Fertility preservation in males with cancer: 16-year monocentric experience of sperm banking and post-thaw reproductive outcomes

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Abstract

Background: Anticancer treatments can impair male fertility. Cryopreservation of semen is an efficient procedure for fertility preservation. The aim of this study was to evaluate pre-freeze semen parameters among the various types of cancer, post-thaw sperm viability and reproductive outcome of samples used for assisted reproductive treatment (ART).

Methods: This study included 721 men with cancer that had their semen cryopreserved in our bank in 1999–2015. Semen analysis and cryopreservation were performed before the start of antineoplastic treatment, according to the World Health Organization recommendations, European Commission and Italian law.

Results: Among the 721 patient, 196 had seminoma of the testis, 173 Hodgkin's lymphoma, 108 mixed testicular tumors, 89 germ cell tumors, 67 other tumors, 46 hematological tumors, and 42 non-Hodgkin's lymphoma. The mean age of patients was significantly lower in Hodgkin's lymphoma compared to other tumors. Statistically significant lower volume, sperm count and number of straws stored were observed respectively in Hodgkin's lymphoma, mixed testicular tumor and hematological tumors. Nineteen patients used their frozen semen for 20 ART cycles. After thawing a significant reduction of motility and vitality was recorded. A lower fertilization rate was observed in patients affected by testicular tumor and lymphoma (35.42% and 50%) compared with other cancers (71.43%). No significant differences were observed in terms of cleavage and implantation rates. A total of five pregnancies and seven healthy newborns were achieved.

Conclusions: Fertility preservation before gonadotoxic therapy is of great importance to patients with cancer and must be indicated before the start of treatment.

Keywords: cancer, assisted reproductive technology, cryopreservation, sperm bank, fertility preservation, IVF outcome with frozen semen

Introduction

Cancer incidence in young men has been increasing: about 6500 men aged 15–39 years are diagnosed with cancer every year in Italy. In particular, malignant testicular neoplasias represent 16% of all new cancer diagnoses (incidence of 10.2/100,000 inhabitants), followed by Hodgkin lymphomas and non-Hodgkin lymphomas (respective incidence of 5.0/100,000 and 6.2/100,000) [AIOM-AIRTUM, 2014]. New and more effective therapies have led to an increase in the number of

cancer survivors. The overall survival rate of patients treated for many types of cancer has improved from 50% in the 1970s to nearly 70% in the 1990s, and the actual cure rates for patients with testicular cancer and lymphoma can be as high as 90% [American Cancer Society, 2012].

Unfortunately, anticancer treatments (i.e. chemotherapy, radiotherapy or surgical removal) can damage the germinal epithelium in men resulting in oligozoospermia or azoospermia.

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Sperm cryopreservation before gonadotoxic therapies is the most valuable and frequently used method to preserve reproductive function in men undergoing cancer treatments such as chemotherapy or radiotherapy. If there is an urgent need to start cancer therapy, fewer semen samples can be cryopreserved, and this can also be the case in the presence of impairments of semen parameters. In fact, single intracytoplasmic sperm injection (ICSI) is now commonly used in assisted reproduction, thus allowing the successful use of samples with few spermatozoa.

In this 16-year retrospective study we evaluated the data on male cancer patients referred for sperm cryopreservation at our institution with the aim to evaluate pre-freeze semen parameters among the various types of cancer, post-thaw sperm viability and reproductive outcome of samples used for assisted reproductive treatment (ART).

Patient population and methods

From June 1999 and December 2015, 778 men affected by cancer were referred to the IVF Unit and Gametes Bank of University Hospital of Bari (Italy) for sperm cryopreservation. Patients were included before the start of antineoplastic treatment.

The type of cancer was determined by oncologist's letter and histological diagnosis. During a consultation with a physician it was explained the adverse effects of cancer treatment on spermatogenesis and the aim of cryopreservation.

The management of donation, manipulation, storage and distribution of gametes was done in accordance with the rules and procedures established by the European Commission (2004/23 CE) and Italian law (D Lgs 6 November 2007, no 191; D Lgs 25 January 2010, no 16 D Lgs 30 May 2012, no 85; Decreto 10 Ottobre 2012, GU no 15 del 18/01/2013). For that reason during the patient counselling, the physician recommended serologic testing for HBsAg, Anti-HBc, Anti-HBe, Anti-HBs, HCV, HIV, VDRL to allow a storage of semen samples in nitrogen tanks dedicated for seropositive or seronegative samples.

Semen analysis was performed manually according to the World Health Organization recommendations [Cooper *et al.*, 2010] and by different trained embryologists, but checked by the same laboratory manager.

The liquefied semen sample was transferred to a sterile, conical centrifuge tube (Falcon DB 15 ml) with a cryoprotectant medium (freezing medium Test Yolk buffer, Irvine Scientific, Santa Ana, CA, USA), added dropwise until a 1:1 sample:medium ratio. The sample-medium mixture was aliquoted in 0.5 ml straws (PET-G sperm straw 0.5 ml sterile CE Criobiosystem, LaigleCedey, France), labelled with name and surname of the patient date of birth and cryopreservation data. Then straws were suspended in vapor-phase nitrogen before being stored in liquid nitrogen.

The number of collections was flexible and adapted to the quality of semen sample and the different situations (time to start antineoplastic treatment).

At the end of the procedure, the embryologist and the physician informed the patient about the sperm parameters and stored straws, and a formal consent was signed by the patient and the physician.

For semen thawing, straws were allowed to thaw at room temperature for 15 min and then cut at the extremity inside a conical tube to allow the sample overflow. Cryoprotectant was removed by centrifugation at 1500g/min per 10 min in Sperm Medium (COOK Medical). Thawed spermatozoa were used for ART [Depalo *et al.* 2009].

Fertilization rate was defined as the percentage of pronucleate oocytes to the number of oocytes injected, and implantation rate as the percentage of implanted embryos to the total of transferred embryos. Pregnancy was confirmed by ultrasound visualization of a gestational sack 4–6 weeks after embryo transfer, and the pregnancy rate was calculated as the percentage of cycles that lead to pregnancy to the number of embryo transfer cycles.

Statistical analysis

In the present study all the data were presented as mean $\pm$ standard deviation. The difference between groups were evaluated using the analysis of variance (ANOVA) test, Mann–Whitney *U* test for nonparametric data; nonparametric data were evaluated with Spearman test.

Mann–Whitney *U* test was used for nonparametric evaluation of the mean differences between independent groups. Dichotomous outcomes

Figure 1. Changes over time in the number of patients referred to our bank for sperm cryopreservation from 1999 to 2015.

were compared by using Fisher's exact or the Chi-square test. In all cases, $p \leq 0.05$ was considered significant.

For the statistical analysis was used software MedCal Statistics® (MedCal Software, Ostend, Belgium).

Results

A total of 778 men with cancer were referred to our bank for fertility preservation. Among them 721 (92.67%) patients had their semen banked successfully, 11 (1.41%) patients were excluded because cancer treatment had been started, 22 (2.83%) were not interested or not able to provide a semen sample and 24 patients (2.96%) were found to be azoospermic and were not included in the study group.

Over the years (1999–2015) there was an increase in number of men that underwent fertility preservation before cancer treatment (Figure 1).

The mean age at the admission was 29.23 ± 7.95 years, 38 patients (5.27%) were 13–17 years of age, 392 patients (50.84%) were 18–30 years old, 233 patients were 31–40 years old (30.22%) and 58 patients were 40 years older (7.52%).

Among the patients that cryopreserved their gametes the most frequent types of cancer were seminoma (196), Hodgkin's lymphoma (173), mixed

testicular tumors (108) and germ cell tumors (89) (Figure 2).

All 721 patients provided at least one semen sample. Patients with mixed testicular tumor had a significant higher number of semen collections (1.99 ± 0.99) than those with germ cell tumors (1.86 ± 1.02), seminoma of the testis (1.84 ± 0.93), Hodgkin's lymphoma (1.64 ± 0.69), hematological tumors (1.63 ± 0.97), other tumors (1.63 ± 1.28) and non-Hodgkin's lymphoma (1.62 ± 0.8 ; $p < 0.05$).

Table 1 shows semen parameters (sperm concentration, normal forms, motility and vitality) for the first ejaculate for the whole study population and according to age and type of cancer.

The mean age of patients was significantly lower in Hodgkin's lymphoma (25.88 ± 7.29) than in hematological tumors (27.91 ± 9.82), mixed testicular tumors (29.11 ± 6.37), seminoma of the testis (30.64 ± 5.39), non-Hodgkin's lymphoma (31.87 ± 10.05) and other tumors (30.7 ± 11.2 ; $p < 0.001$).

According to type of cancer, a statistically significant lower volume was observed in Hodgkin's lymphoma ($p < 0.001$) and sperm count was significantly lower in mixed testicular tumors ($p < 0.05$). No significant difference was observed in the other semen characteristics among the groups.

Figure 2. Cancer types from patients undergoing sperm cryopreservation. The number and percentage of the categories is represented. Other tumors were: sarcoma, prostate cancer, colon cancer, bladder cancer, astrocytoma, lung cancer, kidney tumor, glioma, gastric adenocarcinoma and thyroid cancer.

The overall number of straws stored was 9,517, with a mean number of 13.22 ± 8.17 per patient.

A statistically significant difference was observed in the number of straws stored per type of cancer ($p < 0.05$); the highest number of straws was obtained in mixed testicular tumors (14.68 ± 8.11) and the lowest in hematological tumors (9.76 ± 5.55) compared with the other groups.

Of the 721 cancer patients, 7 (1%) patients decided on disposal of their samples for the following reasons: four got a pregnancy spontaneously and three recovered normal spermatogenesis after 2 years from the end of the therapy. Nineteen patients came to our unit with a fatherhood desire. Of them, nine had already finished cancer therapy at least 24 months before, but obtained an azoospermic result in a post-therapy sperm analysis; for 10 of them, 24 months had not yet passed from the end of chemo/radiotherapy.

The comparison of semen parameters before banking and after thawing is shown in Table 2. As expected, the result of the semen dilution after the addition of cryoprotectant was a significant reduction of spermatozoa concentration (27 million spermatozoa/ml *versus* 10.66 million spermatozoa/ml). A significant reduction of motility a + b + c (44.74% *versus* 26.58%), motility a + b (33.16% *versus* 3.16%) and vitality (60.28% *versus* 35.74%) was recorded after thawing.

In order to normalize sperm number, total motile count was evaluated before freezing and after

thawing, showing a significant reduction (12.08 million spermatozoa/ml *versus* 2.83 million spermatozoa/ml).

Samples from the 19 patients were used for 20 ART cycles, with controlled ovarian hyperstimulations and *in vitro* fertilization (IVF) with or without ICSI. Sixteen out 20 cycles had embryo transfer. The total mean number of oocytes inseminated was 4.73 ± 2.26 .

IVF outcome of the 19 patients, divided in cancer types (testicular tumor, lymphoma and other cancers), is reported in Table 3.

A lower fertilization rate was observed in patients affected by testicular tumors and lymphoma (35.42% and 50%) compared with other tumors (71.43%). No significant difference among the three groups was observed in terms of cleavage and implantation rates.

A total of five pregnancies, three singular and two pairs of twins, was achieved.

The pregnancy rate was 37.5% in testicular tumor group and 33.3% in lymphoma group, and no pregnancy was obtained in the patients affected by other tumors. Seven healthy newborns of two baby boys and five baby girls were achieved after 37 ± 2.28 weeks of gestation with two by spontaneous deliveries and three Caesarian sections, with an average birth weight of $2,465 \pm 582.94$ g. No congenital anomalies were registered at birth.

Table 1. Patients' age and semen parameters at banking stratified by cancer types.

	ST (n = 196)	HL (n = 173)	MT (n = 108)	GCT (n = 89)	OT (n = 67)	HT (n = 46)	NHL (n = 42)	p
Age (years)	30.64 ± 5.39	25.88 ± 7.29	29.11 ± 6.37	27.46 ± 6.66	35.65 ± 11.32	27.91 ± 9.82	31.87 ± 10.05	**
Number of collections	1.84 ± 0.93	1.64 ± 0.69	1.99 ± 0.99	1.86 ± 1.02	1.63 ± 1.28	1.63 ± 0.97	1.62 ± 0.8	*
Paillettes (n)	13.72 ± 8.83	11.78 ± 7.04	14.68 ± 8.11	14 ± 7.13	13.31 ± 7.31	9.76 ± 5.55	13.26 ± 5.57	*
Volume (ml)	3.24 ± 2.05	2.72 ± 1.55	3.26 ± 1.91	3.1 ± 1.57	3.25 ± 1.84	2.12 ± 1.28	2.8 ± 1.74	**
Number of spermatozoa (million/ml)	22.02 ± 24.28	34.41 ± 35.31	16.06 ± 23.09	21.63 ± 24.85	32.37 ± 39.67	48.13 ± 43.47	35.48 ± 34.56	*
Normal forms (%)	24.73 ± 13.77	21.51 ± 12.82	22.4 ± 14.73	23.23 ± 13.8	22.63 ± 12.12	23.33 ± 15.30	25.71 ± 13.88	
Motility a (%)	5.56 ± 7.81	5.80 ± 7.94	4.56 ± 6.66	5.40 ± 8.37	5.81 ± 7.43	5.70 ± 7.81	6.43 ± 9.32	
Motility a + b (%)	31.1 ± 19.74	26.97 ± 18.59	28.26 ± 20.75	31.07 ± 20.73	29.83 ± 17.10	28.41 ± 22.29	28.81 ± 20.40	
Motility a + b + c (%)	46.83 ± 21.65	42.51 ± 20.9	44.66 ± 22.98	48.79 ± 19	44.58 ± 17.65	44.02 ± 25.5	45.07 ± 23.58	
Vitality (%)	68.13 ± 16.56	66.83 ± 17.34	66.49 ± 17.08	70.83 ± 13.58	66.60 ± 16.99	65 ± 20.59	65 ± 18.22	

Demographic characteristics (age, number of paillettes banked, semen parameter) of 642 men that stored their sample, divided in groups.
ST, seminoma of the testis; GCT, germ-cell tumor; MT, mixed testicular tumor; HL, Hodgkin's lymphoma; NHL, non-Hodgkin's lymphoma; HT, hematological tumors; OT, other tumor.
Statistical significance for * $p \leq 0.05$, ** $p \leq 0.001$.

Table 2. Comparison of semen parameters before banking and after thawing.

	Pre-storage	Post-thawing	<i>p</i> value
Number of spermatozoa (million/ml)	27 ± 28.41	10.66 ± 14.37	**
Motility a + b (%)	33.16 ± 20.08	3.16 ± 7.11	**
Total motility count (million/ml)	12.08 ± 21.24	2.83 ± 5.46	**
Vitality (%)	60.28 ± 17.7	35.74 ± 22.86	**
Semen parameters: number of spermatozoa (million/ml), motility a + b (%), total motility count (million/ml) and vitality (%) before banking and after thawing. Statistical analysis was performed with Student's <i>t</i> test and data were considered significant for ** <i>p</i> < 0.001.			

Table 3. Reproductive outcome of 19 patients stratified by cancer type.

	Tumor of testis (<i>n</i> = 8)	Lymphoma (<i>n</i> = 6)	Other (<i>n</i> = 5)	<i>p</i>
Female age (years)	34.75 ± 5.33	35.5 ± 5.35	35.6 ± 3.83	NS
Male age (years)	32.5 ± 6.24	36.5 ± 10.21	34.4 ± 4.03	NS
Duration of storage (years)	3.35 ± 3.24	4.5 ± 3.99	2.6 ± 1.5	NS
Number of oocytes inseminated (<i>n</i>)	6 ± 3.43	4 ± 1.41	4.2 ± 1.94	NS
Number of oocytes fertilized (<i>n</i>)	2.13 ± 2.37	2 ± 1.15	3 ± 2.1	NS
Number of oocytes cleaved (<i>n</i>)	2 ± 2.35	2 ± 1.15	3 ± 2.1	NS
Transferred embryos (<i>n</i>)	1.25 ± 0.66	1.83 ± 1.34	2.2 ± 1.72	NS
Fertilization rate	35.42%	50%	71.43%	NS
Cleavage rate	94.11%	100%	100%	NS
Implantation rate	40%	27.27%	0	NS
Pregnancy rate	37.5%	33.33%	0	NS
Reproductive outcomes of 19 patients who used their banked samples for 20 assisted reproductive cycles, with controlled ovarian hyperstimulations and <i>in vitro</i> fertilization (IVF) with or without intracytoplasmic sperm injection (ICSI). Statistical analysis was performed with Student's <i>t</i> test and data were considered significant for ** <i>p</i> < 0.001.				

Discussion

According to the data of the Italian Cancer Register (AIRTUM) every day 30 male patients younger than 40 years are diagnosed of cancer (365,500 new male cancer patients in 2014-AIRTUM data) [AIOM-AIRTUM, 2014].

Cancer therapy could lead to testicular failure or ejaculatory dysfunction or result in subfertility or sterility due to permanent damage to gonad or to germ cells caused by adjuvant therapy. The risk associated depends on several factors: the age of the patient at the time of treatment, the dose site and the type treatment [Kelleher *et al.* 2001]. These data are supported by Gandini and colleagues [Gandini *et al.* 2006] who investigated short and long chemo/radiotherapy effects on spermatogenesis in 166 patients with testicular cancer and detected a significant reduction of sperm parameters 6 months after the end of treatment.

On the other hand, the survival rate of patients treated for many types of cancer has improved in recent years and the possibility of fertility loss after cancer treatment may lead to psychosocial problems. For these reasons all health care providers have a responsibility to inform patients about the risks that cancer treatment will permanently impair fertility [Loren *et al.* 2013].

Cryopreservation of human spermatozoa, introduced in the 1950s by Bunge and Sherman, became particularly important in cases of preservation of male fertility before radiotherapy or chemotherapy [Bunge *et al.* 1953, 1954].

Semen cryopreservation before cancer therapy is an increasingly common activity; in our study, referrals increased from 5 in 1999–2003 to 88 in 2015. This observation can be related to the incidence of cancer but also to a better awareness of fertility preservation among patients and

oncologists. The low number of patients referred to our unit after having started an antineoplastic treatment indicates good counseling and an optimal network between oncologists and the bank staff.

Moreover, the majority of the patients that stored their samples in our unit were in the age at which the desire for parenthood is strongly present: over 82% of the men were 18–40 years old and 17% included children and teenagers (10–17 years of age) and men older than 40. This data shows that patients of 18–40 years old were more interested in cryopreservation before cancer therapy because this could be their only chance for future paternity.

Previous study reported that some pre-freeze semen parameters were impaired in cancer patients [Bizet *et al.* 2012; van Casteren *et al.* 2010]. Our study confirmed these results showing that semen samples in patients affected by seminoma of the testis were more compromised compared with other groups. In particular, we observed a lower sperm concentration before therapy suggesting that seminoma itself impairs spermatogenesis.

According to Meseguer and colleagues [Meseguer *et al.* 2006] it is important to evaluate the number of straws to freeze per patient in order to allow a good attempt of IVF to achieve pregnancy. For that reason there are two aspects to consider in order to decide the number of samples to store: sperm quality and storage security. Subsequently the number of straws should be higher if semen parameters are abnormal.

The present study noted a lower utilization rate of cryopreserved spermatozoa (2.46%) compared with previous studies that reported utilization rates lower than 5% [Crha *et al.* 2009; Lass *et al.* 2001] or between 5% and 10% [Bizet *et al.* 2012; Agarwal *et al.* 2004; Magelssen *et al.* 2005; Ragni *et al.* 2003; van Casteren *et al.* 2008; Kelleher *et al.* 2001; Muller *et al.* 2016]. Meseguer and colleagues [Meseguer *et al.* 2006] found a utilization rate of 16.3%.

In our practice, the majority of cancer patients were likely to continue sperm storage and paid for keeping their straws in storage, confirming their future reproductive desire.

In our experience only 19 patients out of 721 referred to our unit had a paternity desire. This

dropout is related to a difficult recovery of cancer patients after antineoplastic treatments, the death of the subject, anxiety towards IVF procedures and the uncertainty of long-term prognosis.

All 19 referrals for childbearing had azoospermic results at the follow-up semen analysis and gave consent to use their banked samples to inseminate their partners' oocytes in IVF procedures. In a total of 20 attempts we performed ICSI in 80% of cases and conventional IVF in 15%. ICSI was also the most commonly used technique in a previous follow-up study [Meseguer *et al.* 2006]. The decision on which technique should be used depends on the sperm quality after thawing and it should be evaluated at the time of insemination.

For the majority of cancer patients IVF represents the only opportunity for childbearing after the antineoplastic therapy.

In agreement with data reported in the literature, a significant reduction of sperm motility and vitality was observed after thawing [O'Connell *et al.* 2002]. Recent work showed that the total motile sperm after cryopreservation were 46.4% of the post-wash sample [Oberoi *et al.* 2014]. Lee and colleagues [Lee *et al.* 2012] reported that the freeze–thawing process caused a 66% reduction in rapid–progressive spermatozoa, 45% reduction in slow progressive motile spermatozoa and a 2% reduction in nonprogressive motile sperm. The reasons behind this are that cryopreservation and thawing induce deleterious changes to sperm structure and function due to thermal shock with the formation of intracellular and extracellular ice crystals, cellular dehydration and osmotic shock [Stanic *et al.* 2000]. The intracellular ice crystals thus formed may breach the membranes and affect the functioning of the organelles. This can lead to impaired cell survival and motility by an impairment of mitochondrial activity [O'Connell *et al.* 2002] and a reduction of the fertilization capacity by damage of the acrosomal vesicle [Cross *et al.* 1991].

In our study we evaluated the fertilization rate of the samples thawed stratifying the patients by cancer types and we observed a slightly higher fertilization rate in men from the 'other cancer' group, compared with lymphoma and tumor of the testis.

Molnár and colleagues [Molnár *et al.* 2014] in a follow up of 11 years reported that 86 men underwent semen cryopreservation before starting

chemotherapy or radiotherapy and 11.9% of the patients used their banked sperm, which led to live birth in 57% of the couples (four live births and two abortions).

In 2014, van der Kaaij and colleagues published their data on fertility preservation in 363 patient affected by Hodgkin's lymphoma that cryopreserved semen before the start of potentially gonadotoxic treatment and reported that 48 out of 258 men (19%) who had children after Hodgkin's lymphoma treatment became a father using cryopreserved semen [van der Kaaij *et al.* 2014].

In our study the pregnancy rate was 31.75% and 7 healthy babies were born from 16 couples that had embryo transfer. As already reported in previous studies, semen cryopreservation does not increase the probability of congenital malformation after IVF cycles [Lass *et al.* 2001].

Recently Muller and colleagues [Muller *et al.* 2016] evaluated the effectiveness of ART performed with cryopreserved semen in 898 cancer patients during 30 years of sperm banking and reported that 96 (10.6%) used their frozen sperm for IUI, IVF and ICSI with a cumulative success of 77%; specifically, a total of 94 children (68 singletons and 13 sets of twin) were born.

Our study suggests that discussions about fertility preservation before gonadotoxic therapy are of great importance to patients with cancer and cryopreservation must be indicated before the start of treatment.

Sperm cryopreservation is a simple technique that can be done quickly and also in emergency situations in which the patient has to start the treatment in a very short time. The efficiency of counseling for fertility preservation treatment requires the creation of a multidisciplinary network in which the oncologist, surgical oncologist and reproductive specialist should collaborate in order to guarantee the sperm cryopreservation to all of the cancer patients. Development of local clinical guidelines and the organization of conference to promote the fertility preservation should be encouraged.

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