred from the Department of Urology to the Assisted Reproduction Centre for sperm cryopreservation prior to treatment for testicular cancer. An obligatory examination of HIV 1, HIV 2, HBsAg, anti-HCV, and anti-HBC before storage of cryopreserved semen was introduced in 2003. Sperm counts were evaluated according to the respective WHO (World Health Organisation) laboratory manual [6] using the Neubauer counting chamber. Commercial media, Medi-Cult (Jyllinge, Denmark) or Vitrolife (Kungsbacka, Sweden) were used. The semen was mixed with a cryopreservation medium and placed in 2 ml Nunclon CryoTubes (Roskilde, Denmark) and frozen. Cryopreservation technology and the procedures used in the storage of frozen sperm samples were aimed at minimising the potential risks, including mistaken identity and transmission of infection. Sperm samples were frozen in a programmable Planer Kryo F10 (Sunbury-on-Thames, United Kingdom) instrument using a standard cooling curve. Samples from 1–3 collections before starting cancer treatment were frozen. The cryotubes were stored in liquid nitrogen at a temperature of –196 °C in

Table 1
Characteristics of patients referred for semen cryopreservation (n=166). NSGCT = nonseminomatous germ cell tumours

Age (years)	Mean 27.6	SD±4.4	Range 15 - 45	Median 27
Histological diagnosis	Seminoma: 87 men (52.4 %)	NSGCT: 79 men (47.6 %).		
Stage	I: 121 men (72.9 %),	II: 29 men (17.5 %)	III: 10 men (6.1%)	
	IV: 1 man (0.6 %)	Unknown: 5 men (2.9 %)		

Table 2
Semen analysis values before cryopreservation (n=166). (NSGCT = nonseminomatous germ cell tumours, NS = statistically non-significant)

	Seminoma (n=87)	NSGCT (n=79)	
Sperm concentration (10^6/ml)			
Mean	17,2	19,7	NS
Median	8,5	9,4	
Range	0 - 122	0 - 108	
S.D.	21,4	26,3	
Total sperm count ($10^{6)}$			
Mean	37	43	NS
Median	18,3	19,2	
Range	0 - 414	0 - 522	
S.D.	375	482	
Progressive sperm motility (%)*			
Mean	9,8	12,3	NS
Median	5,2	5,5	
Range	0 - 60	0 - 65	
S.D.	12,9	11,3	

* Nine patients with azoospermia were excluded from these analyses.

Table 3
Clinical results of infertility treatment with cryopreserved semen (n=25 couples).
(ICSI = intracytoplasmic sperm injection, IUI = intrauterine insemination, ET = embryotransfer, N.A. = not applicable)

	No. of couples	No. of cycles	No. of retrieved oocytes	Fertilisation rate	No. of transferred embryos/ET	No. of clinical pregnancies	Pregnancy rate (%)	Deliveries
ICSI	23	36	8.5±3.2	68,70%	1.9±0.3	12	33,3	9
IUI	2	5	N.A.	N.A.	N.A.	1	20,0	1

Table 4
Obligatory examinations before semen cryopreservation and storage according to Act No. 296/2008 (Czech Republic)

anti-HIV-1	Human immunodeficiency virus 1 antibody
anti-HIV-2	Human immunodeficiency virus 2 antibody
HBsAg	Hepatitis B surface antigen
anti-HBc	Hepatitis B core antibody
anti-HCV	Hepatitis C virus antibody
TPHA	Treponema pallidum haemagglutination

an LS 4800 container Tailor-Wharton HARSCO (Husum, Germany), with an indicator of the surface level and an alarm.
The assisted reproduction methods used complied with the respective standards of the centre [7]. The diagnoses and deceases were verified in the database of the National Oncological Register of the catchment area, in compliance with personal data protection. The Faculty Hospital Brno Ethics Committee's approval was obtained.
The study group was described using the basic descriptive statistics, where categorical variables were characterised using the percentage representations of individual categories, while continuous variables (age, sperm concentration and motility) were described using the mean, the median, the range of values (minimum and maximum), and standard deviations.
Statistical testing was used to confirm the hypothesis of whether or not the results of sperm counts correlated with the patient's diagnosis. The differences among groups of patients were tested using the Kruskal-Wallis test. When the influence of the diagnosis on the sperm count was significant, partial hypotheses were tested to see which particular diagnoses differed by their values (i.e. multiple comparisons of mean ranks). The critical limit for the level of significance was set to $p = 0.05$.

RESULTS

Testicular cancer was diagnosed and treated in 226 men (age 27.6±6.4) from 1995 to 2007. A total of 166 men (73.5 %) decided to freeze their semen before cancer treatment. The number of men asking for semen cryopreservation increased gradually from 9.1 % in 1995 to 89.5 % in 2007 (Figure 1). Seminoma was diagnosed in 87 men (52.4 %), nonseminomatous germ cell tumours (NSGCT) in 79 men (47.6 %). Patient characteristics including the stage of the tumour are given in Table 1. Azoospermia was diagnosed in 9 men (5.4 %), thus semen was cryopreserved in 157 patients. Sperm concentration was 17.2±21.4 (median 8.5) mil/ml in the seminoma group and 19.7±26.3 (median 9.4) mil/ml in the NSGCT group – a non-significant difference (Table 2). Statistical analysis did not find any significant difference of sperm count in relation to the stage of the cancer.
Until now, 5 patients (2.9 %) died and 27 testicular cancer survivors (17.2 %) with semen cryopreserved prior to cancer treatment have undergone infertility treatment. The interval between cryopreservation and infertility treatment ranged from 7 to 70 months (mean 22.2±14.7, median 18 months). In these men the mean fresh sperm concentration was 8.4±16.8 (median 4.4) mil/ml, progressive sperm motility 4.2±17.7

(median 3.7) %, azoospermia was found in 4 men (14.8 %). As these examinations were done after different time intervals following cancer treatment and after different chemotherapy protocols, the data were not sufficient for reliable statistical analysis.

A confounding female infertility factor was found in 19 (70.4 %) female partners. Cryopreserved samples were used in 25 couples (5 cycles of intrauterine insemination, 36 ICSI cycles). Clinical data and results of infertility treatment are given in Table 3. Fresh semen was used in 2 cases for 6 ICSI cycles and one clinical pregnancy resulted in spontaneous abortion. After failure of two ICSI cycles 3 couples (11.1 % of men coming for infertility treatment after sperm cryopreservation) decided to use intrauterine insemination with donor sperm, 5 cycles resulted in 2 pregnancies and deliveries.

DISCUSSION

The increasing frequency of testicular cancer in our study corresponds to the increasing incidence of this tumour in the Czech Republic. In 1977 the incidence of testicular cancer was 3.2/100 000 men, in 1995 it increased to 6.5, and in 2005 to 8.8/100 000 men. Approximately 68 % of them were in the range from 20 to 39 years. Advances in the diagnostics and management of this cancer and progress in its treatment contributed to a significant improvement in the survival rate of this very common malignancy of young men [8]. Stage I tumours were only 5 % in 1977 and improved gradually to 50 % in 1995 and 55 % in 2005. Similar findings are also reported by the Bourn Hall Clinic [9] and other clinics [10, 11].

Azoospermia was found in 5.4 % of the males referred for sperm cryopreservation, as compared to Lass et al. [9], who reported 17.3 %, and Kelleher et al. [12], who detected only 3.3 %. Severe abnormalities in sperm concentration and progressive motility were detected in both seminoma and NS-GCT groups, similar to other studies [13, 14].

The aetiology of impaired spermatogenesis in testicular cancer patients is not fully understood. Damage to the DNA of the sperm due to malignancy was confirmed [15]. However, the extent of damage to spermatogenesis does not correlate with the severity of the cancer [16]. The correlation between sperm pathology and testicular tumours is also known [17]. The impaired quality of sperm production is probably associated with disturbed differentiation of the testicle during the embryonic development of the gonad – the Testicular Dysgenesis Syndrome hypothesis [18]. This syndrome is manifested by the increased incidence of developmental defects of the genital (cryptorchism, hypospadias), spermatogenesis disorders, and testicular carcinomas. Testicular dysgenesis is caused by alteration in the development of the testicle by factors affecting endocrine regulation ("endocrine disruptors"). As spermatogenesis disorders correlate well with testicular carcinoma, close urological examination of men with severe sperm abnormalities is of importance [17]. When analysing impaired spermatogenesis in relation to the type of malignancy, we did not find any significant difference like Agarwal et al. [5].

After the completion of gonadotoxic therapy, the quality of sperm was significantly impaired. The resulting function of the gonad is affected by a number of factors, such as early diagnosis of the malignant disease, the chemotherapy regimens used, and the sperm count as determined prior to the start of therapy [19]. The recovery of spermatogenesis is usually very slow – for example, the mean period of time to achieve the best level of the sperm count is 51 months in patients who completed chemotherapy for leukaemia [20]. In the case of azoospermia, the methods of assisted reproduction based on the surgical collection of sperm provide worse results [21, 22]. Sperm cryopreservation performed prior to cancer therapy is therefore a prerequisite for the successful treatment of subsequent infertility. The main requirement of this programme is to establish a special cryobank to allow safe long-term storage of sperm samples. The operation of such a cryobank requires an exact database of patients and its own records. A proper examination complying with the law of the Czech Republic (Table 4) is a prerequisite of cryopreservation and storage of frozen semen. Another important aspect is that the patients should be given clear and relevant information on the possibilities and conditions of sperm cryopreservation and its use in future.

The fulfilment of this task requires close co-operation between the respective department of urology, the assisted reproduction centre capable of providing this kind of treatment, and the tissue bank. In the Brno Faculty Hospital, this interdisciplinary co-operation is facilitated [23, 24]. Thanks to a strong awareness among urology specialists of the possibilities and easy availability of sperm cryopreservation in our centre, the number of patients referred for this procedure has increased.

In our study 17.2 % of the males have come in for infertility treatment so far. This finding is higher than the data from the literature, for example 7.7 % published by Kelleher [25].

The reasons are not only in the area of patient health, but also in the social area, i.e. patients usually plan to start a family long after they have successfully completed therapy [26]. Another important aspect is that patients are afraid of the increased risk of congenital defects and malignant tumours in their offspring. Many detailed studies which have investigated this risk [3, 16, 27] have failed to prove its increase [28, 29]. Most males from our group who came in for infertility treatment had undergone successful treatment for testicular cancer and usually came 18 months after sperm cryopreservation.

Intrauterine insemination was performed in our clinic much less frequently (17.0 %) as compared to Schmidt et al. [29]; ICSI was used in 83.0 % of the treatment cycles.

The mortality rate of our group of patients was also analysed. According to the data obtained from the Oncological Register, only 2.9 % of males referred for sperm cryopreservation died, which corresponds to the total survival rate in patients with the early stage of testicular seminoma, which exceeds 95 % (30).

CONCLUSION

Sperm cryopreservation prior to gonadotoxic therapy is the basic method used to preserve reproductive potential for the survivors of cancer treatment. Cancer patient sperm banking programmes require close co-operation between the respective oncology department, the assisted reproduction centre, and the tissue bank. Sperm cryopreservation should be offered to every patient before therapy that could damage spermatogenesis.

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