

Sperm cryopreservation in oncological patients: a 14-year follow-up study

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Objective: Oncologic treatments can destroy spermatogenic dividing cells and cause azoospermia which could be irreversible. Sperm banking is the best option to preserve male fertility after these treatments. It is easy, inexpensive, and safe. To date, few clinical data are available about large series of cancer patients. Our objective was to determine the usefulness of these preventive sperm freezing protocols.

Design: Prospective study.

Setting: University-affiliated private fertility center.

Patient(s): One hundred eighty-six cancer patients who banked sperm samples at our center before surgery or chemo- or radiotherapy treatments from 1991 to 2004.

Intervention(s): Conjugal status, age, type of cancer, treatment, and future use (if any) of the cryopreserved sperm samples for assisted reproduction technology (ART), and cycle results were recorded, analyzed, and compared with a control group.

Main Outcome Measure(s): Basic sperm analysis of semen samples from cancer patients prior to freezing, after thawing, and after capacitation for ART.

Result(s): A total of 320 semen samples were frozen before antineoplastic treatment. Six months later, 27% of the males recovered normal sperm production. From all frozen samples, 8.7% were discarded; the reasons were pregnancy achievement (55%), normal sperm production (28%), and patient death (18%). Finally, 5 IUI cycles and 30 ICSI cycles were done from frozen samples, with 1 and 15 pregnancies, respectively; results were comparable with those obtained in a control group.

Conclusion(s): A significant number of males who cryopreserved semen samples before receiving antitumoral treatments have employed them. The results obtained showed that this is the strategy of choice, aiming to preserve fertility for the future, because the cost/benefit ratio is favorable. Patients should be counseled accordingly. (Fertil Steril® 2006;85:640–5. ©2006 by American Society for Reproductive Medicine.)

Key Words: Cancer, sperm bank, cryopreservation, infertility, chemotherapy, assisted reproduction technology

Multimodal cancer therapies have significantly improved survival rates for young patients suffering from the commonest malignancies within the reproductive age range: testicular cancer, Hodgkin's lymphoma, and leukemia (1).

However, cancer therapies are frequently aggressive and unwanted side effects are common. Chemotherapy and radiotherapy adversely affect spermatogenesis (2) and retroperitoneal lymphadenectomy may impair normal ejaculation (3). These consequences are particularly relevant in young men without offspring.

Harmful effects of chemotherapy on spermatogenesis are variable, depending on the type of chemotherapeutic agents used, their dosage, and treatment length. Moreover, it is not possible to predict with certainty if spermatogenesis will return to normal parameters after the therapy (4).

It has been established that 15%–30% of the males where the cancer has been cured still remain sterile after several months or even years (5). Therefore, sperm banking before starting chemotherapy is highly recommended in young cancer patients (6, 7).

A relevant but variable percentage (13%–30%) of cancer-diagnosed patients wishing to freeze semen samples are already azoospermic when trying to produce ejaculates before treatment (8). Nevertheless, recent data reveal that a high percentage of the azoospermic males before therapy (87%) recover normal production several months after antineoplastic treatment, whereas 12%–13% will never recover functional spermatogenesis (8). The tumor can be causing the involuntary absence of testicular germ division, thus indirectly protecting stem cells from cytotoxic agents.

With the introduction of IVF and intracytoplasmic sperm injection (ICSI) many patients with poor semen characteristics or low sperm survival after a freezing-thawing protocol can father their own genetic children.

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Several years ago, given the poor recovery of frozen samples and the possibilities that IUI offered, many patients were not counseled to leave frozen sperm. Nevertheless, with IVF and ICSI, even the poorest samples are apt to be frozen with high success rates.

Lamentably, for years and even today, few cancer patients are recommended or allowed to bank their semen samples by their oncologist (4).

Several reports support the lack of information about sperm banking and future fertility possibilities in newly diagnosed cancer males, provided from oncologists, because they had a total absence of knowledge about assisted reproduction technology (ART) (9–11), or even because nearly 50% of the patients did not follow the recommendations (12, 13).

Many patients become severely oligozoospermic after treatment in the worst circumstances, but they can be successfully treated by routine IVF/ICSI. Even those becoming azoospermic who did not freeze semen samples before the antitumoral protocols still can decide on initiating a pregnancy by ICSI or testicular sperm extraction (TESE)–ICSI, but couples should be aware of the unknown potential genetic risks and low pregnancy rates of the procedures (14).

Our aim in this study was to describe males following cancer treatments who banked sperm samples for future employment, the use rate, and the results obtained when using these stored samples to determine the usefulness of banking semen before antitumoral treatments.

MATERIALS AND METHODS

Institutional Approval

This project was approved by the Institutional Review Board on the Use of Human Subjects in Research at the Instituto Valenciano de Infertilidad and complies with the Spanish Law of Assisted Reproductive Technologies (35/1988).

Patients

We retrospectively assessed the databases in two of our clinics, Instituto Valenciano de Infertilidad in Valencia and Madrid, Spain, searching for all male cancer patients who were referred to our unit to cryopreserve sperm during the period from January 1991 to October 2004, yielding a total number of 186 men, whose histories were studied. Patients with sperm obtained by the intrusive method were excluded from this work and only the first attempt to produce the ejaculate has been considered.

All patients were counseled by a specialized biologist from the Andrology Laboratory and fully informed about sperm banking (costs, future possibilities, negative effects of the freezing protocols for the samples, etc.) and afterwards provided written consent before freezing.

The recorded parameters were male marriage status, age, sperm features, type of cancer and treatment, length of

sample cryostorage, and the results and type of ART on those cancer males with the previously cryopreserved sperm. Some patients decided to discard cryopreserved samples for various reasons. Our study also examined the causes for disposing sperm specimens and whether sperm quality or patient features are associated with a specific type of cancer or treatment.

All samples were obtained by masturbation after three days of sexual abstinence. After 10–30 min of liquefaction at 37°C with 5% CO₂, semen samples were examined for concentration and motility in a Mackler chamber according to WHO guidelines (1992 and 1999). Semen samples were frozen by dropwise addition of a glycerol-based cryoprotectant with continuous shaking (Sperm Freezing Medium; MediCult, Jyllinge, Denmark) as previously described (15, 16). The sperm freezing protocol has been employed since 1996, when our group reported the first full-term pregnancy achieved with frozen sperm obtained by TESE. To date thousands of live births have been achieved in our facility (17).

Mean, SD and SEM for age, sperm count ($\times 10^6$), motility (%), and volume were calculated using the Statistical Package for Social Science (SPSS, Chicago, IL).

Thawing protocols were performed as previously described (15, 16).

Ovarian stimulation in the assisted reproduction cycles. For ovarian stimulation, both GnRH agonist and antagonist protocols were used. For GnRH agonist, long protocol was employed as previously described (18). GnRH antagonists were used following the low-dose daily protocol (19). Recombinant FSH (Gonal-F; Serono, Valencia, Spain; or Purigon; Organon Española, Spain) and hMG (Lepori; Farma Laboratories, Valencia, Spain; or Menopur; Ferring, Valencia, Spain) were used for ovarian stimulation. Initial doses were determined according to patient's age and basal serum FSH and E₂ levels. On stimulation day 3, serum E₂ level was assessed and gonadotrophin doses adjusted according to a step-up or step-down protocol. The hCG (10,000 UI Profasi; Serono) was administered when three or more follicles reached 18 mm in diameter, and oocyte retrieval was scheduled 36 h later.

ICSI. The microinjection was performed as previously described (20).

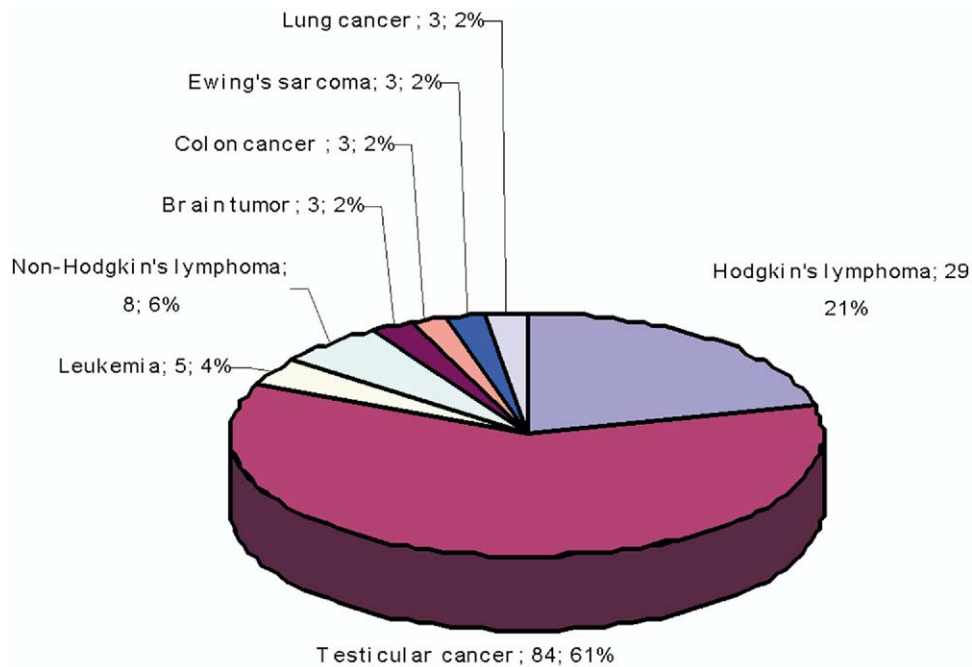
Injected oocytes were incubated in 20- μ L drops, and fertilization was assessed after 18 hours and embryo cleavage 24 hours thereafter. Embryos were transferred into the uterine cavity 48–72 h after ICSI. Clinical pregnancy was determined by observing a gestational sac with fetal heart-beat at seven weeks of pregnancy.

Control Group

We included a control group to compare ART outcomes with our group of oncologic patients. To do that we selected a

FIGURE 1

Cancer types from patients undergoing sperm cryopreservation. The number and percentage of each of the categories is represented.



Meseguer. Cryopreservation in oncologic patients. *Fertil Steril* 2006.

group of females with tubal infertility undergoing the first ICSI cycle with frozen ejaculated sperm. A total of 97 cycles were considered. Male's age and abstinence delay matched our study's group.

Statistical Analysis

Age, sperm count ($\times 10^6$), motility (%), and volume were expressed as mean \pm SEM. Statistical analysis was performed using analysis of variance, and for multiple post hoc comparison DMS and Bonferroni tests were performed. $P < .05$ was considered to be significant. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, Chicago, IL) and MedCalc software (Ghent, Belgium).

RESULTS

A total of 186 patients were referred to our unit. Among them, 184 were able to produce sperm cells (98.9%), and the remaining were diagnosed as azoospermic.

A total of 320 sperm samples were frozen, with a mean number of 1.7 ± 0.5 per patient (range 1–10). The mean age was 27.1 ± 6.4 years old (range 15–58), and we must emphasize that more than half (54.3%) of our patients had no reproductive couple at the time of cryopreservation. After sperm freezing, 17.9% of the patients received only radio-

therapy treatment, 69.5% only chemotherapy treatment, 15.2% both treatments, and 2.5% bone marrow transplantation. Finally, although it is not recommended, 4.0% of the patients had already received some chemotherapy sessions before the sperm freezing. Following freezing, 15.7% of the patients were unilaterally orchidectomized.

The distribution of disease's diagnoses is shown in Figure 1 and included Hodgkin's lymphoma, testicular cancer, leukemia, non-Hodgkin's lymphoma, brain tumor, colon cancer, Ewing's sarcoma, and lung cancer. There were no differences in marital status (single 46.4% vs. 53.6%), mean age (25.9 vs. 27.9 years), and number of sperm specimens banked (1.6 vs. 1.9) between testicular cancer and Hodgkin's lymphoma patients, respectively. Sperm features of our patients depending on cancer type are shown on Table 1; no statistical differences were found between groups.

Sixteen patients decided on disposal of their samples (8.6%), for the following reasons: 55% got a pregnancy spontaneously, 18% died during the study period because of cancer, and 27% recovered normal sperm production. A total of 41 semen analyses were performed at least six months after treatment, with the following results: 30% recovered normal sperm production, 10% were oligozoospermic, 20% presented cryptozoospermia, and 40% were azoospermic.

TABLE 1**Comparison of sperm motility and total sperm count in patients according to cancer type.**

Sperm characteristics	Testicular cancer (n = 112)	Hodgkin's disease (n = 38)	Non-Hodgkin's lymphoma (n = 11)	Leukemia (n = 7)	Other (n = 16)	Total (n = 184)	P
Volume	2.9 ± 0.1	3.5 ± 0.2	3.1 ± 0.6	3.3 ± 0.1	5.7 ± 0.3	3.6 ± 0.2	n.s.
Total sperm count	34.2 ± 3.7	48.9 ± 3.9	30.1 ± 5.0	16.4 ± 2.1	37.0 ± 12.0	32.5 ± 1.9	n.s.
Progressive motility	41.3 ± 1.5	50.5 ± 2.3	50.8 ± 6.5	54.8 ± 5.2	49.2 ± 6.3	46.3 ± 1.1	n.s.
Nonprogressive motility	10.4 ± 0.4	10.4 ± 0.3	9.5 ± 2.37	11.60 ± 2.01	9.6 ± 2.1	10.7 ± 0.4	n.s.

Note: Values are mean ± SEM. $P < .05$ was considered significant by analysis of variance test. n.s. = not significant.

Meseguer. Cryopreservation in oncologic patients. *Fertil Steril* 2006.

Only 30 patients (16.3%) underwent ART, and 35 cycles were performed: 30 ICSI (including 5 frozen embryo transfer) and 5 artificial insemination (AI) (Table 2). A total of 16 pregnancies were achieved (14 by ICSI, 1 by frozen embryo transfer, and 1 by AI) and 12 healthy newborns (with three pregnancies ongoing at time of writing). We compared the data with a control group selected from women with tubal infertility that underwent ICSI treatments with frozen sperm. Statistical data did not reveal any difference between the groups in terms of fertilization, cleavage, and implantation rates. Mean age was comparable in both groups.

Thawed sperm characteristics are presented in Table 3. As observed in ICSI cycles, parameters were similar in both groups.

The average time that the samples were cryopreserved in our banks until used was much longer in our group of oncologic patients compared with the control group. Obviously, the reasons for cryopreservation are completely different in both groups.

DISCUSSION

According to the data of the Spanish National Epidemiology Center (Centro Nacional de Epidemiología) 97,000 new male cancer patients are diagnosed each year in Spain and more than 57,000 people die from cancer. About 800 are diagnosed with testicular cancer and 1,500 with Hodgkin's lymphoma, and a significant percentage of the men are of reproductive age. Amazingly, the number of males banking sperm under these circumstances is extremely low in comparison with the number of newly diagnosed tumors in men younger than 40–45 years of age.

The usefulness of sperm banking before cancer treatment can be explained by either describing the possibilities of becoming sterile after the treatment or analyzing the rate of frozen samples used.

In the majority of the semen analysis, oncologic patients present sperm cells in their ejaculates available for freezing. They are a young population (usually <30 years old). Youthful patients would easily manage to pay for the costs

TABLE 2**ICSI results.**

Procedure	Cancer ICSI	Control ICSI	AI
No. Cycles	30	97	5
Mean age	30.9 ± 2.9	31.6 ± 2.5	30.2 ± 3.1
No. metaphase II oocytes injected	352	1027	—
No. 2-pronuclear oocytes (fertilization rate)	272 (77.2%)	683 (66.5%)	—
No. fertilization failures	0	1	—
No. cleaved embryos	228 (84.1%)	499 (73.3%)	—
No. embryo transfers	30	95	—
Mean embryos/transfer ± SD	2.7 ± 1.1	2.7 ± 1.2	—
No. cryopreserved embryos	35	155	—
No. clinical pregnancies (pregnancy rate)	15 (50.0%)	46 (47.4%)	1
No. live births	12	40	0

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TABLE 3**Post-thaw sperm characteristics.**

Sperm characteristics	Cancer patients		Control patients	
	Pre-thaw	Post-thaw	Pre-thaw	Post-thaw
Fresh volume (mL)	3.1 ± 0.2	1.4 ± 0.2	3.3 ± 0.3	1.4 ± 0.3
Total sperm count (×10 ⁶ /mL)	34.5 ± 1.9	13.4 ± 3.8	37.6 ± 2.1	19.4 ± 4.9
Percent thawed sperm progressive motility	41.3 ± 1.1	7.2 ± 3.1	49.3 ± 2.1	8.9 ± 3.9
Total capacitated sperm count (×10 ⁶ /mL)	—	1.2 ± 0.6	—	1.7 ± 0.9
Percent capacitated progressive motility	—	20.3 ± 6.8	—	22.2 ± 7.9
Length of storage (days)	—	1,022.8 ± 235.7	—	45.7 ± 15.5 ^a

^a *P* < .05.Meseguer. Cryopreservation in oncologic patients. *Fertil Steril* 2006.

of sperm freezing and maintenance because this is probably the last option of future paternity.

It is obvious that patients with children will not consider an azoospermia an important problem, and sperm freezing before chemo- or radiotherapy will not be contemplated. These patients are suggested to bring a sperm sample for a semen analysis 7–8 months after the end of the treatment to compare the results with the semen before freezing. Our aim with this procedure is to counsel the couple about future reproductive options.

Concerning sperm production after the treatment, in our study only 30% of our patients recovered normal levels, 33% presented concentrations below normal parameters, and 37% did not recover spermatogenesis and sperm cells were not found on their ejaculates. Except for the first, the other circumstances would probably need ART cycles to initiate a pregnancy. In the second condition, sperm quality could be worse than pretreatment frozen sperm, and in the last situation TESE and ICSI could be performed but pregnancy expectancies are lower than for other TESE patients (14).

The second way to determine if banking sperm is worthwhile is by analyzing the percentage of the samples that have been afterwards employed in ART as a result of the impossibility to achieve pregnancy.

In our work, we found that approximately 15% of the males needed the sample several years later, and this is even higher than the rates found in the literature, which ranged from 4.7% to 12.5% (6, 12, 13, 21, 22). Up to now, this paper reports the largest number of patients diagnosed with cancer using ICSI treatment with frozen spermatozoa. This is an unequivocal indicator that sperm banking before chemotherapy is the best choice.

Mean number of sperm samples frozen per patient is around two. Two important aspects are considered in order to decide the number of samples stored: sperm quality and storage security. We should freeze enough sperm cells to allow sufficient ART procedures to achieve a pregnancy, and

subsequently this number will be higher if semen quality is low. Where normal sperm production is found, a minimum of two samples must be recommended, trying to be stored in different banks. This would prevent handling accidents (i.e., sample missing) or liquid nitrogen bank breakdown. Sometimes, the first option is not possible because patients had no more days before the antineoplastic treatment and also some of them can not afford, or did not accept, a second sperm freezing.

Cancer-diagnosed men receive chemo- or radiotherapy depending on the cancer type, and only few patients perform sperm freezing after having started an antineoplastic treatment; this is probably caused by the lack of medical-oncologic information or the urgency in the initiation of anticancer treatment.

Keeping in mind the low mean age of this population, testicular cancer and Hodgkin's disease are the most prevalent malignancies, i.e., testicular cancer represents 0.8% (23) of all new neoplasms in men and 64% of our oncologic population. Hodgkin's disease, testicular cancer, leukemia, and non-Hodgkin's lymphoma are the most common malignancies seen among men of reproductive age (Spanish National Center of Epidemiology).

Apparently, there is no difference in the sperm production dependent on the cancer type. Nevertheless we can not discard severe stages of the illnesses in which the general bad health status could be affecting the normal sperm production.

Some of our patients decided to destroy their samples, as reported by others (24). Reasons for disposal of frozen semen samples are quite common, although the proportion of each of them is variable. In our study, the main reason is spontaneous pregnancy.

Despite our recommendations, some patients destroyed their samples when reaching normal sperm production; unfortunately we can not be sure whether the sperm production is not genetically or structurally affected, and consequently

until pregnancy is reached samples should be kept frozen (25, 26).

In the worst cases patients could die during or after the anticancer treatment. In these situations ethical and legal questions are the main issue. In Spain, a frozen semen sample can still be used by the man's wife in an IVF cycle in the six months after his death but only if he had authorized it.

Finally, when frozen sperm cells were used for a cycle, good outcome results were obtained, comparable with a control group of infertile couples with tubal infertility and much better than patients with permanent azoospermia after chemotherapy who have not frozen sperm samples before therapy in which testicular spermatozoa were successfully retrieved for ICSI. Moreover, these results are comparable or even better than what has been previously reported in the international literature (27–29). Few cycles of AI were performed, and as shown in Table 3 total capacitated sperm count average was around 1 million cells. Consequently, not many circumstances were adequate for AI, and ICSI was then the commonly chosen procedure.

In summary, all the above-related reasons are enough to conclude that sperm freezing before treatment is the best option in patients with cancer to preserve fertility, and that we must encourage oncology specialists to recommend it.

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